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LAPAROSCOPIC ASSISTED VAGINAL HYSTERECTOMY (LAVH) VERSUS HAND ASSISTED LAPAROSCOPIC HYSTERECTOMY (HALH) IN GYNECOLOGICAL TUMOURS

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Abstract

Objectives and Background: *Hysterectomy is one of the most commonly performed major gynecological procedures required for the treatment of a number of gynecological disorders. The use of laparoscopic techniques now permits combination of benefits of both abdominal and vaginal routes of hysterectomy. Hand assisted laparoscopic surgery was first described in the early 1990s as a surgical method designed to facilitate the performance of challenging laparoscopic procedures while maintaining the advantages of a minimally invasive approach.*

Our present study aims to: *(1) Evaluate laparoscopic assisted vaginal hysterectomy as regard operative time, blood loss, flatulence relief time, postoperative pain, analgesic requirement, early and late operative complications. (2) Compare short and long term clinical results of laparoscopic assisted vaginal hysterectomy and hand assisted laparoscopic hysterectomy. (3) Evaluate the value of hand piece in laparoscopic hysterectomy.*

Materials and Methods: *This randomized prospective study was held at Oncology Center, Mansoura University (OCMU) included sixty one sequential patients scheduled for hysterectomy at Oncology Center, Mansoura University (OCMU) were divided randomizally (patient by patient) into three groups; group 1(control) included 20 patients who un-*

derwent open hysterectomy, group 2 included 21 patients underwent laparoscopic assisted vaginal hysterectomy (LAVH) and group 3 included 20 patients who underwent hand assisted laparoscopic hysterectomy (HALH) From August 2010 to March 2013. Patients were excluded from this study if they had contraindications to either vaginal hysterectomy, such as several priorabdominal surgeries, vaginalstenosis or severe endometriosis, or to laparoscopy,including underlying medical conditions that could be worsened by pneumoperitoneum or the Trendelenburg position. Body mass index (BMI) was not a limiting factor forpatient inclusion in the study.

Results: *The clinical characteristics of the 61 patients were similar as regard follow up duration, age, parity and uterine size.The indications for hysterectomy among the study groups were nearly similar. No statistically significant difference between the two laparoscopic groups in the operative time. Operative time decreased progressively for both laparoscopic groups but more in the HALH group. Operative blood loss was higher in the LAVH group. Two cases in the LAVH group were converted to laparotomy to control bleeding and to repair a urinary bladder tear. The HALH group showed less analgesic consumption, earlier ambulation, shorter hospital stay and earlier regain of daily and coital activities. On the contrary, much more direct costs.*

Conclusion: *According to our study; much more scope should be concentrated in the future towards HALH as our results had shown that the HALH group had less analgesic consumption, earlier ambulation, shorter hospital stay and earlier regain of daily and coital activities. On the contrary, much more direct costs; which requires much effort to be directed towards this fruitful technique and more training programmes to our surgeons to increase their experience enriching hand skills in that emerging technique.*

Key words: *Hand-assisted laparoscopy surgery (HALS), Hysterectomy, Laparoscopic assisted vaginal hysterectomy (LAVH).*

Introduction

Hysterectomy is a procedure in which the uterus is removed surgically for the treatment of a number of gynecological disorders and is one of the most commonly performed major gynecological procedures.(1)

Approximately 494,000 hysterectomies are performed annually in the United States, making this procedure one of the most commonly performed in women of reproductive age.(2)

The optimum approach to hysterectomy would retain the advantage of abdominal route which include clear visualization and ease of manipulation of the adenexial structures, and to combine these features with principle advantage of vaginal hysterectomy namely avoidance of a large abdominal incision. The use of laparoscopic techniques now permits combination of these benefits. But, Laparoscopic hysterectomy has been associated with a higher risk of urinary tract injury compared with abdominal and vaginal procedures, and the risks of these minimally invasive approaches must be balanced with the benefits.(3)

Currently, there are several methods of laparoscopic hysterectomy including laparoscopically assisted vaginal hysterectomy (LAVH), hand assisted laparoscopic hysterectomy (HALH), total laparoscopic hysterectomy (TLH), and more recently, robotic hysterectomy. Three main types of hysterectomy are now used: abdominal, vaginal, and laparoscopic. Laparoscopic assisted vaginal hysterectomy (LAVH) has already gained widespread acceptance since it was first reported by Reich et al in 1989.(4)

Laparoscopic assisted vaginal hysterectomy has become a popular alternative to abdominal hysterectomy in cases difficult to manage via vaginal route alone.(5)

LAVH is now regarded as a safe and feasible technique for managing uterine diseases, because it offers minimal postoperative discomfort, less blood loss, shorter hospital stay, rapid convalescence, and an early return to the activities of daily living.(6)

Hand assisted laparoscopic surgery was first described in the early 1990s to facilitate the performance of challenging laparoscopic procedures while maintaining the advantages of a minimally invasive approach.[7]

In this technique, the surgeon's non-dominant hand is introduced into the abdominal cavity by means of a hand-port device while maintaining pneumoperitoneum. The dominant hand is then used to manipulate instruments in concert with a surgical assistant. Hand-assisted laparoscopy combines the benefits of laparoscopy with advantages of a conventional laparotomy, allowing for improved exposure, manual exploration, blunt dissection, and immediate control of hemostasis.[8]

Materials And Methods

This randomized controlled prospective study was held at Oncology Center, Mansoura University (OCMU) in which 41 patients with uterine tumours in addition to 20 patients control; matching with age were classified into three groups: Group 1: control group, open hysterectomy was done for them(20). Group 2: laparoscopic assisted vaginal hysterectomy (LAVH) was done for them; (21 patients). Group 3: hand assisted laparoscopic hysterectomy (HALH) was done for them; (20 patients).

Patients were excluded from this study if they had contraindicationsto either vaginal hysterectomy, such as several priorabdominal surgeries, vaginalstenosis or severe endometriosis, or to laparoscopy,including underlying medical conditions that could be worsened by pneumoperitoneum or the Trendelenburg position. Body mass index (BMI) was not a limiting factor forpatient inclusion in the study.

Full history,general,abdominal and vaginal examinationswere conducted for every patient. Complete blood count,liver and renal functions and electrocardiography were ordered too. An informed consent for every patient was obtained. All patients underwent the same standard preparation prior tosurgery, including antibiotic prophylaxis and administration of low molecular weightheparin (LMWH).

Group 2 (LAVH) A peritoneal access is performed with a 10-mm sheath placed supraumbilically using closed (Veress needle) or open (Hasson trocar) technique. CO2 is insufflated with a high-flow (>3 L/min) insufflator at pressures <15 mm Hg. The laparoscope is inserted and upper abdominal contents are visualized. The patient is placed in 20º to 30º Trendelenburg position for visualization of the pelvic structures. Additional sheaths are placed under laparoscopic guidance with transabdominal illumination and avoidance of the major vessels. Two 5-mm sheaths are placed approximately 3 to 4 cm medial to and slightly above the level of the anterior superior iliac spines. The inferior epigastric vessels should be avoided when these sheaths are being placed. Additional 10-mm sheath is placed in the suprapubic location.

The bowel is manipulated out of the pelvis with atraumatic forceps to visualize the anatomical landmarks.The course of every pelvic urerter is visualized through the medial leaf of the broad ligament, and its position is verified during each portion of the procedure.

The uterus is placed on lateral traction (with the help of uterine manipulator), and the round ligament and infundibulopelvic on each side is elevated and divided with the endoscopic scissors using monopolar electrocautery. The vaginal phase consists of posterior colpotomy, followed by clamping, cutting,and suture-ligating the remaining paracervicaltissues. The uterine vessels are sought and controlled. After completing the vaginal phase of LAVH, the uterus is removed vaginally and the stump is closed vaginally.

Group 3 (HALH) The procedure is like group 2, but the intra-abdominal hand does most of the retracting action and also tactile sensation of the ureters.After freeying the whole uterus the hand device is removed and the vagina is opened and the specimen is retrieved through the abdomen .the vaginal stump is closed with continuous vicryl sutures. Closure of LAP DISC® wound in two layers first the rectus sheath by vicryl 1-0 then skin

anda pneumoperitoneum is re-created to confirmhomeostasis and re-check for peristalsis of theureters.

Both umbilical and supra-pubic ports are closed in two layers first the rectus sheath by vicryl 1-0 then skin, while the other ports are closed only by skin stitches.

Results

During the period between August, 2010 and March, 2013 (a total of 31 months), 61 patients were enrolled in the study, of these; 21 patients were treated with laparoscopic assisted vaginal hysterectomy (LAVH) and 20 patients were treated with hand assisted laparoscopic hysterectomy (HALH). Together with 20 patients were treated with open hysterectomy(control group).

In our study the clinical characteristics of the 61 patients were similar as regard follow up duration, age, parity and uterine size, the indications for hysterectomy among the study groups were nearly similar Uterine fibroids and endometrial carcinoma had the highest percentage comprising 82% of indications in all groups.

The operative time: (from the insertion of the Veress needle in LAVH group or making the abdominal incision for the hand piece in HALH group to skin closure at the last trocar incision site). The mean operative time of HALH was shorter than that of LAVH, (123.50 min and 131.67 min respectively) but this variation was not statistically significant.

A very important observation was that for both procedures, there was a decline of the operative time with progress of the study (160 min to 105 min in first group and 190 min to 95 min in second group). We compared the operative time between the first half of cases and the second half in both groups. The second group (HALH) showed a statistically significant more decrease in the operative time.

Estimated blood loss, the need for blood transfusion, and haemoglopin reduction were higher in LAVH group, but the difference is

not statistically significant.

Two cases (9.5%) of LAVH group needed laparotomy to control bleeding in one case and bladder injury was detected intra operatively during bladder dissection in one case of LAVH group.No difficulty was met in delivering the uterus in any case in both groups. We did not do any morcellation for the specimens. No bowel or ureteric injuries occurred.No conversion was need in the HALH group.

Hospital stay in the HALH group was shorter (3.45 days) than the LAVH group (4.57). This difference was statistically significant (p=0.007). Five cases (12.2%) had fever: four in the LAVH due to urinary tract infection (three cases) and wound infection (one case, this case was that one who had laparotomy to control bleeding) and one case in the HALH group due to wound infection.

No statistically significant difference was found between both groups as regard resumption of ordinary daily activities. But the mean duration of resumption of coital activities (if there were) was significantly lower in the HALH group (47.67 days) versus the LAVH group (58.00 days).

We found no statistically significant relation between uterine size and operative time and estimated blood loss. On the other hand, both time to begin ambulation and to regain daily activities are strongly related to operative time (p=0.001, p=0.006 respectively).

Discussion

In most studies about laparoscopic hysterectomy, dysfunctional uterine bleeding is a major indication. This is different from our study which is restricted to cases with tumors.In our study, the indications for hysterectomy among the study groups were nearly similar with uterine fibroids and endometrial carcinoma had highest percentage comprising 78% of indications in both groups with no statistically significant difference.

Our series of laparoscopically assisted vaginal hysterectomy with mean operative time of 131.5 minutes is comparable to other studie:178.0 min[9], 253.8 min[10], 270min[11], 120min[12], 144 min

[13], 12.5min[14], and102 min[15].

Estimated blood loss, the need for blood transfusion, and haemoglopin reduction were higher in LAVH group, but the difference is not statistically significant. Mean estimated blood loss in the LAVH group was 532.2 ml which is higher than other studies: 105.13 ml [9], 433.6ml[10], 500ml[11], 200 ml[12], 457ml[13] and 314ml[15].

In our study, there were no relations between the uterine size and the operative time or the rate of complications. But our study cannot efficiently address this issue because from the start the patient group is selected with avoidance of relatively large uterei. At our institution, we are not familiar with morcellation because most of our patients have malignant or potentially malignant conditions.

Shiota et al. compared the surgical results (blood loss, operative time, rates of conversion to laparotomy, and intraand postoperative complications) among 9 groups classified by uterine weight. Statistically significant differences in surgical outcomes were found between the group with a uterine Weight ≥ 800g and the other groups.So when the uterine weight was ≥ 800g, TAH was more appropriate because significant blood loss and/or complications would be expected during LAVH. A removed uterus weighing 800g is reportedly equivalent to a preoperative uterine size of approximately 12cm. Therefore, LAVH may be safely indicated for patients with a uterine size ≤12cm (approximately equivalentto the uterine size at 16-weeks' gestation).[16]

Strategic considerations are in the majority of cases (69%) the reason for converting laparoscopic hysterectomy to the conventional abdominal approach. Visibility and/or mobility problems are the main reason for this type of conversion, while uncontrollable bleeding is the main adverse event leading to a reactive conversion. As reported in other studies, BMI and uterus weight are confirmed to be independent risk factors for conversion.[13]

Hospital stay in the HALH

group was shorter (3.45 days) than the LAVH group (4.57 days). This difference was statistically significant (p=0.007). Duration of hospital stay in our study is comparable to other studies 5 days [12], 4.5 days[17] and 3.79 days [14]. Asian, especially, Korean studies reported longer durations of hospital stay: 7 days[10] and 10 days[11].

We also found no statistically significant difference between both groups as regard resumption of ordinary daily activities (mean time is 24 days). But the mean duration of resumption of coital activities (if there were) was significantly lower in the HALH group (47.67 days) versus the LAVH group (58.00 days). Yi et al., in a meta-analysis,found this period to vary between 21 to 30 days (mean is 25 days).[18]

For all malignant cases in the study, there were no residual or recurrent tumors. The relatively small number and the short interval of follow up make this study inappropriate to discuss the effect of various laparoscopic approaches on the oncologic aspects.

Key Messages:

The hand assisted laparoscopic technique was successfully developed and manual access to the laparoscopic field facilitated completion of anotherwise minimally invasive procedure.

We demonstrated that hand assisted laparoscopic hysterectomyis technicallyfeasible, and in selected cases may provide an alternative to conventional techniques of hysterectomy.

Fewer complications were attributable directly to the HALH technique.

We feel that modifications in the technique that reduce surgical time would be beneficialand that careful case selection and preparation is important for a successful outcome.

We anticipate that the HALS technique could be readily modified for development of other minimally invasive procedures of theestablished laparoscopic procedures.

In our study the direct cost of hand assisted laparoscopic hyster-

ectomy was much more than laparoscopic hysterectomy, because the LAP DISC® alone costs about 850 bounds. So we recommend its usage in patients with large uterie as the indirect costs of conventional laparotomy may exceed the direct costs of hand assisted surgery.

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PREVALENCE OF OCCUPATIONAL BURNOUT AMONG MANSOURA UNIVERSITY HOSPITALS' RESIDENTS AND ASSISTANT LECTURERS

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Abstract

Background: *Burnout syndrome occurs frequently amongst health-care workers. It has a detrimental effect on the patient–physician relationship. Little is known about the prevalence and causes of burnout amongst Mansoura university hospitals' residents and assistant lecturers.*

Methods: *An anonymous questionnaire was distributed to a representative sample of Mansoura university hospitals' residents and assistant lecturers (n = 182). It included demographical data, burnout level (Maslach Burnout Inventory). Validated scales were used when available.*

Results: *The response rate was 77% (140/182). Emotional exhaustion (EE) and Depersonalisation (DP), the major components of burnout, were reported, respectively, by 80 % (n = 112) and 45.7 % (n = 64) of the residents and assistant lecturers.*

Conclusion: *The burnout level is high amongst Mansoura university hospitals assistant lecturers and residents. Interventions are needed and could include support groups, more intense coaching by senior*

Introduction

Burnout is a professional psychological stress-induced syndrome defined by the three dimensions: emotional exhaustion, depersonalisation and low personal accomplishment.[1–3]

Its prevalence is high amongst physicians. Whippen and Canellos randomly surveyed 1000 oncologists and showed that 56% of them reported being burnt out.[4]

In a recent meta-analysis of 10

observational studies in oncology, the overall prevalence of high emotional exhaustion, depersonalization and low personal accomplishment were, respectively, 36% (95% confidence interval (CI): 31 41), 34% (95% CI: 30–39) and 25% (95% CI: 16–34).[5]

Burnout has a detrimental effect on the physician's quality of life and is associated with an increased risk of suicidal ideation.[6] It has also been linked to poorer quality of care, increased medical errors and lawsuits, decreased empathy[6,7], job withdrawal and absenteeism.[8]

Some medical specialties are at higher risk of burnout. Although a study comparing burnout amongst residents in various medical specialties in the United States reported no significant differences between specialties9, two Finnish studies.[10,11]

Reported more burnout amongst doctors who more often treat chronically ill, incurable or dying patients. Oncology was one of these specialties. The factors associated with stress and burnout in Oncology are insufficient personal or vacation time, a sense of failure, unrealistic expectations of patients, cognitive or ethical dissonance, repeated losses and grieving or problems concerning managed care.[12]

Burnout is highly prevalent amongst medical residents. Reported levels of burnout attained 76% amongst the residents in an internal Medicine programme7 and 49.6% amongst US medical students.6 However, the prevalence and causes of burnout amongst oncology residents have never been properly studied.

The aims of this study were to quantify the frequency of burnout amongst oncology residents and assistant lecturers, to determine demographical and psychological factors associated with burnout.

Methods

A descriptive cross sectional study carried out targeting resident physicians and assistant lectures at Mansoura university hospitals to estimate the prevalence of burnout syndrome among residents in the hospital.

1. Sampling and Sample Size:

A stratified proportional sampling technique was used to withdraw the estimated sample 182 residents and assistant lecturers from different departments in different Mansoura university hospitals in the first phase of the study. The number of the responders was 140 (77%) residents and assistant lecturers.

2. Measurement Instrument:

The survey included eight demographic questions before the Maslach Burnout Inventory-Human Services Survey MBI-HSS. The survey was designed by the researcher to collect specific demographic information from the Residents and Assistant Lecturers.

The items on the demographic portion of the survey consisted of personal and professional information such as age, gender, clinical department, number of Experience years, number of working hours per week, monthly income and Residence. The demographic portion of the survey contained a small number of questions (eight). This survey intentionally collected only minimal personal information about the participants to avoid identifying the participant and to maintain strict confidentiality.

3. Procedure:

The Maslach Burnout Inventory-Human Services Survey pencil, self-report measure designed to assess the three components of the burnout syndrome: emotional exhaustion, depersonalization, and reduced personal accomplishment (Maslach & Jackson, 1986). There are 22 items which are divided into three subscales and the items are written in the form of statements about personal feelings or attitudes such as “I feel burned out from my work,” “I don’t really care what et al., 1996). Respondents answer using a seven-point Likert-type scale, with the two extremes of “never feel the effects” (0) and “feel the effects every day” (6). Scores are generated for each subscale by adding the numeric responses for the items corresponding with each scale. Subscale scores range from a low of 0 to a high of 54 on the EE subscale, from 0 to 30 on the DP subscale, and from 0 to 48 on the PA subscale (Maslach & Jackson, 1986). A high degree of burn-

out is reflected by high scores on the Emotional Exhaustion and Depersonalization subscales and a low score on the Personal Accomplishment subscale. The scores for each subscale are considered separately and are not combined into a single, unitary score, and subsequently the three scores are computed for each respondent (Maslach et al., 1996).

The specific categorization of low, Moderate, or high burnout for medical occupations is shown in Table 1 below and by definition a high degree of burnout is reflected by high scores on the Emotional Exhaustion and Depersonalization subscales and a low score on the Personal Accomplishment scale (Maslach et al., 1996).

4. Data Collection:

The research for this study was conducted at Mansoura University Hospitals. To garner support for this study the researcher first engaged in a series of requests and approvals.

The survey was distributed to the Residents and Assistant Lecturers in Mansoura University Hospitals for 182 Participants. The researcher provided the needed information about the survey to all participants so that they would have a clear understanding of the study and would understand their voluntary participation in this study. Each participant completed the survey in paper and pen format. The survey was expected to take approximately 10 minutes to complete.

The participants were informed that the information provided in the instrument would be kept confidential.

Results

Table 1: socio-demographic Characteristics of the studied group (n=140 subjects).

	Overall	Surgery	Medicine	Pediatric
Physicians: N (%)	140	47 (33.6%)	70 (50%)	23 (16.4%)
Mean Age: (SD)	28.9 (2.5)	29.1 (2.9)	28.9 (2.3)	28.6 (2.3)
Median Age:	29	29	28	28
Gender: N (%)				
Male	76 (54.3%)	47 (100%)	20 (28.6%)	9 (39.1%)
Female	64 (45.7%)	0 (0%)	50 (71.4%)	14 (60.9%)
Residence: N (%)				
Urban	128 (91.4%)	43 (91.5%)	64 (91.4%)	21 (91.3%)
Rural	12 (8.6%)	4 (8.5%)	6 (8.6%)	2 (8.7%)

Table 2: Burnout Level According to Exhaustion Levels in the studied group (n=140 subjects).

Burnout LeveL (Exhaustion)	Frequency	Percent	Cumulative Percent
Low	8	5.7	5.7
Moderate	20	14.3	20.0
High	112	80.0	100.0
Total	140	100.0	

Table 3: Burnout Level According to Depersonalization Levels in the studied group (n=140 subjects).

Burnout Level (Depersonalization)	Frequency	Percent	Cumulative Percent
Low	28	20.0	20.0
Moderate	48	34.3	54.3
High	64	45.7	100.0
Total	140	100.0	

Table 5: Burnout Level According to Personal Achievement Levels in the studied group (n=140 subjects).

Burnout Level (Personal Achievement)	Frequency	Percent	Cumulative Percent
High	60	42.9	42.9
Moderate	44	31.4	74.3
Low	36	25.7	100.0
Total	140	100.0	

Discussion

This study demonstrated that the prevalence of burnout are high amongst Mansoura University Hospitals residents and Assistant Lecturers. An excessive workload, psychosomatic disorders or anxiolytics intake were independently associated with increasing emotional exhaustion scores.

Although this study provides a number of important contributions, it is important to point out its limitations. First, as the design of the study was cross-sectional, it does not allow causal interpretations between job characteristics and health-related variables. Respondents with poor psychological well-being, i.e. high burnout, may have reported a negative work environment. Nevertheless, longitudinal studies have shown that perceived work characteristics were predictive of psychological distress and not the reverse.[22,23]

Second, the present study, like most burnout and stress studies, is based on self-reported measures.[24]

Which could influence the statistical analysis. Indeed, the independent and dependent variables are based upon a single source of information, the participants.[25]

This can result in an overestimation of the main effects by inflating the association between perceived work environment factors and strain indicators. However, as pointed out by Spector,[26] there is a high consistency between objective and subjective ratings of variables such as those used in our study.

Despite the limitations discussed above, this study has many strengths. Firstly, it is the first multi-institutional study on the subject. Secondly, the response rate is high, somewhat higher than those usually found in studies on medical students.[27] thirdly; the study included residents and assistant lecturers in medical, surgical and pediatric fields, and thus evaluated the three clinical specialties of healthcare service.

The prevalence of burnout in this study is consistent with the levels reported in other studies

amongst healthcare workers[5] or amongst residents and medical students[6,8] or with levels expected due to the internal validity of EE or DP scores.[2] However, there is a lot of heterogeneity between burnout levels reported by these studies. Indeed, virtually all the studies used the MBI to quantify burnout levels, but the cut-offs applied were usually those published in the initial study,[2] which are probably not relevant for all of these populations. Cut-offs should be adapted to take into account social and cultural differences.[1]

Factors associated with burnout have previously been studied in oncology[12,28] and reported in a meta-analysis.[5]

The most important ones are related to workload, insufficient personal or vacation time, feeling of being fallible as a doctor, excessive number of deaths, emotions and particularly emotional dissonance and problems related to the working environment (excessive paperwork team communication-Difficulties...).

As residents are the future oncology physicians, their needs and aspirations should be taken into account, especially these days when cancer incidence is on the increase[29,30] and the crude number of oncologists may become too low in some countries. Indeed, some countries are facing a demography crisis in oncology and should take notice of the fact that, as shown in our study, burnout is strongly associated with a desire to change specialty, or even to quit Medicine.

In other studies, burnout was associated with job absenteeism, intention to leave the organization and job turnover.[8]

Fatigue and depression have been associated with increased perceived medical errors.31,32 Interestingly, burnout was not always associated with higher rates of medical errors.[32,33]

Burnout is detrimental to the patient–physician relationship in general Medicine.[34] As such, they should be taken into account by university hospitals to improve both student wellness and patient care. To limit the incidence of

stress and burnout, Shanafelt et al have proposed a multistep process including the identification of professional goals, the choice of the most fitting type of practice and the management of the stressors specific to that practice type. This process allows to determine how to balance competing personal and professional goals.[35]

Intervention studies are needed, e.g. support groups, more intense coaching by senior physicians, training programs on 'breaking bad news' and teaching of stress management skills to study how to prevent or reverse burnout. Such courses are feasible and have proven their ability to reduce the levels of burnout in the short and long term, to improve physicians' well-being and attitudes associated with patient-centered care.[37]

Recovery from burnout is possible and was associated with a reduction of suicidal ideation in a population of U.S. medical students, confirming that burnout is a reversible phenomenon.[6]

In brief, this study shows that the prevalence of burnout is high amongst Mansoura University Hospitals Assistant Lecturers and residents and is associated with a poorer perceived health status and the will to quit Medicine or change specialty.

Medical schools and University hospitals should consider this a cause for concern and develop screening strategies and intervention programs to improve residents' wellness. The results of our study need to be confirmed and confronted other major western countries. Nevertheless, a close and confident relationship between teachers and junior doctors needs to be maintained and enhanced during this intense period of training. Comparisons with senior oncologists or residents of other specialties are ongoing to evaluate factors related to burnout that could be specific to oncology or to residents.

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PREVALENCE OF OCCUPATIONAL BURNOUT AMONG MANSOURA UNIVERSITY HOSPITALS' RESIDENTS AND ASSISTANT LECTURERS

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GLYPICAN-3 EXPRESSION IN HEPATOCELLULAR CARCINOMA IN RELATION TO THE GRADE OF DIFFERENTIATION

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Abstract

Background: *Glypican-3 (GPC-3) is an oncofetal protein normally expressed in fetal liver and placenta but is not found in normal adult liver. GPC-3 expression has been reported in 75–100% of hepatocellular carcinoma (HCC). It has also been suggested that poorly differentiated hepatocellular carcinomas are more likely to express GPC3. The aim of this study was to assess the diagnostic value of GPC-3 immunostaining in HCCs and to analyze its expression profile in relation to the grade of differentiation.*

Material and methods: *This study was performed on 58 cases of formalin-fixed, paraffin-embedded cases of HCC obtained from the files of pathology laboratory of GastroEnterology Center, Mansoura University from 2009 to 2012. The H&E slides were reviewed to confirm the diagnosis and assess the grade of differentiation. The following cases were studied: well differentiated- HCC (WD-HCC) (n=16), moderately differentiated HCC (MD-HCC) (n=22), and poorly differentiated HCC (PD-HCC) (n=20)*

All cases were immunostained with GPC-3.

Results: *Among the 58 cases of primary HCC, GPC-3 expression was observed in 84.4% of cases. The staining was diffuse in 62% of cases and focal in 22.4 % of cases. GPC3 expression was significantly higher in PD-HCC than in WD- and MD-HCC (P value, 0.017).*

Conclusion: *Our data demonstrate that GPC-3 has high sensitivity to HCC and is more expressed in poorly differentiated tumors. As such, we recommend that this marker should be included in any antibody*

panel used to distinguish HCC from cholangiocarcinoma and metastatic carcinoma to increase diagnostic accuracy.

Introduction

Hepatocellular carcinoma (HCC) is the most common malignant primary tumor of the liver[1]. HCC affects about a million people every year worldwide[2]. In Egypt the incidence of HCC has doubled in the past 10 years, thus becoming the second most incident and lethal cancer in men[3].

Accurate diagnosis is critically important to appropriate clinical management of the patients and assessment of the prognosis. The histologic diagnosis of HCC is relatively straightforward when the tumor recapitulates the cytoarchitectural appearance of the normal liver. However, HCC exhibiting a pseudoglandular or poorly differentiated morphology may be difficult to distinguish from cholangiocarcinoma or metastatic adenocarcinoma involving the liver [4].

The presently employed immunohistochemical panels have greatly facilitated the diagnosis of HCC. However, there are several situations where these markers are of limited use. Hep Par 1 is a highly sensitive and specific marker of benign and malignant hepatocytes (80%–90% sensitivity, nearly 100% specificity). However, expression is decreased in poorly differentiated HCC and in the sclerosing variant of HCC, which can show immunoreactivity in only 50% of cases. Similarly, polyclonal CEA have low sensitivity (~50%) for the diagnosis of poorly differentiated hepatocellular carcinoma [4,5]. Hence, Hep Par 1 and polyclonal CEA may be less helpful in the setting of a poorly differentiated hepatic neoplasm in distinguishing hepatocellular carcinoma and metastatic adenocarcinoma.

Glypican-3 (GPC-3) is an oncofetal protein and is a member of the membrane-bound heparin sulfate proteoglycans. This protein is normally expressed in fetal liver and placenta but is not found in normal adult liver. It plays a role in cell growth, differentiation, and migration[6]. GPC-3 is highly expressed, both at the mRNA and

protein level, in HCCs. It has also been suggested that poorly differentiated hepatocellular carcinomas are more likely to express GPC-3[7]. The aim of this study is to assess the diagnostic value of GPC3 immunostaining in hepatocellular carcinomas and to analyze its expression profile in relation to the grade of differentiation.

Materials and Methods

Cases

This study was performed on 58 cases of formalin-fixed, paraffin-embedded cases of HCC obtained from the files of pathology laboratory of GastroEnterology Center, Mansoura University from 2009 to 2012. Twelve specimens were trucut needle biopsy, and 46 were from partial hepatectomy specimens. The available paraffin blocks were sectioned at 4-5 microns and stained with haematoxylin and eosin. The slides were reviewed to confirm the diagnosis according to the guidelines of the WHO[8]. The studied tumors included: Well differentiated- HCC (WD-HCC) (n=16), moderately differentiated HCC (MD-HCC) (n=22), and poorly differentiated HCC (PD-HCC) (n=20).

Immunohistochemistry

Monoclonal antibody to GPC-3 (Clone 1G12, 1:300, Biocare, Concord, CA), was used for staining all cases of the study. Previously validated slides were used as positive control samples. Slides using phosphate-buffered saline instead of primary monoclonal antibody were regarded as negative control samples. The sections were deparaffinized and rehydrated in graded alcohol. Endogenous peroxide was blocked by 3% hydrogen peroxide treatment, and the antigen was retrieved using sodium citrate treatment in a microwave oven at 98℃ for 10 minutes. Following peroxidase block and incubation with primary antibody for 30 min at room temperature, the sections were incubated with horse-radish peroxidase-labeled secondary antibody (UltraVision ONE HRP Polymer; Catalog no.TL-015-HDJ; Thermo, Fermont, CA) for 30 minutes at room temperature, DAB Plus substrate chromagen for 15 min, and counterstained with hematoxylin.

Staining was considered positive when immunoreactivity was present in at least 5% of lesional

hepatocytes and located in the cytoplasm and/or membrane. The results of immunohistochemical staining with GPC-3 in hepatocellular lesions were recorded as negative (0–4% of tumor cells), focal positive + (5–50% of tumor cells) and diffuse positive ++ (>50% of tumor cells) based on visual estimation of the entire tumor on the slide[9].

Statistical Analysis

Data were described as number and percentage. Categorical data were compared with the chi-square test or Fisher exact test when appropriate. P values <0.05 were considered statistically significant. Statistical analyses were performed with SPSS 16.0 software.

Results

Among the 58 cases of primary HCC, GPC-3 expression was observed in 49 cases (84.4%). All positive cases showed cytoplasmic pattern of staining, with additional membranous accentuation in 10 cases. Three cases expressed prominent staining adjacent to canaliculi. The staining was diffuse in 36 cases (62%) and focal in 13 cases (22.4%).GPC3 expression was significantly higher in PD-HCC than in WD- and MD-HCC (P=0.017) whereas growth pattern was not significantly related to GPC-3 expression (Table 1).

Table 1: Relation between GPC3 Expression and histologic features in conventional HCC.

Histological feature	GPC-3 staining				P-value
	Negative		Positive		
	No.	%	No.	%	
Grade					
WD-HCC	6	37.5	10	62.5	
MD-HCC	2	9	20	91	0.017*
PD-HCC	1	5	19	95	
Growth patterns					
Trabecular	4	19	17	81	
Compact	1	5	19	95	0.263
Pseudoglandular	4	23.5	13	76.5	

WD: Well-differentiated, MD: Moderately-differentiated,
PD: Poorly-differentiated *(P<0.05= significant).*

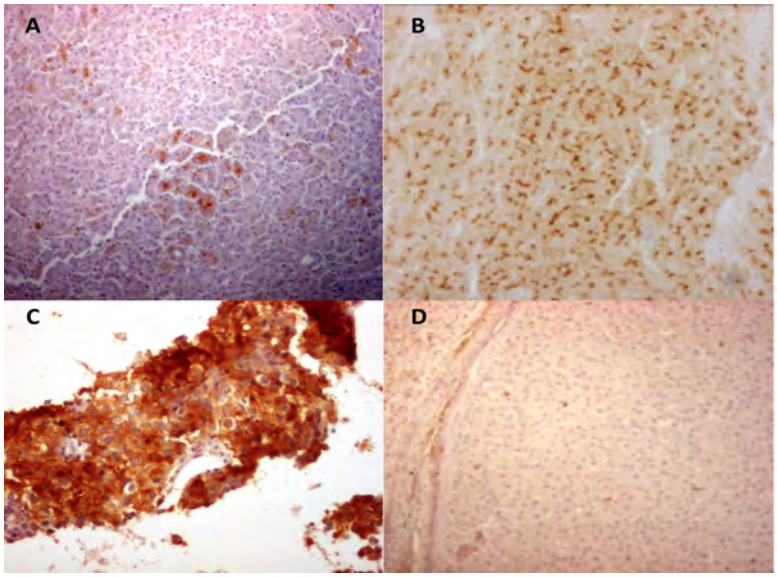

FIG.1: A): WD-HCC showing focal positive GPC-3 immunostaining (GPC-3 100x), **B):** diffuse positive GPC3 with prominent staining adjacent to canaliculi (GPC-3 100x), **C)** PD-HCC showing diffuse cytoplasmic GPC-3 staining (GPC-3 200x), **D):** MD-HCC showing negative GPC3immunostaining (GPC3 100x).

Discussion

The differential diagnosis of HCC varies, depending on the degree of tumor differentiation. Work-up often requires immunohistochemical stains, which should be judiciously selected based on the H&E morphological pattern and the differential diagnosis.

Several studies have demonstrated the efficacy of GPC-3 as a diagnostic tool in HCC. The reported sensitivity ranges from 75-100%, with figures of 75-5% in larger series[10,11,12,13]. Our results are similar to the literature, with 84.4% of hepatocellular carcinoma being positive for GPC-3. The staining was diffuse in 62% of cases and focal in about 22%; indicating that the focal staining detectable in a fraction of HCCs might cause false negative results in tiny liver biopsies, a finding that was previously reported by Shafizadeh et al[7].

Poorly differentiated hepatocel-

lular carcinomas pose a diagnostic problem as they can mimic metastatic neoplasms. As mentioned previously, many earlier studies have demonstrated that the commonly used hepatocellular markers like Hep- Par 1 and pCEA have low sensitivity for poorly differentiated hepatocellular carcinoma [4,14,15].

The results of the current study showed that the extent of GPC-3 immunoreactivity was significantly related to the tumor grading, because less differentiated HCCs had a greater immunoreactivity. This finding is consistent with that reported by Di Tommaso et al [16] and Shirakawa et al[11]. In other studies, the opposite was described[12], or there was no association with grade[7,10&13]. This discrepancy may be related to variation in number of studied cases, different cutoff point of GPC-3 positivity (5% vs. 10%), or to the type of sample (whole sections vs. Tissue microarray).

In conclusion, our data confirm previous observations regarding the high rate of expression of GPC-3 in HCC, particularly poorly differentiated tumors. As such, we recommend that this marker should be included in any antibody panel used to distinguish HCC from cholangiocarcinoma and metastatic carcinoma to increase diagnostic accuracy.

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GLYPICAN-3 EXPRESSION IN HEPATOCELLULAR CARCINOMA IN RELATION TO THE GRADE OF DIF-FERENTIATION

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EFFECTS OF TAMOXIFEN ON LIPID PROFILES IN POST-MENOPAUSAL BREAST CANCER PATIENTS

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Abstract

Background & Objective: *The risk of cardiovascular mortality increases dramatically in women after menopause because of lipid-metabolism alterations that are attributed to estrogen deprivation. Tamoxifen is the usual endocrine (anti-estrogen) therapy for hormone receptor-positive breast cancer in pre-menopausal women, and is also a standard in post-menopausal women although aromatase inhibitors are frequently used in that setting. The long-term use of anti-estrogen agents showed a potential to improve lipid profiles in post-menopausal breast cancer patients. The present study has been undertaken to assess the effect of tamoxifen therapy on plasma lipid profile in postmenopausal breast cancer patients.*

Patients & Methods: *The study population consisted of 36 postmenopausal, primary operable breast cancer patients treated with surgery in the form of a total mastectomy or a breast-conserving resection with axillary dissection. The patients were adjusted for adjuvant chemotherapy and or radiation therapy and allocated to tamoxifen 20 mg daily . Serum lipid profiles evaluated were total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG). Time points for blood collection were before the start of administration of tamoxifen, 3 and 6 months after the start of its administration.*

Results: *The mean level of plasma total cholesterol was significantly decreased (P<0.001) after 3 and 6 months of tamoxifen treatment compared with mean baseline levels. Also , significant decreases were observed in mean LDL-C (P<0.001). At 3 and 6 months' evaluation, a trend toward increase of plasma triglycerides and HDL cholesterol levels*

were observed but, it didn't reach statistical significance.

Conclusion: *In conclusion, favorable changes of lipid profiles were detected in postmenopausal patients with breast cancer treated with tamoxifen.*

Keywords: *Tamoxifen, lipid profiles, post-menopause, breast cancer.*

Introduction

Breast cancer is the most frequently diagnosed invasive cancer in women, with more than 1.3 million women worldwide are diagnosed with breast cancer each year, making it the second most common form of cancer behind lung cancer[1]. The increased number of breast cancer diagnoses along with improvements in initial treatments, have led to an increase in the number of breast cancer survivors[2].

In postmenopausal women with endocrine-responsive early breast cancer, adjuvant hormonal therapy is the established standard of care. In postmenopausal women, the two most commonly used strategies of endocrine treatment are either the interference with estrogen signaling by binding to the estrogen receptor protein with a selective estrogen-receptor modulator(SERM), such as tamoxifen, or the inhibition of endogenous estrogen production by using an aromatase inhibitor (AI)[3].

Tamoxifen is the usual endocrine (anti-estrogen) therapy for hormone receptor-positive breast cancer in pre-menopausal women, and is also a standard in post-menopausal women although aromatase inhibitors are also frequently used in that setting[4].

The long-term use of anti-estrogen agents showed a potential to improve lipid profiles of post-menopausal breast cancer patients ,which attracted attention in both research and clinical settings[5].

The risk of cardiovascular mortality increases dramatically in women after menopause because of lipid-metabolism alterations that are attributed to estrogen deprivation[6]. Levi etal. suggested that the greatest cause of death in women with early-stage breast cancer is heart disease[7].

The role of low-density lipoprotein cholesterol (LDL) in the pathogenesis of atherosclerosis and subsequently in coronary heart disease is well known. Evidence suggests that increased levels of LDL are highly correlated with increased risk of heart disease, even while total cholesterol remains within normal range[8]. At the same time, high-density lipoprotein (HDL) cholesterol is known to have a protective effect against coronary heart disease[9]. The role of triglycerides is less clear, but increased levels have been associated with risk of cardiovascular diseases in both women and men[6].

Some trials demonstrated that tamoxifen shares the beneficial effects of estrogens on cardiovascular risk factors by decreasing total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) both in pre-menopausal and postmenopausal women[10]. It produces no significant change in triglycerides (TGs), high density lipoprotein cholesterol (HDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels in premenopausal and postmenopausal women[11].

Cholesterol also is the precursor to steroid hormone synthesis and endogenous sex steroid hormones which are directly related to breast cancer risk[12]. On the other hand, an inverse association was observed between high-density lipoprotein cholesterol (HDL-C) level and breast cancer [13].

The present study has been undertaken to assess the effect of tamoxifen therapy on plasma lipid profile in a sample of egyptian postmenopausal breast cancer patients.

Materials and Methods

This prospective, study was conducted from June 2010 to December 2012 at the Clinical Oncology and Nuclear Medicine Department and the Clinical Pathology Department, Mansoura University Hospital.

A total of 36 postmenopausal patients with primary operable breast cancer between 48 and 70 years of age were included in this study.

Patients included in the trial satisfied the following entry criteria: (1) all demonstrated a postmenopausal status (defined as either no menses for more than 1 year or shorter duration of amenorrhea with follicle-stimulating hormone [FSH] levels in the postmenopausal range); (2) all had undergone breast surgery (either lumpectomy or mastectomy) and were considered to be potentially curable; (3) none had received either radiation or chemotherapy before breast surgery; (4)estrogen receptor ER positivity was confirmed by histopathology; (5) none had either diabetes mellitus, renal or hepatic disease; (6) none had received drugs known to affect the lipid and lipoprotein levels; and (7) all were instructed to follow their usual diet and maintain weight during the study period.

All patients underwent surgery in the form of a total mastectomy or a breast-conserving resection with axillary dissection. The patients were adjusted for adjuvant chemotherapy and or radiation therapy and allocated to tamoxifen 20 mg daily .

Serum samples were collected after overnight fasting (12-14 hours). Time points for blood collection were before the start of administration of tamoxifen, 3 and 6 months after the start of its administration. Serum lipid profiles evaluated were total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG). Lipid profile assay was measured by standard enzymatic methods (Human GmbH Germany). LDL-C values were calculated using the equation of Friedewald[14]. Diagnostic criteria for lipid abnormalities are as follows: hyper-LDL-cholesterolemia, ≥140 mg/dl; hypo-HDL-cholesterolemia, <40 mg/dl; and hypertriglyceridemia, ≥150 mg/dl.

Statistical Methods

Basic descriptive statistics, including means, standard deviations (SD), ranges, and percentages, were used to characterize the study participants. Changes in lipid profiles were analyzed using Student's paired t-test based on differences between mean values before administration of tamoxifen

(baseline) and mean values of 3 and 6 months after administration (significance level ≤ 0.05). Statistical analysis was done using Statistical Program for Social Sciences (SPSS 17.0).

Results

Patient characteristics, including age, weight, stage, prior adjuvant chemotherapy or radiotherapy, and ECOG performance status are presented in Table 1.

A total of 36 patients underwent lipid profile evaluation at baseline, but decreased to 32 and 31 patients at 3 and 6 months evaluation, respectively.

Table 2 shows the mean lipoprotein levels at baseline, 3 months, and 6 months in patients receiving tamoxifen.

The mean level of plasma total cholesterol was significantly decreased (P<0.001) after 3 and 6 months of tamoxifen treatment compared with mean baseline levels. Also, similar decreases were observed in mean LDL-C (P<0.001).

At 3 and 6 months' evaluation, a trend toward increase levels of HDL cholesterol was observed, but these values were statistically non significant in comparison to pre tamoxifen treatment (P =0.32), (P =0.06) respectively.

Similarly, a trend toward increase levels of plasma triglycerides were detected at 3 and 6 months' evaluation but , it didn't reach statistical significance(P =0.14), (P =0.09) respectively.

Tamoxifen significantly decreased T-C by 8.06 mg/dl at 3 months and by 21.45 mg/dl at 6 months (p< 0.001) and also decreased LDL-C by 10.89 mg/dl at 3 months and by 22.45 mg/dl at 6 months (p< 0.001) (Table 2).

Table (1): Patients Characteristics.

Patient characteristics	No of patients = 36	Percent=100%
Age (years)		
Median	59	
Range	48 – 70	
Weight (Kg)		
Median	84	
Range	69-105	
ECOG performance status		
0	14	38.89%
1	22	61.11%
Stage*		
I	3	8.33%
IIA	5	13.89%
IIB	13	36.11%
IIIA	15	41.67%
Adjuvant chemotherapy		
Yes	31	86.11%
no	5	13.89%
Adjuvant radiotherapy		
Yes	29	80.56%
no	7	19.44%

* Tumor extent based on the 7th edition of the American Joint Committee Cancer Staging Manual.

Table (2): Effect of tamoxifen on plasma lipids in postmenopausal breast cancer patients .

Parameters	0 day (baseline) No of patients = 36 mean ±SD	3months No of patients = 32 mean ±SD	6months No of patients = 31 mean ±SD
TC(mg/dl)	207.56±10.04	199.5±8.66*	186.11±5.18*
LDL(mg/dl)	157.28±4.99	146.39±6.08*	134.83±5.28*
HDL(mg/dl)	42.89±1.32	43.50±2.18	44±2.06
TG(mg/dl)	148.89±6.30	149.72±6.66	150±5.25

*=significant

Discussion

Epidemiologic studies have provided data to support the finding that elevated LDL-C and triglycerides levels, and reduced HDL-C levels are important risk factors for developing cardiovascular disease[15]. Overall, a 1 mg/dL increase in HDL cholesterol decreases the risk of CHD by 2-3%[16]. A 10 mg/dL increase in LDL cholesterol increases the risk of cardiovascular disease by 12% [17].

Hypercholesterolemia and hypertriglyceridemia are more common in post-menopausal women compared with pre-menopausal women, and the primary cause is thought to be decreased blood estrogen concentrations[18].

It is generally accepted that adjuvant hormonal treatment with tamoxifen has a beneficial effect on serum lipids of postmenopausal breast cancer patients, by decreasing plasma total cholesterol and low density lipoprotein (LDL) cholesterol levels[19]. Tamoxifen also may increase plasma triglyceride (TG) levels [20].

In the present study, we evaluated the effects of tamoxifen on plasma lipid profile in postmenopausal patients with breast cancer. Tamoxifen was found to cause significant reduction in TC and LDL-C, while non significant increased levels of plasma triglycerides and HDL cholesterol were observed. These results are in accordance with those of Sawada et al., who demonstrated that tamoxifen significantly decreased T-C by 13.4% (p=0.001) and LDL-C by 23.5% (p<0.001)[21].

Our results agree also with those previously reported by Gupta et al where postmenopausal patients after tamoxifen therapy showed significant reduction in total cholesterol levels by 10.42 mg/dl at 3 months (P<0.001) and by 16.42 mg/dl (P<0.001) at 6 months. LDL-c also significantly decreased by 12.5 mg/ dl at 3 months (P<0.001) and by 21.26 mg/dl at 6 months (P<0.001), with the peak decrease in the LDL-c at 6 months. Triglycerides levels increased by 1.00 mg/dl at 3 months and by 2.6 mg/dl at 6 months but these values were statistically non significant in comparison to pre drug treatment. Similar increase in HDL-c levels from baseline value was observed after 3 and 6 months respectively, but it was statistically non significant[19].

Final findings from another study confirm the protective effect of tamoxifen on the lipid profile and indicate an overall trend for increasing cholesterol levels after cessation of tamoxifen[22].

In a study comparing the effect of toremefine and tamoxifen on

lipid profile of postmenopausal breast cancer patients ,Tominaga et al showed the beneficial effect of tamoxifen to lower TC and LDL-C, where the tamoxifen group (n=120) showed significantly decreased total cholesterol (P<0.001) and low-density lipoprotein cholesterol levels (P<0.001); no significant changes occurred in high-density lipoprotein cholesterol (P=0.297) or triglyceride levels (P=0.120)[23].

In a study by Patil et al, postmenopausal breast cancer patients, after tamoxifen therapy expressed a highly-significant reduction in TC and LDL-C levels, there was no significant increase in TGs and HDL-C levels in comparison to baseline values[24].

Tamoxifen has been associated with a cardioprotective effect as confirmed by data from a meta-analysis of 32 studies in more than 50.000 patients, and was also associated with a significant reduction in myocardial infarction-linked deaths and a trend toward decreased incidence of myocardial infarction[25].

In general, our present results are consistent with other previous reports.

While tamoxifen remains an important endocrine therapy for breast cancer, third generation AIs [anastrozole, letrozole, and exemestane] are increasingly replacing tamoxifen as the preferred treatment agent for endocrine-responsive early breast cancer in postmenopausal women[26].

Hozumi et al studied the effect of tamoxifen, exemestane and anastazole on lipid profile of postmenopausal breast cancer patients ,he found that TC and LDL-C rapidly decreased in patients treated with tamoxifen at 3 months. Compared with anastrozole and exemestane patients, TC and LDL-C were significantly lower at all assessment time points in tamoxifen patients (P<0.05). HDL-C did not change significantly in tamoxifen patients and slightly decreased in exemestane patients. Compared with anastrozole patients, HDL-C was significantly lower in exemestane patients at 3 months and 1 year (P = 0.0179 and 0.0013, respectively)[27]. Ta-

moxifen also has experienced lipid-lowering and cardioprotective effect which are not evident with AI[28].

By taking these documented positive effects of tamoxifen on lipids and cardiovascular outcomes into account ,we can 't ignore an effective and cheap hormonal treatment like tamoxifen specially in our developing country.

Conclusion

The results of the present study confirmed the beneficial effects of tamoxifen on lipid profiles of post-menopausal women with breast cancer. The favorable effects of tamoxifen might offer benefits for patients with dyslipidemias or with histories of atherosclerosis and ischemic heart disease .

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EFFECTS OF TAMOXIFEN ON LIPID PROFILES IN POST-MENOPAUSAL BREAST CANCER PATIENTS

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EFFECTS OF INSULIN AND VITAMIN E ON THE EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN AND OXIDATIVE STRESS IN THE CEREBELLUM OF DIABETIC RATS

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Abstract

Background: *Uncontrolled diabetes is associated with increased risk of the central nervous system complications. Poorly uncontrolled diabetes leads to cellular changes in the cerebellum particularly for astrocytes and Bergmann cells, the alterations in activity of these cells could contribute to diabetes-related disturbances and affect neurons.*

Material and methods: *60 adult male rats were divided into 5 groups (12 rat each): -ve control, Streptozotocin (STZ) induced diabetic, diabetic treated with insulin, diabetic treated with vitamin E and diabetic treated with both insulin and vitamin E groups. Animals were sacrificed after 8 weeks of induction. Cerebellum was removed and processed for measurement of oxidative stress markers and stained with cresyl violet and GFAP immunohistochemical stain.*

Results: *There was a significant decrease in the GFAP expression in the cerebellum of the diabetic rats with minimal dystrophic changes in the cerebellar neurons associated with significant elevation of the malondialdehyde (MDA) and significant decrease in the superoxide dismutase (SOD). Treatment with insulin significantly improved GFAP expression and decreased the alterations in MDA and SOD and the dystrophic neuronal changes. The treatment with vitamin E Improved the GFAP expression and decreased the changes in MDA and SOD and the dystrophic neuronal changes but less than insulin. The effects of the combined treatment with both insulin and vitamin E were better than treatment with any of them.*

Conclusion: *Insulin and vitamin E had protective roles on astrocytes in diabetic status by normalizing the hyperglycaemic state and decreasing the oxidative stress.*

Key words: *Astrocytes; glial fibrillary acidic protein; diabetes; cerebellum; oxidative stress*

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Introduction

Diabetes mellitus (DM) is one of the most common endocrine disorders affecting almost 6% of the world's population[1]. Uncontrolled (DM) led to CNS complications concerning with neurotransmitter metabolism, cerebral blood flow, the blood–brain barrier (BBB) and microvascular function. These changes most likely underlie the increased risk of stroke, seizures and dementia, learning and memory alterations and increased neuronal apoptosis in the cerebellum of diabetic rats[2]. The cerebellum is important for a number of motor and cognitive functions, including motor learning, time perception and precise movement[3,4,5].

Astrocytes play critical roles in a number of CNS activities including production of growth factors[6], maintenance of the extracellular environment[7], regulation of synaptic activity and synaptogenesis[8], formation of the BBB[9,10], neuronal transmission and metabolism [11], protection from reactive oxygen species[12], regulation of the cerebral microcirculation[13]. Astrocytes are particularly important in glutamate uptake and metabolism [7]. The alterations of astrocytes number are possibly due to oxidative stress and free radical formation. Hyperglycemia causes a reduction in levels of protective endogenous antioxidants and increases generation of free radicals [14].

Glial fibrillary acidic protein (GFAP)—is an intermediate cytoskeletal filament protein specific for astrocytes. The alteration in the expression of GFAP is a key indicator of astrocyte activity[15,16]. It was proved that the number of GFAP +ve astrocytes increased in

the hippocampus of STZ-diabetic mice and rats[17,18].

This study aimed to investigate the effect of diabetes mellitus on the expression of GFAP, Lipid peroxidation and the neuronal insults in the cerebellum of STZ induced diabetic rats after 8 weeks of induction. It also aimed to elucidate the protective effect of insulin and vitamin E.

Material and Methods

Animals Model:

Sixty adult male albino rats (Sprague Dawly), weighting 175-185 gm aging 10-12 weeks were purchased from Mansoura experimental research center (MERC), Egypt. They were housed in stainless steel mesh cages under control condition of temperature (23ºC±3), and relative humidity throughout acclimatization and experimental periods, with ad libitum access to food and water and fixed 12:12-hours light/dark cycle. All rats were maintained under specific pathogen-free conditions in the animal house. All the experiments were carried out according to the rules and regulations lay down by the committee on animals' experimentation of Mansoura University.

Induction of diabetes:

To induce experimental diabetes, streptozotocin (Sigma Chemical Co.) was dissolved in 0.9% NaCl (normal saline) (pH: 7.4) and injected intrapetinoneally (IP) in a dose of 55 mg/kg, within 15 min of preparation[19]. At 24h after the STZ injection, the blood glucose level was determined by Accu-Chek blood glucose meter (Roche Diagnostic, Germany) and animals with blood glucose level measured >350 mg/dl were considered as diabetic[20].

Experimental design:

After one week of acclimatization, the rats were randomly divided into 5 groups (one control and 4 experimental groups), 12 rats each:

1. **Group I (-ve control group):** received a single intraperitoneal injection of one ml 0.9% NaCl (normal saline) (pH: 7.4).

2. **Group 2 (diabetic group):** STZ induced diabetic animals.

3. **Group 3 (insulin group):** diabetic animals received subcuta-

neous injection of 4U/Kg/day of Mixtard 30/70 insulin (Novo Nordisk Co.) in 2 divided doses at 8 am and 6 pm throughout the duration of the study.

4. Group 4 (vitamin E group): diabetic animals received vitamin E in a dose of 125 mg/kg/day[14]. Vitamin E was dissolved in olive oil and administrated by oral gavage[21].

5. Group 5 (vitamin E and Insulin): diabetic animals received SC injection of 4U/Kg/day of Mixtard 30/70 insulin in 2 divided doses at 8 am and 6pm and vitamin E by oral gavage in a dose of 125 mg/kg/day.

Monitoring Blood Glucose Level:

A drop of blood from the tail vein was obtained once per week and the glucose level was checked by using ACCU-CHEK glucose test strip and evaluated with the ACCU-CHEK glucose meter, (Roche Diagnostic Co., Germany)[22].

Specimens collection:

At the assigned time (8 weeks after induction of diabetes), the rats of each group were weighted, anaesthetized with ether inhalation, decapitated and cerebellar hemispheres were carefully dissected out of the skull. The cerebellar samples were divided into two subgroups; 6 specimens each.

In the first subgroup, mid sagittal sections from the cerebellum were fixed in 10% neutral buffered formalin for 2-3 days and used to prepare paraffin sections for cresyl violet and GFAP immunohistochemical stains, the rats in this subgroup subjected to intracardiac fixation after anesthesia and before dissection of the cerebellum.

The other subgroup was used to obtain fresh cerebellar specimens for colorimetric assessment of oxidative stress markers MDA [23] and SOD[24].

Cresyl violet staining for Nissl bodies:

The cerebellar samples were stained for Nissl bodies [25] to evaluate the viability of the neurons. The tissues were deparaffinized by keeping the slides in a

60º C oven for 30 min then immersed in xylene and rehydrated through a grading serial of alcohol concentrations. Tissues were covered with filtered 1% cresyl fast violet diluted in distilled water (Fluka Chemika, Switzerland), kept for 15 min., rinsed in distilled water and passed through the alcohol series for dehydration. Finally, tissues were rinsed in xylene and mounted in DPX.

Immunostaining for GFAP protein:

Mounted tissue sections were deparaffinized and hydrated to distilled water followed by exposure of to 0.9% hydrogen peroxide for 20 min at room temperature to abolish endogenous peroxidase-like activity. Sections were placed in 10 mm sodium citrate pH 6.0, and were heated to boiling for 10 min. Sections were then incubated for 20 min in 5% normal serum of the species in which the secondary antibody was made and 2.5% bovine serum albumin (BSA) in PBS. GFAP antibody (Abcam co.) was applied in a dilution 1/1000, and the tissue sections were incubated overnight at room temperature. Sections were treated with biotinylated secondary antibodies, then with horseradish peroxidase enzyme (HRP), and chromogen 3,3'- Diaminobenzidine (DAB). Lastly, sections were stained with hematoxylin[26].

Quantitation of GFAP positive immunostained astrocytes: The tissue sections from the cerebellum were examined under the light microscope (Zeiss) at magnifcations. X100 For morphometric analysis, the percentage area ratio of the positively stained astrocytes were done using program NIH Image J program (National Institutes of Health, Bethesda, MD, USA).[18].

Statistical Analysis

Data were analyzed by one-way Analysis of Variance (ANOVA) using the statistical program Graph Pad Prism version 6 for Windows (Graph Pad Software, San Diego, CA). Differences between treatment groups within a given time period were assessed by the Tukey–Kramer Multiple comparison test (Graph Pad Software, San Diego, CA). Data were reported as means ±SEM.

Results

1. Effects of Diabetes on Body

Weight (table 1):-

The initial body weight was 181±2.25 gm at the start of the experiment. The body weight of the control group was 345±2.07 gm after 8 weeks from the start of the experiment. Diabetes resulted in a significant decrease in body weight ($P<0.05$) after 8 weeks in the diabetic groups, as compared with the control group. The body weight in the STZ induced diabetic groups was 249±2.1gm at 8 weeks.

Treatment of the diabetic animals with insulin only prevented the marked decrease in the body weight with a significant difference compared to the diabetic group, but still significantly different from the control groups ($P<0.05$), while In treatment of the diabetic animals with vitamin E only, there was a significant decrease in the body weight compared to the control group with no significant difference compared to the diabetic groups at 8 weeks ($P<0.05$). The combined treatment with insulin and vitamin E improved the decrease in the body weight more than insulin or vitamin E treatment alone. There was no significant difference between the –ve control group and group treated with both insulin and vitamin E at 8 weeks.

2. Blood Glucose Level (table 1):

During the eight weeks of the experiment, Streptozotocin administration caused a significant increase of the blood glucose level (596.3±9.681 mg/dl) compared to the –ve control group ($P<0.05$). The blood glucose level of the –ve control group was (125±8.7 mg/dl). Treatment with insulin significantly prevented the increase in the blood glucose level (231.5±11.5). Vitamin E treatment alone did not protect against the elevation in the blood glucose level. The mean blood glucose level of this group was 536±23 mg/dl. Treatment with both insulin and vitamin E prevented the marked elevation in the blood glucose level. The mean blood glucose level of this group was 188±5.2 mg/dl. These levels were significantly lower than that of the diabetic group and not significantly different from that of the control group.

3. Measurement of oxidative stress markers SOD and MDA

(tables 2):

The levels of MDA and SOD in the -ve control group of the cerebellum were 0.154 nmol/gm tissue and 28.7 U/gm tissue at 8 weeks respectively. Diabetes resulted in a significant increase in tissue MDA and significant decrease in SOD in cerebellum at 8 weeks versus the –ve control groups at the same time. Treatment with insulin or vitamin E significantly improved the changes of both MDA and SOD after 8 weeks versus the diabetic groups. The combined treatment with insulin and vitamin E significantly improved the changes in the levels of MDA and SOD after 8 weeks versus the diabetic groups in a way better than insulin or vitamin E alone.

4. Cresyl violet staining for Nissl bodies in neurons:

In the cresyl violet staining of the -ve control cerebellum, the cerebellar cortex was formed of superficial molecular layer and deeper granular layer with the purkinje cells layer clearly visible in between the two layers. Nissl granules appeared within the cell body cytoplasm of the Purkinje cells surrounding the large prominent centrally located nuclei indicating viability of the neurons (Fig. 1 A&B). Some degenerating and dystrohic changes had been noticed in a few numbers of neurons in the STZ induced diabetic groups after 8 weeks including neuronal hypoplasia (shrunken hyperchromatic dark stained neurons). Few neurons were hypertrophic with abnormal distribution of Nissl granules (Fig. 1 C&D). These dystrophic changes were less visible in vitamin E group after 8 weeks (Fig. 2 F) and minimal in insulin group (Fig. 2 E). In the combined vitamin E and insulin group, almost all the cells appeared normal and viable (Fig. 2 G).

5. Immunohistochemical staining for GFAP protein:

Immunohistochemistry for the expression of the GFAP in the cerebellum of the –ve control rats showed GFAP +ve immunostained Bergmann cells radiating into the molecular layer of the cerebellum and immunostained astrocytes in the granular layer, purkinje cell layer, around the blood vessels and forming the glia limitans

membrane (Fig. 3 A). In STZ induced diabetic group there was significant decrease in GFAP +ve immunostained Bergmann cells and astrocytes in both molecular and granular layer respectively compared versus the –ve control (Fig. 3 B). Treatment of diabetic rats with insulin resulted in significant increase in GFAP +ve immunostained Bergmann cells and astrocytes compared versus the diabetic group (Fig. 3 C). While treatment of diabetic rats with vitamin E only didn't change significantly the amount of GFAP expression by Bergmann cells and astrocytes versus the diabetic group (Fig. 3 D). Treatment of diabetic rats with insulin and vitamin E resulted in significant increase in +ve GFAP immunostained Bergmann cells and astrocytes versus the diabetic group (Fig no. 4).

The area ratio % of the GFAP expression was measured in different regions of cerebellum (superior vermis and inferior vermis) in both molecular layer and granular layer (table 3). There was significant difference in this ratio of the diabetic group and vitamin E group compared to the –ve control group. The ratio % of the expressed GFAP protein in the insulin group and the combined insulin and vitamin E group was significantly different compared to the diabetic group while there is no significant difference compared to the -ve control group (Graph no. 1).

Table (1): Body weight and the blood glucose in the experimental rats before sacrifice (mean ±SEM)

Groups	Body weight (gm)	Blood glucose (mg/dl)
Initial weight	181±2.25	
Control	345±2.07	125 ±8.7
Diabetic*	249±2.1	596.3 ±9.681
Insulin*#	314±1.54	231.5 ±11.5
Vitamin E*	253±1.06	536 ±23
Insulin &Vit. E #	340±2.70	188±5.2

* Indicates a significant difference compared to control groups ($P < 0.05$).
# Indicates a significant difference compared to the diabetic groups ($P < 0.05$).

Table (2): levels of lipid peroxidation markers MDA and SOD in the cerebellum of the experimental rats (mean ±SEM)

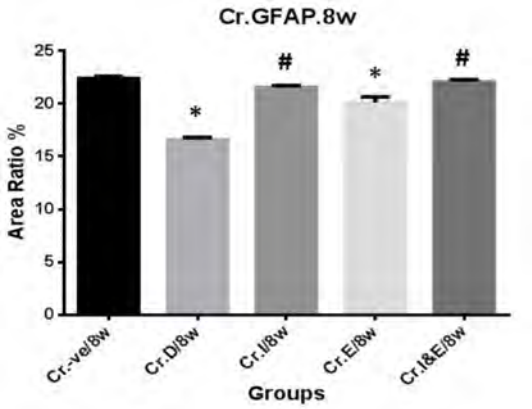
Groups	MDA (nmol/g.tissue) †	SOD (U/g.tissue) ‡
Control	0.154±0.0014	28.53 ±0.12
Diabetic*	0.274±0.0019	11.80 ±0.05
Insulin#	0.164±0.0010	20.80 ±0.05
Vitamin E#	0.188±0.0030	16.90 ±0.05
Insulin& Vit. E#	0.159±0.0006	23.70 ±0.07

* Indicates a significant difference compared to the –ve control groups
# Indicates a significant difference compared to the diabetic groups
† $P < 0.001$
‡ $P < 0.05$

Table (3): Analysis of the area ratio % of the GFAP expression in cerebellum of the experimental rats

Groups	area ratio %
Control	22. 34 ±0.09
Diabetic*	16.58 ±0.08
Insulin #	21.51 ±0.08
Vitamin E*	20.07 ±0.22
Insulin&Vit. E #	22.02 ±0.10

*Indicates a significant difference compared to the –ve control group ($P < 0.05$).
# Indicates a significant difference compared to the diabetic group ($P < 0.05$).



Graph (1): area ratio % of the GFAP expression in cerebellum of the experimental rats
* Indicates a significant difference compared to the –ve control group (P value < 0.05).
# Indicates a significant difference compared to the diabetic group (P value < 0.05).

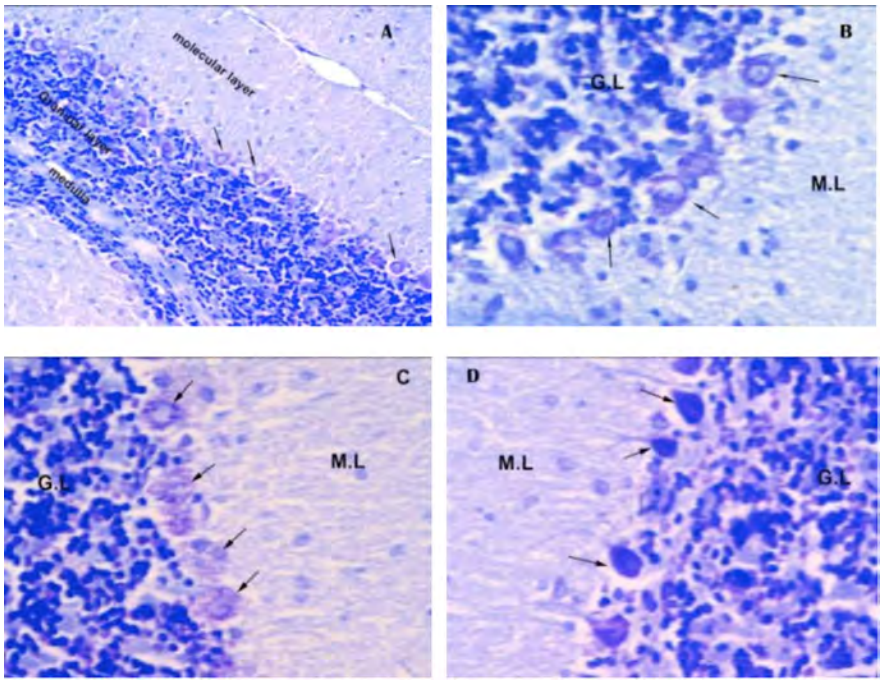


Fig. (1): A photomicrograph for cresyl violet staining of a sagittal section in a rat cerebellum showing: **A:** -ve control rat cerebellum showing molecular layer (M.L), Purkinje cell layer (thin arrows), granular layer (G.L) and medulla. **B:** -ve control rat cerebellum showing molecular layer (M.L), granular layer (G.L) and Purkinje cell layer (thin arrows) with normally appearing neuron with big central nucleus and peripheral dark stained Nissl granules, **C:** abnormally shaped, hypertrophied Purkinje cells in diabetic cerebellum. **D:** neuronal hypoplasia with hyperchramatic shrunken Purkinje cells in diabetic group, Magnification in A; 100x, in B, C, D; 400x.

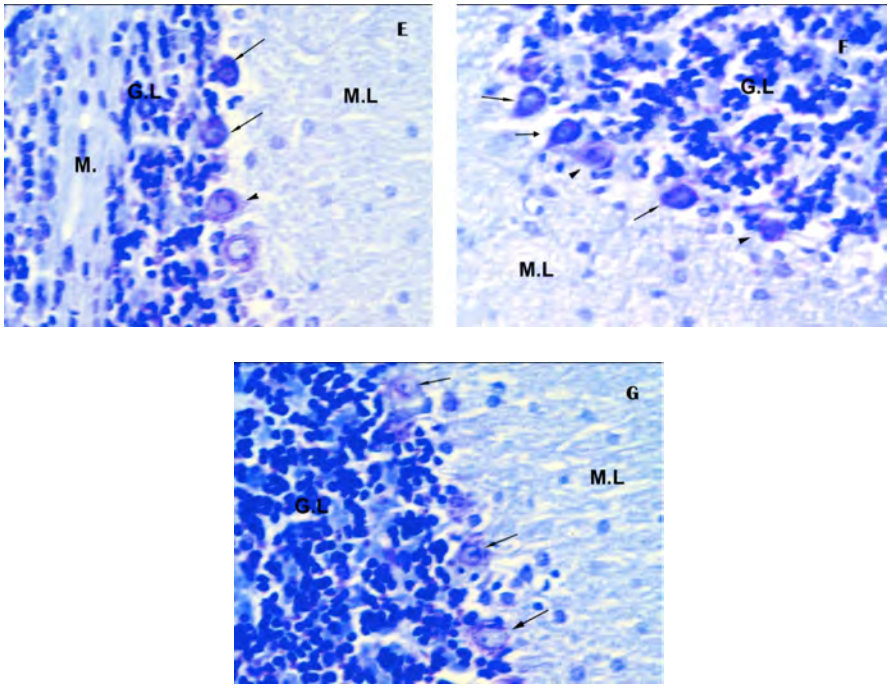


Fig. (2): A photomicrograph for cresyl violet staining of a sagittal section in a rat cerebellum showing molecular layer (M.L), Purkinje cell layer (thin arrows) and granular layer (G.L) **E:** insulin treated diabetic rat showing minimal changes with multiple pattern purkinje cells; dark stained cells (thin arrows) and normally appearing purkinje (arrow heads). **F:** vitamin E treated group, showing dark stained shrunken neuron (thin arrows) and normally appearing neurons with rounded with central nucleus and peripheral Nissl granules (arrows heads). **G:** combined insulin and vitamin E treatment showing normally appearing Purkinje cells with normal pattern of Nissl granules and large central nucleus. Magnification 400x.

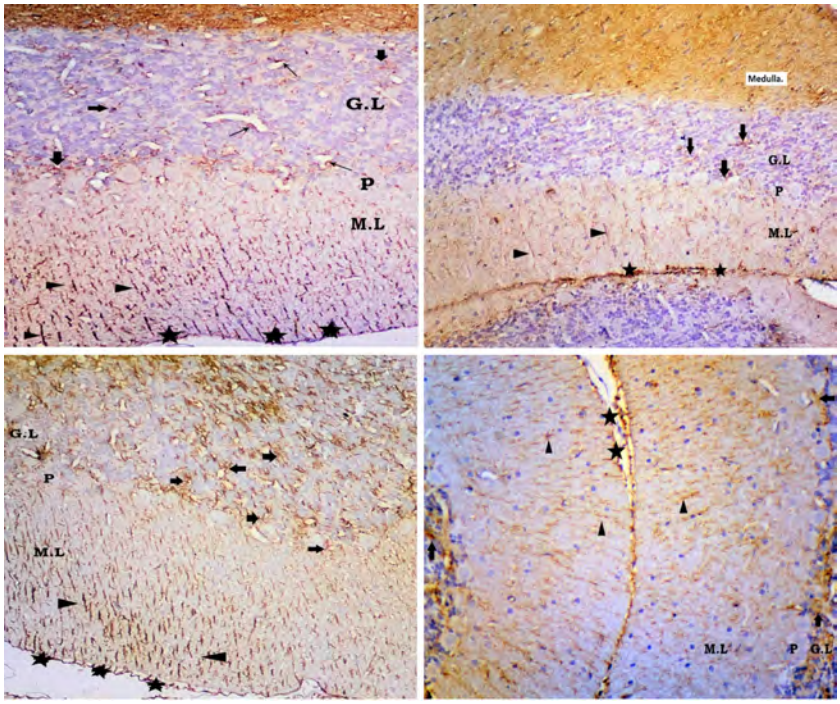


Fig. (3): Photomicrograph of sagittal section in the rat cerebellum of A: -ve control group showing: +ve GFAP stained Bergmann cells in the molecular layer (M.L) (arrow heads) and astrocytes in the granular (G.L) and Purkinje cell (P) layers (broad arrows). Astrocytes form the glia limitans membrane just below the Pia mater on the surface of the cerebellum (asterisks). +ve GFAP stained astrocytes around the blood vessels (thin arrows). B: STZ induced diabetes showing a significant attenuation of the GFAP expression in astrocytes in the granular layer and in Bergmann glial processes in the molecular layer. C: Insulin treatment caused apparent increase in the expression of GFAP in astrocytes in the granular and Purkinje cell layer (broad arrows) and Bergmann glial cells processes in molecular layer (arrows heads). D: Treatment of diabetic rat with vitamin E only slightly improved the decrease in the GFAP expression. GFAP immunoperoxidase; *100.

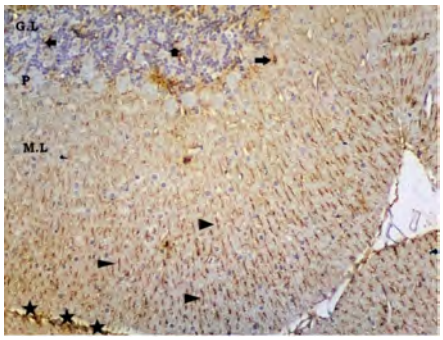


Fig. (4): Photomicrograph of sagittal section in the rat cerebellum of diabetic group treated with insulin and vitamin E showing: marked increase of +ve GFAP Bergmann cells in the molecular (M.L) (arrows heads) and astrocytes in the granular (G.L) and Purkinje cell (P) layers (broad arrows) versus the diabetic group. Astrocytes glia limitans membrane (asterisks). GFAP immunoperoxidase; *100.

Discussion

Following the STZ induction of diabetes, the diabetic animals lost weight throughtout the 8 weeks duration of the experiment and had a significant increase in the blood glucose levels compared to -ve control animals. These changes had been observed for 8 weeks following STZ induction of diabetes. Insulin treatment prevented the marked diabetes-induced body weight decrease and blocked the blood glucose increase during the 8 weeks of the experiment to a level which is considered non diabetic (below 350 mg/dl). These results are in agreement with insulin therapy's overall amelioration of complications associated with diabetes and effect of insulin on the expression of GFAP[27-22].

Treatment of the diabetic animals with vitamin E alone didn't protect neither against the body weight loss nor the increase in the blood glucose level, which were not significantly different from the diabetic animals, these results are in agreement with previous studies[28,29]. While combined treatment with insulin and vitamin E significantly improved the body weight and the blood glucose level with no significant difference compared to the -ve control group, this had been previously reported in previous study[30].

The pathophysiology of the CNS complications in diabetes is very complex. Astrocytes play a vital role for maintaining normal neuronal activity, metabolism and survival through a variety of mechanisms, yet their functional role in diabetes has not been

clearly defined[31].

In this study, neuronal degeneration could be seen in the cerebellum of STZ induced diabetic animals, these changes were in the form of neuronal hypoplasia (hyperchromatic shrunken neurons) or abnormal pattern of distribution of Nissl granule with large hypertrophied vacuolated neurons. These results are in agreement with[32] who reported that neurons tend to degenerate and show a relative inability to grow and proliferate in diabetes. Changes in the dentritic process of neurons in frontal lobe and hippocampus have been reported in diabetes[33]. Studies showed that neurons suffer oxidative damage and undergo apoptosis in diabetes[34]. These changes are minimal with insulin treatment but more visible in groups treated with vitamin E only. This neuronal degeneration was prevented by the combined treatment with insulin and Vitamin E.[35] proposed that vitamin E is especially potent as neuroprotective agent.

The change in astrocyte number and morphology could be the consequence of unviable extracellular conditions such as hyperosmolarity, low nutrient availability, or increased oxidative stress. The lack of insulin could be involved because insulin influences astrocyte morphology, differentiation, and GFAP expression[36]. Glial cells are widely exceeding in number the neurons in the adult brain representing about 90% of human brain cells [37]. Astrocytes are the most numerous cells in the CNS [38], which occupy up to 50% of the total brain volume[39]. One of the subfamily of the astrocytes is the Bergmann cells of the cerebellum[40].

There are many discrepancies concerning changes in GFAP levels in diabetic animals in the previous studies due different response of astrocytes and its subtype Bergmann glial cells to diabetes after variables periods [41,17,14,18]. A second possibility that may account for the difference is that not all astrocytes are identical[42] and astrocytes in different brain regions respond differently to diabetes[22].

In the present study STZ dia-

betes led to a significant decrease in the expression of GFAP protein by astrocytes in the granular layer of cerebellum and the processes of Bergmann glial cells in the molecular layer of the cerebellum. This decrease in GFAP expression could be due to changes in the amount of this structural protein per cell and/or decrease in the number of GFAP-positive cells. These observations are consistent with the results of [18,22,2]. On contrary Baydas et al.,[14] stated that diabetes induced a glial reactivity by increasing the expression of GFAP protein in many parts of the brain. On the other hand Luchuga-Sancho[2] reported that Bergmann glial cells were not affected by diabetes, however, in the current study Bermann Glial processes had been apparently affected as GFAP expression was significant decrease after 8 weeks diabetes. Penky and Nelson[43] reported that there the increase in the GFAP immunoreactivity and proliferation of astrocytes is a general response to neuronal injury. Luchuga-Sancho[2] had stated that there is initial increase in GFAP expression after one week of diabetes induction which pointing to active astrogliosis associated with induced diabetic cerebellar injury, followed by significant decrease in the expression of GFAP probably as a result of both decreased cell proliferation and increased cell death.

The decrease in the GFAP expression in the cerebellum was improved by the treatment with either insulin or vitamin E but still significantly lower than that of the -ve control group. The combined treatment with insulin and vitamin E prevented this decrease and almost restore the GFAP expression up to the -ve control level at 8 weeks. This is in agreement with previous study of[22]. The response of the cerebellar astrocytes and its subfamily Bergmann cell may vary according to the severity of the diabetes[31].

In many neurodegenerative diseases, oxidative stress both initiates and drives the progression of the pathogenic process[44] and many of the diabetic complications such as diabetic neuropathy are believed to be a result of excessive accumulation of reactive oxygen species and of a decreased

antioxidant defense system[45]. Excessive production of free radicals is believed to be involved in many diabetic complications, including diabetic neuropathy in diabetes mellitus[46]. In the current study STZ induced diabetes caused oxidative stress as it significantly elevated the MDA marker and decreased the SOD marker of the lipid peroxidation in the cerebellum after 8 weeks of diabetes induction. These finding are in agreement with previous results of [47,48,49,14]. It is known that astrocytes have more antioxidant capacity than do neurones [50,51,52]. The glial cells protect neurones against oxidative stress and promote neuronal survival as they express a variety of neurotrophic factors and cytokines[14].

The current study demonstrated that STZ induced diabetes leads to a decrease in GFAP levels in astrocytes and its subtype Bergmann glial cells of the cerebellum at 8 weeks of diabetes together with increased oxidative stress. These might contribute to the underlying patho-physiology of diabetes-induced CNS disorders and neuronal changes that had noticed in Purkinje cell layer of the cerebellum at 8 weeks. These changes were prevented by treatment with both insulin and vitamin E.

Astrocytes have an expanding list of functions in supporting the neurons and maintain the neuronal environment, the role of astrocytes in diabetes-induced CNS disorders clearly warrants further investigations and studies to evaluate the effects of diabetes on the functionality of the astrocyte, glutamate transporters, insulin receptors in astrocytes and neurons, the effect of diabetes and insulin treatment for longer periods on neurons and the molecular changes to assess the effects of insulin therapy on diabetes-related astrocyte alterations.

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EFFECTS OF INSULIN AND VITAMIN E ON THE EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN AND OXIDATIVE STRESS IN THE CEREBELLUM OF DIABETIC RATS

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WNT/β-CATENIN SIGNALING PATHWAY IN BREAST CARCINOGENESIS IN RATS

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Abstract

Background: *Breast cancer is a common worldwide malignancy among women. De-regulation of Wnt/β-catenin signalling is increasingly being implicated in both experimental and human carcinogenesis; however, its role in breast cancer is unclear.*

Aim of work: *The goal of this study was to elucidate the role of Wnt/β-catenin signaling in breast carcinogenesis, and to test for a potential relationship between Wnt/β-catenin pathway activation and expression of Glioma-associated oncogene homolog 1 (GLi1), a marker of Hedgehog pathway activation.*

Material & Method: *We used a methyl-nitrosourea (MNU)-induced rat breast carcinogenesis model that mimics many essential elements of human breast cancer to investigate the expression pattern of two of the main key players in Wnt signaling (β-catenin and Axin2) by semiquantitative reverse transcription polymerase chain reaction (RT-PCR) analysis. Also, Gli1 mRNA was analyzed in relation to both genes in order to reveal any possible crosstalk.*

Results: *The expressions of target genes were significantly upregulated in the diseased groups than in control group. There was no significant correlation between the expression of β-catenin, Axin2, and Gli1.*

Conclusion: *These results indicate that overexpression of β-catenin, Axin2, and Gli1 may be associated with the malignant transformation of mammary cells, suggesting a new target for breast chemoprevention.*

Key words: *Breast cancer; Wnt signaling; β-catenin; Axin2; Gli1; rats.*

Introduction

Breast cancer is one of the most common cancers around the world with approximately 1.6 million new cases and 425 thousand deaths from the disease in 2010 [1]. It is a highly heterogeneous disease represented by tumors that have a diverse natural history, complex histology and a variable response to therapy. Although the molecular events that trigger breast cancer progression, including its initiation, promotion and progression to a fully malignant state, are not fully understood[2]. The role of Wnt/ß-catenin signaling in human breast cancer has been subject to much debate[3,4]. Although, the first mammalian Wnt gene, Wnt1, was originally identified as a locus activated by retroviral insertion of mouse mammary tumor virus (MMTV), and transgenic Wnt1 overexpression was subsequently shown to drive mammary tumor formation in mice[5,6]. The definitive evidence linking Wnt signaling to human breast cancer has been slow to emerge[7]. Moreover, historical failure to identify substantial frequencies of Wnt ligand overexpression in human breast tumors hindered appreciation of the relevance of Wnt signaling to the human disease. In contrast, there is abundent evidence that hyperactive Wnt signaling contributes to the genesis of a wide range of other human cancers. Striking frequencies of aberrant nucleocytoplasmic β-catenin accumulation have now been recorded in multiple human neoplastic conditions, most notably in colorectal cancers [8]. However, mutation of pathway components, including the APC, Axin, and CTNNB1 genes (encoding β-catenin), leading to β-catenin stabilization, and hence activation of the Wnt/β-catenin pathway, is now recognized as a common event in human tumorigenesis [9,10]. Because such mutations are comparatively rare in human breast carcinomas, excepting fibromatoses and metaplastic tumors[11].

Aberrant reactivation of Hh signaling has also been reported in breast cancer[12,13]. Hh signaling is also thought to contribute to invasiveness[14]. Glioma-associated oncogene homolog 1 (GLi1) is thought to be a marker of Hh pathway activation[15]. Although

the Hh and Wnt pathways can be activated concurrently during breast tumorigenesis, the functional significance of signaling crosstalk in tumor initiation and progression has not been established[16].

Our goal in the present study was to further investigate the frequency of Wnt/β-catenin signaling pathway activation in breast neoplasia using means of semiquantitative reverse transcription polymerase chain reaction (RT-PCR) of both mRNA of β-catenin and Axin2, a specific target gene. An additional goal was to assess the relationship between canonical Wnt pathway activation and Gli overexpression based on several lines of evidence indicating that Wnt and Hedgehog (Hh) signaling pathways can interact[16-18].

Materials and Methods

* Animals

A total of 55 female Sprague-Dawley rats, aged 14 days and weighing (35±5g) were obtained from and housed in Medical Experimental Research Centre (MERC). The animals were housed 4-5 per cage and maintained at 23±2ºC and 12 h light:dark cycle. They were fed a purified diet (AIN-76A) and had access to water ad libitum.

* Experimental Design

After a 7 days acclimatization period, the animals were randomly divided into two groups (experimental and control group). For induction of breast cancer in the experimental group (n=43), MNU (Sigma-Aldrich, St. Louis, USA), (50 mg/kg body weight) was injected i.p. on the day 21 and 65 of age. The MNU was always dissolved immediately before use in 0.9% NaCl adjusted to pH 4 with acetic acid. The solubility of MNU in water at room temperature was 1.4 % (w/v)[19]. The animals were weighed once a week. Ten rats of the experimental group died during the experiments. Sex and age matched control rats (n=12) were maintained free access to tap water and basal diet without any treatment until scarification. The experiment was terminated on the 130 th day of the animals age.

* Tissue sampling

The abdominal-inguinal mammary glands (AIMG) on both sides,

left and right were evaluated for the presence of grossly detectable mammary tumours and the dissected animals with tumours were photographed to provide identification record on the location and gross morphology of lesions (Figure.1.b). The mammary tissues were excised from the subcutaneous tissue with scissors along with the fat pads (Figure.1.a), then cut into 2 pieces, the proximal region including a lymph node (abdominal gland) and the distal region (injuinal gland). For histopathological study, specimen of the breast (tumour and tumour adjacent) tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut at 5ml thickness and stained with hematoxylin and eosin (H&E) after processing. The rest of the breast tissues were snap frozen in liquid nitrogen for subsequent molecular analysis. Mammary tumors were classified based on the criteria described by Russo[20] into hyperplasia, carcinoma insitu (CIS) and adenocarcinoma which shows obvious invasion. Also, The normal non tumored mammary gland from rats bearing tumor were collected and analyzed.

*** Molecular Biology Assays in the Breast:**

RNA Isolation and Semiquantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) Analysis:

Total RNA was extracted from breast tissue specimen using both Trifast Reagent (Peqlab, Germany) followed by GeneJET RNA Purification Kit (Fermentas Lifesciences, U.S.A.). Purity and concentration of extracted RNA was assessed by measuring the optical density at wave lengths of 260, and 280 nm. Absorbance ratio at 260/280 was used to determine the purity of the RNA samples. DNA contamination was assessed using formaldehyde agarose gel electrophoresis. The RNA is then was converted into cDNA using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, U.S.A). Two micrograms of total RNA were reverse transcribed into first strand cDNA in a volume of 20 µl at 10 min at 25°C followed by 15 min at 50°C and heated at 85ºC for 5 min to terminate the reverse transcription reaction. β-catenin, Axin2, Gli1 and GAPD (housekeeping gene) genes were amplified from 2 µl cDNA mixtures in a final vol-

ume of 20 µl PCR reaction mixture by adding 18 µl of DreamTaq™ Green PCR Master Mix (2X) (Fermentas Lifesciences, U.S.A). Reaction mixtures were first denatured at 94ºC for 5 min, and amplification was performed for 35 cycles, at 95ºC for 45 s, annealing (57ºC for β-catenin, Gli1 and GAPD, while 62ºC for Axin2) for 1 min, and at 72ºC for 2 min, followed by an extension for 10 min at 72ºC. The PCR primer pairs used were enlisted in Table 1.

After amplification, the PCR products were electrophoresed on agarose gel. The marker used was 50 bp DNA Ladder (GeneRuler™, Fermentas, Canada) ranging from 1000-50 bp with two reference bands at 500 and 250 bp. The resulted photos were analyzed with scion image® release Alpha 4.0.3.2 software for windows® (Scion image, Scion corporation, USA) which performs bands detection and conversion to peaks. Areas under each peak were calculated in square pixels and used in quantification. Semiquantitative gene expression was determined by calculating the ratio between the square pixel value of the target genes in relation to the housekeeping gene. Each target gene expression was evaluated by comparison of the results at each experimental stage with those of the normal control.

Results

Expression of β-catenin mRNA, Axin2 mRNA and Gli1 mRNA:

Comparative analysis of β-catenin expression (post hoc test) showed a significant increase in β-catenin expression in the different histopathological stages of MNU-induced breast cancer (hyperplasia, CIS & adenocarcinoma) when compared with the control group ($P<0.01$). There was no statistically significant difference between these 3 groups. Also, there was a highly significant increase in β-catenin expression in normal mammary tissue from tumor- bearing rats when compared with control ($P<0.001$).

Comparative analysis of Axin2 expression (post hoc test) showed a significant increase in Axin2 expression in the three pathological groups when compared with the

control group (P<0.01). There was no statistically significant difference between these 3 groups. Also, there was a significant increase in Axin2 expression in normal mammary tissue from tumor-bearing rats when compared with control (P<0.001).

Comparative analysis of Gli1 expression (post hoc test): shows a high significant increase in Gli1 expression in hyperplasia, CIS and adenocarcinoma groups when compared with the control group (P<0.001). There was significant difference between these 3 groups. Also, there was a significant increase in Gli1 expression in normal mammary tissue from tumor-bearing rats when compared with control (P=0.01).

Correlation between expression of β-catenin, Axin2 and Gli1 mRNA: Pearson correlation coefficient testing revealed a significant positive correlation between the expression of β-catenin and Axin2 in adenocarcinoma group (r=0.6). It revealed no significant correlation between neither β-catenin nor Axin2 and Gli1 expression in the different groups.

Table (1): Sequence of all primers used in the experiment (Sigma, Germany).

Gene	Primer sequence	PCR product size (bp)
β- catenin		
	F: 5′- ACTGCTAAATGACGAGGACC- 3′	292
	R: 5′- GGCGTAGAACAGTACGGAAT- 3′	
Axin2		
	F: 5 - AGTGAGCGTCAGAGCAAGTC-3	510
	R: 5 - GCTGAGCTGCTCCTTGAAGT-3	
Gli1		
	F: 5′- AGCTCCTGTGTAATTACGTTCAGTC- 3′	132
	R: 5′- GGCTCTGACTAACTTGAGAACCTC - 3′	
GAPDH		
	F: 5′- GGCTCTCTGCTCCTCCCTGTTCTAG - 3′	469
	R: 5′- ACCCATCACAAACATGGGGGCAT -3′	

Table (2): Gene expression levels (relative mean ratio to the corresponding GAPD) of the parameters studied in breast tissues in the different histopathological groups.

Parameter	Control	Hyperplasia	CIS	Adenocarcinoma	NTMG
β-Catenin	0.57±0.06	0.74±0.03$^{a*b\#}$	0.81±0.05$^{a\#b*}$	0.83±0.06$^{a\#b*}$	1.03±0.09$^{a\#\#}$
Axin 2	0.08±0.02	0.25±0.04$^{a\#b\#\#}$	0.26±0.04$^{a\#b\#\#}$	0.31±0.03$^{a\#\#b*}$	0.48±0.03
Gli 1	0.1±0.02	0.34±0.05$^{a\#}$	0.43±0.05$^{a\#\#c\#}$	0.7±0.08$^{a\#\#c\#\#d\#}$	0.29±0.05$^{a*c\#\#}$
n	11	15	12	11	10

Data are represented as mean ± SEM; n number of observations for each group. **CIS:** carcinoma insitu, **NTMG**: non tumored mammary gland from tumored rat.

a compared to Normal
b compared to NTMG
c compared to Hyperplasia
d compared to CIS
e compared to Adenocarcinoma
* $p < 0.05$; # $p < 0.01$; ## $p < 0.001$

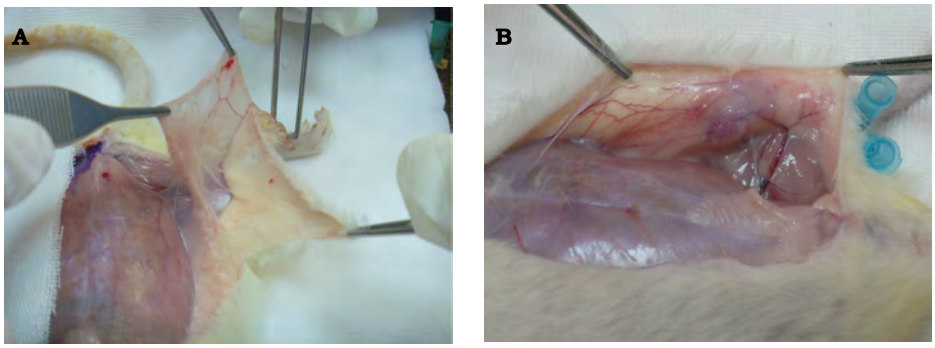

Fig. (1): A: Dissection of the right AIMG from the abdominal skin of a normal rat. **B:** palpable adenocarcinoma in a 130 day old rat.

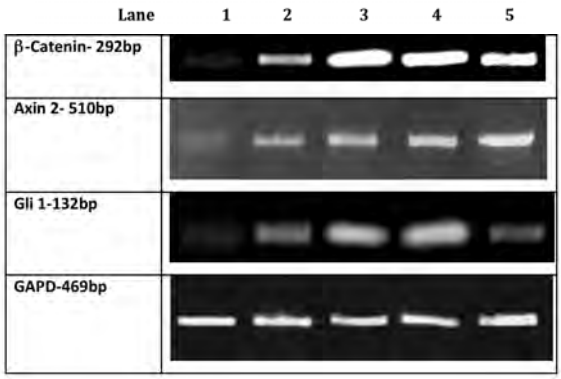

Fig. (2): Representative agarose gel electrophoresis profiles of mRNA amplification of β-catenin, Axin 2, Gli1 and GAPD obtained by RT-PCR of breast tissues from 1 normal (n=11), 2 hyperplasia (n=15), 3 CIS (n=12), 4 adenocarcinoma (n=11),and 5 NTMG (n=10).

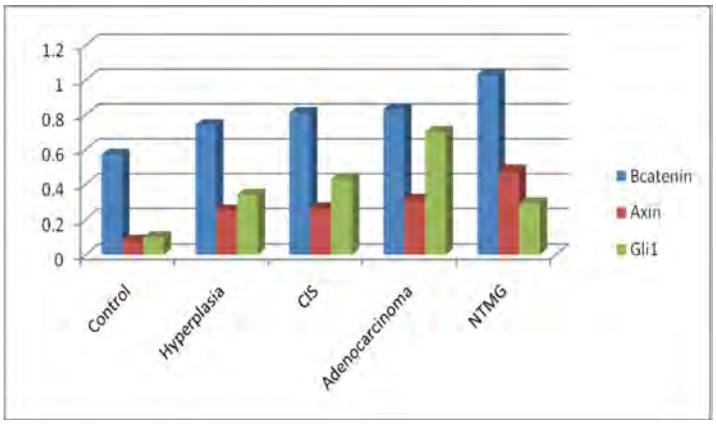

Fig. (3): mRNA expressions of β-catenin, Axin2 and Gli1in different studied groups. Data are represented as means±SEM.

Discusssion

Breast cancer is the most frequent malignancy among women worldwide[1]. Experimental models mimicking this disease in rodents, such as methylnitrosourea (MNU)-induced carcinogenesis, provide a tool for the understanding of the molecular alterations arising in human breast cancer[2]. Disregulation of Wnt pathway via genetic mutations of certain components of this pathway are rare in human breast cancer, yet activation of the pathway is evident from the mislocalization of β-catenin[21]. Instead, Wnt activation seems to occur at the level of Wnt ligand-receptor interaction, through their upregulation and/or downregulation of secreted inhibtors[22]. In our study we tried to investigate the altered Wnt signalling at two main levels: the cytosol (β-catenin) and the nucleus (axin2).

β-catenin is a transcriptional cofactor and is also an essential component of E-cadherin mediated cell–cell adhesion complexes. β-catenin is the central player of the Wnt signalling pathway[9]. The defining feature of activated canonical Wnt pathway is the stabilization and accumulation of cytosolic β-catenin, which enters the nucleus and binds to transcription factors of the TCF/LEF family. This binding activates Wnt target genes that encode for proteins associated with cellular transformation[23]. Accordingly, our study evaluated

the mRNA expression of β-catenin in the different histopathological stages of MNU-induced breast cancer (hyperplasia, CIS & adenocarcinoma), showing significant increase when compared to the control normal rat tissue. This overexpression of β-catenin mRNA indicates mostly transcriptional activation occurring during breast cancer initiation and progression. Little is known about regulation of β-catenin gene expression. However, TCF/LEF and AP1 transcription factors were found in the promoter region, suggesting possible direct feedback mechanism for augmenting β-catenin expression by β-catenin /TCF/LEF pathway and one of its downstream targets, c-Jun[24].

Also, the upregulation of β-catenin in this study is suggesting that transcriptional deregulation of the β-catenin gene itself might be important during tumor development. This suggestion is supported by other studies. One study demonstrated the β-catenin oncogenic effect, as transgenic mutant β-catenin expressed in mouse mammary glands leads to carcinoma[25]. Also, Ryo et al.,[26] and Johnnson et al.,[27] confirmed elevated β-catenin levels by Western blotting of tumor lysates. Meanwhile, several studies observed elevated levels of nuclear and/or cytoplasmic β-catenin detected by immunohistochemical staining in the majority breast tumor tissue samples, and even that high β-catenin activity was significantly correlated with poor prognosis of breast cancer[28-30].

Axin2 is a direct and specific transcriptional target of the Wnt/β-catenin signaling pathway. It is well recognized that the expression level of Axin2 is the signature of the activation of Wnt/β- catenin signaling[31]. Our study revealed upregulation of Axin2 mRNA expression in the different histopathological stages of breast cancer when compared with the control group. The overexpression of Axin2 mRNA could be explained by activation of Axin2 gene transcription. The effectors of the Wnt/βcatenin pathway (TCF/LEF) and E2F1 have shown to be involved in regulation of Axin2 gene activity[32,33]. Beside this, chromatin immunoprecipitation/microarray analysis of many genes coding for

Wnt inhibitors, including Axin2 showed that they were occupied by EZH2, a subunit of the polycomb repressor complex 2 (PRC2). EZH2 is a methyl transferase that deposits the repressive H3K27me3 marks on chromatin[34].

The increased expression of Axin2 in this study is in agreement with other studies who confirmed that Axin2 was markedly upregulated upon mRNA analysis of high-grade breast tumours [35,36]. The oncogenic effect of Axin2 was a point of question since Axin2 is known as a negative regulator of canonical Wnt signaling in normal cells. However, rather than functioning as a tumor suppressor, Axin2 was found acting as a potent promoter of carcinoma behavior by upregulating the activity of the transcriptional repressor, Snail1, inducing a functional epithelial-mesenchymal transition (EMT) program and driving metastatic activity[37]. Also, Axin2 induced chromosomal instability in CRC has been reported[38].

Nevertheless, it's worthwhile to note that our findings involving the increased Axin2 mRNA expression in the normal tissue from the tumored rats are supported by the recent study of khalil et al.,[39], that demonstrated a finite frequency of positive nucleocytoplasmic staining in normal human breast tissues, indicating an active wnt/ β-catenin signaling pathway in these tissues. Accordingly , We hypothesized that Wnt/β-catenin pathway activation observed in benign breast tissue could result from precancerous changes in morphologically normal breast, and further reasoned that breast tissue adjacent to invasive tumors would be more likely to contain such protumorigenic molecular alterations.

Gli1 has been reported extensively as a universal marker for Hh-pathway activation. Elevated Gli1 expression and its role in a number of cancers has been widely reported, including a recent study that the conditional expression of Gli1 in mouse mammary glands results in mammary tumours[40]. This is consistent with our findings as the Gli mRNA expression showed significant increase during the different histo-

pathological stages of MNU-induced breast cancer (hyperplasia, CIS & adenocarcinoma), when compared to the control normal rat tissue. The elevated expression of Gli1 mRNA could be due to transcriptional activation via both canonical Hh-pathway-dependent [41,42] and non-canonical Hh-independent activation of Gli1 including TGF-β[43], PI3K/AKT[44], Ras/Mek[45], and a Gli1-CDK2-dependent mechanism[46]. Our results revealed statistically significant elevation of Gli1 expression in the adenocarcinoma compared to the other histopathological stages of cancer, suggesting the involvement of Gli1 in cancer progression. This is consistent with other studies which reported that deregulated Hh supports more aggressive phenotypes of human breast cancers, such as progression, metastasis, and therapeutic resistance[47].

A cross talk between the Hh and Wnt signaling in cancer was reported in gastric, colon, endometrial and skin cancer[16]. Depending on the type of tissue Hh and Wnt signalling pathways can have cross talk as a positive feedback or negative feedback activity. For example, Hh signalling has inhibitory effects on Wnt signalling in stomach adenocarcinoma[17]. Meanwhile, it has a stimulatory effect in endometrial cancer[18]. The present study showed that Wnt/β-catenin and Hh pathways are activated concurrently during breast carcinogenesis, although in the present study, Pearson correlation coefficient testing revealed no significant correlation between expression of β-catenin, Axin2 and Gli1 mRNA in the histopathological groups. This finding could be explained by the wide variability in the rate of increase of the different assayed genes as well as heterogeneity of expression of genes between rat samples of the same histopathological group. The heterogeneity of gene expression and interactions of the Wnt and the Hh pathway activation is possibly one of the reasons for drug resistance in breast cancer.

In summary, the Wnt signalling pathway may be a logical focus for novel chemopreventive and therapeutic strategies because of a likely role as one of the "driver pathways" in breast tumourigenesis.

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WNT/β-CATENIN SIGNALING PATHWAY IN BREAST CARCINOGENESIS IN RATS

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BELOW 2 YEARS CHILDREN CAREGIVERS' KNOWLEDGE, ATTITUDE AND BELIEFS TOWARDS IMMUNIZATION

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Abstract

Background: *Uptake of vaccination services is dependent not only on provision of these services but also on other factors including knowledge and attitude of mothers.*

Aim: *To determine the attitude of children below 2 years' caregivers towards immunization in an Egyptian community and detection of the underlying causes of vaccination delay, to evaluate the association between their attitude towards vaccines with both their beliefs and knowledge and to determine their satisfaction regarding aspects of care.*

Methods: *This cross sectional study was carried out on caregivers attending immunization setting in 5 urban and rural health facilities in Mansoura center, Egypt. They fill the questionnaire that asks about, Socio-demographic characteristics and different factors related to their attitude towards immunization.*

Results: *We included 1000 caregiver in the study. We found that no caregiver refused to immunize his children and 10% only delayed their immunization which was mainly due to deficient information about the importance of vaccination. caregivers who delayed vaccines were less likely to believe that vaccines are necessary to protect the health of children, that their child might get a disease if they aren't vaccinated, to read and watch stories about health, to agree with the statement, "vaccines are safe", to believe that they had a good relationship with their*

child's health-care provider, compared with caregivers who regularly vaccinated their children. At the same time, they reported lower satisfaction regarding different aspects of care except for insurances of proper vaccine administration.

Conclusion: *Our results suggest that modifiable determinants for a negative attitude could probably be based on a lack of specific knowledge and this reflect the importance of health education programs to explain different vaccines related worry and improve different aspects of care that enhances their satisfaction.*

Introduction

Vaccination has been shown to be one of the most effective public health interventions worldwide, through which a number of serious childhood diseases have been successfully eradicated (WHO, 2009).

The WHO recommends vaccination against a number of serious infectious diseases, including diphtheria, tetanus, pertussis, HBV, measles, pneumococcal disease, yellow fever, and rotavirus disease for children in some areas as part of their EPI (Gentile et al, 2010).

Despite increase in global immunization coverage, many children around the world especially in developing countries are left unimmunized. In 2007, approximately 27 million infants worldwide were unimmunized against common childhood diseases and 2-3 million children die of vaccine preventable diseases (WHO, 2010). Globally, immunization coverage has increased during the past decade to levels of around 78% for diphtheria, tetanus and pertussis, but in African Regions including Nigeria, it is about 69% (CDC, 2010).

Over the past years, the Egyptian Ministry of Health and Population (MOHP) has implemented a national program for childhood immunization. Health authorities in Egypt have also taken important steps to maintain high levels of immunization coverage among children through routine immunization and implementation of supplementary immunization activities (DCD, 2005; MOHP, 2005).

Parental satisfaction with pediatric care is an indicator of provider quality that has been relatively unexplored in relation to childhood immunization. Research on parental health beliefs and attitudes often assumes that parents decline immunization or are simply less knowledgeable and persistent in the health care setting without also examining their access and timely utilization of well-child care (Ashley et al, 2007).

The four psychosocial domains that influence parents' decisions to vaccinate their children are: susceptibility; seriousness; efficacy and safety & social pressures. These factors soon became the basis for the celebrated Health Belief Model that has been used throughout public health to explain why people adopt behaviors that lead to healthy lives (Glanz et al, 1997; Strecher et al, 1997).

Our study was conducted to determine the attitude of children below 2 years' caregivers towards immunization in an Egyptian community and detection of the underlying causes of vaccination delay, to evaluate the association between their attitude towards vaccines with both their beliefs and knowledge and to determine their satisfaction regarding aspects of care.

Subjects and Methods

This cross sectional study was carried out on caregivers attending immunization setting in 5 urban and rural health facilities in Mansoura center, Egypt during three months from March 2012 to May 2012. Sample size was calculated online (www.dssresearch.com). A pilot study was done on 50 caregivers in order to test the questionnaire, detect any difficulties and also to give an idea about the prevalence about delaying and refusal of child immunization, from which the percent of vaccination delay was found to be 11.5 % and by considering the worst acceptable value as 8.5, the sample size was 613 with 95% confidence level and 80% study power. We increased the sample to reach 1000. The authors gave brief explanations of the objectives of the questionnaire. Caregivers were also assured of their anonymity and the confidentiality of their responses. Systematic random sample meth-

od was used where every 10th was included in the study in order to study the problem of refusal and delay of vaccination.

Study tools:

The attendants asked to fill the pre-constructed questionnaire after taking oral consent from the director of the primary health center and the caregivers. This questionnaire measures the different factors related to immunization delay or refusal such as:

Personal history of the caregiver which included; name, residence, marital status and child order.

Socioeconomic status according to modified (Fahmy and El-Sherbini, 1983) which included Father education, Father occupation, Mother education, Mother occupation, Percapita monthly income in "Egyptian pounds", and the person that is responsible about taking decision in immunization.

Knowledge of caregivers about vaccination such as importance of vaccines to children's health, side effects can a child get after vaccination and the severity of the illnesses of diseases prevented by vaccines.

Caregivers' attitudes and belief: On a scale of 0 to 5 with "0" being "strongly disagree" and "5" being "strongly agree," how much do caregivers disagree or agree with the statements such as if vaccines are necessary to protect the health of children, children receive too many vaccines, if vaccines are safe and if they have a good relationship with child's health care provider, these were finally coded into agree and disagree only.

Caregivers' satisfaction about different aspects of care as if they were told about the benefits of childhood vaccinations, told about the possible side-effects of childhood vaccinations, if they feel that they had given enough time to discuss issues that concerned about the vaccinations, wait long time during vaccination session, If health worker tell them about vaccine schedule (time, dose, next visit, If the health worker confirm that thier child swallowed the vaccine, and etc....).

The completed questionnaire were subjected to revision and the collected data were coded, processed and analyzed through SPSS (Statistical Package for Social Sciences) (Standard version release 16.0). A descriptive analysis of the collected data was done in the form of frequencies and percent. Chi Square was used for testing significance of discrete and categorical data. The significance level was considered at $P < 0.05$.

Results

Our studied group included 1000 children attending the immunization cession; the mean age of the children was 9.21±6.06 months. About 53.4% of these children were from urban areas versus 46.6% from rural areas. 69.8% of the children came to their immunization cession with their mothers. In more than 80% of the children, the mother is the main caregiver (81.8%) and the father is the responsible financially (87.7%). Both father education and mother education achieved tertiary level in about half of the studied group (47.1%, 47.0% respectively), 63.6% of our group had enough income with only 5.2% of the fathers and 48.3% of the mother not working.

In this study, we found that no one refused to immunize his children and the frequency of delayed vaccination was 10% only in which more half of them reported that the delay was for DPT (60%) and the least percent was for MMR (9%) (Figure 1). This delay was mainly due to deficient information about the importance of vaccination at the timing of vaccination (56%), child illness (52.5%), negative knowledge about the vaccines (32%) and also about one quarter due to vaccine deficiency in the health offices (figure 2).

Regarding the relationship between vaccine related knowledge and caregivers' attitude towards vaccination, it was found that significant higher knowledge detected between caregivers who regularly vaccinated their children compared with caregivers who delayed vaccine regarding the great importance of the vaccines (87% vs. 79%, $p<0.001$) and the liability to catch the diseases if the child not immunized (83.3% vs.72%, $p<0.001$) respectively. Also the

same detected for the knowledge about the severity of vaccine preventable diseases (76.7% vs 71% respectively) but with no significant difference.

The association between caregivers' delay and caregivers' beliefs and attitudes about vaccines, showed that caregivers who delayed vaccines were significantly less likely to believe that vaccines are necessary to protect the health of children compared with caregivers who regularly vaccinated their children, (91% vs. 99.3%, p<0.001); that their child might get a disease if they aren't vaccinated (76% vs.100%, p<0.001) and also they were less likely to read and watch stories about health (93% vs.100%, p<0.001).

With respect to influences that discourage caregivers from having their child vaccinated, compared with caregivers who regularly vaccinated their children, caregivers who delayed vaccination were significantly less likely to agree with the statement, "vaccines are safe" (85% vs. 100%, p<0.001). Also caregivers who delayed vaccines were significantly less likely to believe that they had a good relationship with their child's health-care provider (85% vs. 100%, p<0.001) and that medical professionals in charge of vaccinations have their child's best interest at heart (82% vs. 100%, p<0.001). Caregivers who delayed vaccines were significantly more likely to believe that if they vaccinated their child, he/she might have serious side effects (100% vs. 78%, p<0.001) that children receive too many vaccines (100% vs. 92%, p<0.001); and that vaccination should be delayed if a child has a minor illness (100% vs. 80%, p<0.001) (table 2).

Caregivers' satisfaction about perceived knowledge related to vaccines from physician showed that those who regularly immunized their children reported significant higher satisfaction compared to those who delayed the vaccination regarding vaccine safety (53.1% vs 38%), fever development (62.4% vs 59%), illustration time (30.4% vs 14%) vaccine schedule (72% vs 60%), additional vaccines (71% vs 55%). However the reverse observed regarding insurances of proper vaccine administration where those with delayed

vaccination showed significant higher satisfaction than those with regular vaccination (64% vs 51.9% respectively) (table 3).

Table (1): Distribution of studied groups according to their knowledge about importance of vaccination to child heath, hazards of non vaccination and the Severity of vaccine preventable diseases.

Vaccines related knowledge		Vaccination status				Test of significance
		FULL N=900		DELAYED N=100		P value
		N	%	N	%	
Importance of Vaccination to child health	very imp	783	87.0%	79	79.0	0.000
	Imp	111	12.3	12	12.0	
	not at all	3	0.3	2	2.0	
	do not know	3	0.3	7	7.0	
Hazards of non vaccination	Do not know	119	13.2	16	16.0	0.0001
	No Hazards	31	3.4	12	12.0	
	Catch Disease	750	83.3	72	72.0	
Severity of vaccine preventable diseases	severe	691	76.7	71	71.0	0.096
	moderate	114	12.7	16	16.0	
	mild	57	6.4	4	4.0	
	Do not know	38	4.2	9	9.0	

Table (2): Caregivers' concerns about vaccination.

Caregivers' concerns about:	Asked questions	Vaccination status				Test of significance
		FULL N=900		Delayed N=100		
		N	%	N	%	P value
child's risk of getting a VPD*	Vaccination is important to protect the health of the child	894	99.3%	91	91.0%	0.000
VPDs are a sufficient health concern to make vaccinations relevant	Interest in health related topics in media.	900	100.0%	93	93.0%	0.000
	Disease development if the child not vaccinated.	900	100.0%	76	76.0%	0.000
Influences that facilitate or discourage parents from having their child vaccinated.	The child receives too many vaccines.	828	92.0%	100	100.0%	0.000
	Vaccination safety	900	100.0%	85	85.0%	0.000
	Good relationship with the child's health care provider	900	100.0%	85	85.0%	0.000
	Medical professional in charge of vaccinations have the child best interest at heart	900	100.0%	82	82.0%	0.000
	Serious side effect following vaccination.	702	78.0%	100	100.0%	0.000
	Vaccination delay due to minor illness of the child.	720	80.0%	100	100.0%	0.000

***VPD: Vaccine Preventable Disease.**

Table (3): Distribution of studied groups according to caregivers' satisfaction about different aspects of care including perceived knowledge from physician.

Different aspects of care		Vaccination status				Test of significance
		FULL N=900		Delayed N=100		P value
		N	%	N	%	
Knowledge from physician	Vaccine safety	478	53.1%	38	38.0%	0.000
	Vaccine benefits &importance	378	42.0%	33	33.0%	0.156
	Vaccine side effects					
	• Fever development	562	62.4%	59	59.0%	0.016
	• Fever management	598	66.4%	56	56.0%	0.077
	• others	503	55.9%	53	53.0%	0.489
	Vaccine schedule	648	72%	60	60.0%	0.012
	Ensure proper vaccine administration	467	51.9%	64	64.0%	0.007
	Additional Vaccination	639	71%	55	55%	0.000
Time related satisfaction	Illustration Time	274	30.4%	14	14.0%	0.000
	Waiting Time	346	38.4%	32	32.0%	0.358

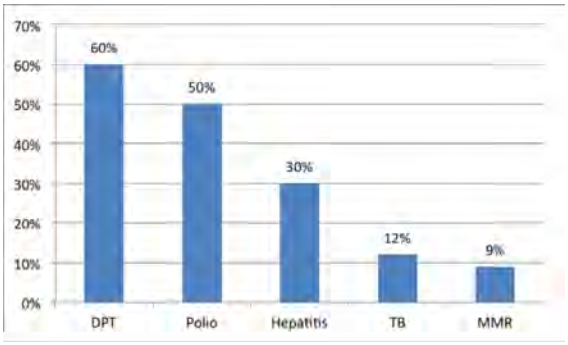

Fig. (1): The percent distribution of different delayed vaccines.

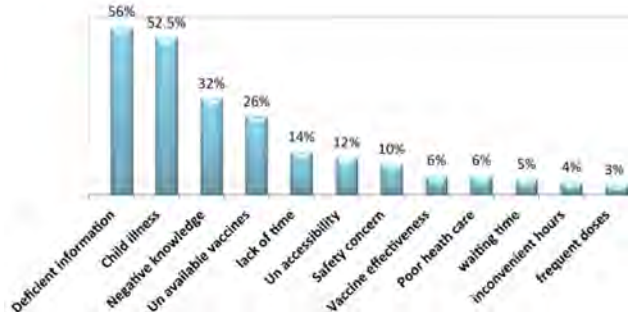

Fig. (2): The percent distribution of different causes of vaccine delay.

Discussion

Immunization is a proven tool for controlling and eliminating life-threatening infectious diseases and is estimated to avert 2 to 3 million deaths each year. It is one of the most cost-effective health investments, with proven strategies that make it accessible to even the most hard-to-reach and vulnerable populations (WHO, 2009). Despite this, vaccine preventable diseases remain the most common cause of childhood mortality with an estimated three million deaths each year (Centre for Global Development, 2005).

Uptake of vaccination services is dependent not only on provision of these services but also on other factors including knowledge and attitude of caregivers (Torun and Bakirci, 2006), density of health workers, accessibility to vaccination clinics and availability of safe needles and syringes. Assessing and evaluation of immunization coverage helps to evaluate progress in achieving program objectives and in improving service delivery (Bonu et al, 2003).

Among the studied children, 90.0 % had their full scheduled immunizations by the age of two years. This rate is markedly higher than that reported by Odusanya et al, (2008) and Jani et al, (2008) who detected that the full immunization coverage in children below 2 years was only 61.9 % and 71.8 % respectively. On the other hand, the vaccination rate reported by the present study is lower than that reported in the United States (94.8%) (Centers for Disease Control and Prevention, 2012). This difference may be due to different economic level between different countries that may affect accessibility, availability of vaccines and health care services.

In the present study, caregivers' perception of vaccination importance for child health was significantly associated with full vaccine uptake. This is in accordance with Wilson et al, (2008) who concluded that the parental decision to vaccinate was due to recognizing the importance of preventing disease.

We also found that, caregivers who regularly immunized her child had better knowledge than

caregivers who delayed child immunization. This is in agreement with Joseph et al, (2011) who found that parents' knowledge about the disease and the vaccine is a predictor of higher vaccination compliance. Consistently, De Courval et al, (2003) and Davis et al, (2001) declared that lack of knowledge about the importance of vaccines has been identified as a main barrier to immunization, and the provision of information about a disease, the adverse sequelae of the disease, and the effectiveness of the vaccine have been shown to increase uptake, so receiving vaccine-information materials during pregnancy or at a well-child visit before the vaccination visit is very essential.

In our study, the delay vaccination was mainly due to deficient information about the importance of vaccination at time or the timing of vaccination, however there were other causes of delayed vaccination as fear of child illness as a side effect of vaccine. Consistently, Ozkaya et al, (2010), declared that some mothers refused to complete child vaccination due to high anxiety levels about vaccine side effect. This agreed with Ritvo et al, (2003), who revealed that fear of vaccination side-effects may be a barrier for immunization. We also found that delayed vaccination may be due to vaccine deficiency in the health offices. In agreement with us, De Serres et al, (2002) reported that the unavailability of the vaccines may be a cause of delayed immunization.

We have declared that, caregivers who delayed vaccination were significantly less likely to agree with the statement, "vaccines are safe" this matched with Freed et al, (2010) who declared that, parents who delayed vaccine were significantly anxious about vaccines safety . They believed that if they vaccinated their child, he/she might have serious side effects.

Limitation of study:

Nevertheless, potential biases should be considered before generalizing these results to all caregivers of Egyptian young children. The most important possible bias is that the caregivers who agreed to participate in the study may be

those who were most in favour of vaccinations and therefore the most inclined to vaccinate their children with recommended vaccinations.

Conclusion and recommendation:

Our results suggest that modifiable determinants for a negative attitude could probably be based on a lack of specific knowledge. In fact, there is still uncertainty surrounding the safety of recommended vaccines, so it is recommended to minimize different barriers to vaccinations by improving health education of caregivers in the vaccination programs and stepping up awareness campaigns using known and effective communication channels to convey messages to communities with a large number of at-risk families. Clearly, the Ministry of health (MOH) needs to do more in informing people about the process of immunization in order to allay fears (e.g., information in the antenatal period may enable caregivers to make more informed decisions about recommended vaccinations) and develop ways of addressing common uncertainties about immunization, including the safety of combining antigens and the need for boosters. Training of the physician about proper communication with caregivers and response to their needs and concerns may increase caregivers' satisfaction.

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BELOW 2 YEARS CHILDREN CAREGIVERS' KNOWLEDGE, ATTITUDE AND BELIEFS TOWARDS IMMUNIZATION

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THE ROLE OF MR DIFFUSION IN DIFFERENTIATION OF MALIGNANT AND BENIGN HEPATIC FOCAL LESIONS

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Abstract

Aim: *The objective of this study was to determine if focal liver masses could be differentiated as benign or malignant on the basis of diffusion-weighted MR imaging (DWMRI) and ADC maps.*

Methods and Materials: *Between June 2011 and December 2012, a total of 60 consecutive patients (43 men, 17 women; age range 20-70, mean age, 45 years) with 60 focal liver lesions were scanned using 1.5 T magnetic resonance imaging (MRI). Respiratory-triggered single-shot echo-planar diffusion weighted imaging (DWI) was performed with b 0, b 500 and b 1000 gradients with ADC measurements. Comparison of mean ADC values between each benign and malignant lesion was done . Reference standard of diagnosis was obtained by correlating DWI with histopathologic findings, characteristic MR sequences and imaging follow-up. The accuracies of DWI and ADC values in differentiating benign and malignant focal liver lesions were assessed with the Student t test, and cut-off values were determined with receiver operating characteristic curve analysis (ROC). The analyzed lesions were hemangioma (n=8), cysts (n=8), adenoma (n=5), focal nodular hyperplasia (FNH) (n=5), hepatocellular cacinoma (HCC) (n=20), cholangiocarcinoma (n=5) and metastases (n=9).*

Results: *The mean ADC value (at b1000) of malignant focal liver lesions was 0.858±0.18X 10-3mm2/sec and of benign focal lesions was 1.55±0.0.43X 10-3mm2/sec. There was statistically difference in mean ADC values between malignant and benign focal liver lesions (p<0.0001). When apparent diffusion coefficient value of 1.0 X 10-*

3mm2/sec was used as a threshold value for differentiation of malignant tumors from benign lesions, sensitivity was 90.3%, specificity 78.57%, accuracy 86.7%, positive predictive value 90.3% and negative predictive value of 78.6%. The best result was obtained with the use of ADC cut off value (at b500) of 1.5 x10-3mm2/sec and ADC cut off value (at b1000) of 1.0 x10-3mm2/sec, with 90.3% sensitivity, 92.86% specificity, 91.1% accuracy, 96.6 % positive predictive value and 81.3 % negative predictive value.

Conclusions: *adding DWI to routine abdominal MRI and ADC measurements at least at 2 different gradients is a useful tool in differential diagnosis of malignant from benign liver lesions and may be useful for differentiation of different subtypes of either benign and malignant lesions.*

Introduction

The differential diagnosis between malignant and benign focal liver lesions remains a diagnostic challenge for every radiologist. For detection and characterization of focal liver lesions, many different modalities have been proposed; including multi-phase contrast-enhanced CT[1] and MRI [2], CT portography[3] and perfusion studies using dedicated ultrasound or Computed tomography(CT) or MRI contrast agents[4]. Of these modalities, magnetic resonance imaging is considered the most accurate imaging technology because it has high resolution for soft tissue and has the potential to characterize a lesion on various data acquired, such as T1, T2, and early and late post-gadolinium images[5] and[6].

Magnetic resonance imaging (MRI) has been used in both the detection and characterization of focal hepatic lesions. With the advent of the echo-planar MR imaging technique, diffusion weighted imaging (DWI) of the abdomen has become possible with fast imaging times which minimize the effect of gross physiologic motion from respiration and cardiac movement[7]. Thus, DWI became a valuable technique for evaluating focal hepatic lesions in addition to conventional MRI sequences[8]. More recently, apparent diffusion coeffi-

cient (ADC) value has been introduced in quantitative measurements as an adjunct to DWMRI. ADC is a quantitative parameter measuring the rate of diffusion of water molecules in biological tissues. There are several reports regarding the use of ADC in diagnosis and characterization of focal hepatic lesions[7,8,9], and[10]. However, the efficacy of ADC values in diagnosing and characterization of solid benign and solid malignant lesions has not been well described. Similarly, lesion and diffusion gradient variabilities were also limited in these studies [8,9,10]. Usage of ADC measurements in various types of focal hepatic lesions at different diffusion gradient values may define the role of ADC values in radiological evaluation of focal hepatic lesions [11].

The expanding role of DWI in evaluation of liver lesions can increase confidence in differentiation between benign and malignant lesions. DW sequences can be performed on most modern MRI machines with relative ease, in a short time period and without the need for contrast medium[12].

Patients and Methods

1. Patients:

Between June 2011 and December 2012, a total of 60 focal hepatic lesions in 60 consecutive patients were evaluated by abdominal MRI. Informed consent was taken from all. Patients with hepatic neoplasms who had undergone chemotherapy or radiation therapy within the last 3 months prior to the MR examination were excluded from our analysis in order to ensure that ADC measurements were reflective of the natural state of the liver lesions. In addition, patients without sufficient confirmation of the nature of the lesions were excluded. The final study population consisted of 60 patients (43 men, 17 women; age range 20–70 years, mean age 45 years).

There were 9 patients with history of an extra-hepatic primary malignancy and suspected liver metastases (colorectal cancer [n=3], breast cancer [n=2], gastric cancer [n=1], and pancreatic cancer [n=3]. There were 28 patients with chronic liver disease (including chronic hepatitis and cirrhosis related to hepatitis C and old bil-

harziasis) with suspected malignancy. Finally, there were 23 patients with no history of malignancy or chronic liver disease who underwent MR imaging for evaluation of presumably benign or indeterminate, incidentally diagnosed focal liver lesion(FLL). In patients with multiple FLL, the largest ones of each lesion type were randomly selected for further analysis by the study coordinator.

Thirty four lesions were malignant tumors (20 hepatocellular carcinoma (HCCs), 5 cholangiocarcinomas & 9 metastases). Benign liver lesions were twenty six (8 cysts, 8 hemangiomas, 5 adenomas and 5 focal nodular hyperplesia (FNH).

The standard of reference for characterizing FLL was evaluated by two radiologists (GE & MA), with experience in MR imaging of 20 and 10 years respectively. Malignant nature of lesions was confirmed by pathologic findings following biopsy or surgery for 13 HCCs, 5 cholangiocarcinomas and 5 metastases. The diagnosis of the remaining 7 HCCs lesions was confirmed by using the established imaging criteria and follow up after chemoembolization. The remaining 4 metastatic lesions were confirmed on the basis of new occurrence of a lesion compared to a prior MR study, follow up of lesion size after the start of chemotherapy in patients with known extra-hepatic primary malignancies. Three FNHs and two adenomas were confirmed pathologically. The remaining benign lesions were diagnosed by using established imaging criteria[13,14,15] in conjunction with stable appearance and size at follow-up imaging in equivocal cases with a minimum follow-up interval of six months (range: 6-12 months).

2. MR imaging & image analysis:

Magnetic resonance imaging examinations were performed on 1.5 tesla (T) system (Philips, Achieva) with a sixteen-channel body coil.

Before DWI, breath-hold T1-weighted image, fat-saturated fast spin echo T2 weighted image, dual echo fast spoiled gradient-echo (FSPGR) and single shot fast spin echo (SSFSE) T2 weighted images

were obtained in axial and coronal images. Diffusion weighted images were obtained before contrast administration with b values of 0, 500, and 1000 s/mm2. Breathhold, dynamic 3D T1 weighted sequence was performed after DWIs (bolus injection of 20 ml gadopentetate dimeglumine 1.5 ml/s). All DWIs were obtained in transverse plane using single-shot echo-planar spin echo sequences. Imaging parameters for DWIs were: Repetition time (TR): 1100 ms; TE: 67–91 ms; FOV: 35 Centimeter (cm) x 35 cm (change according to body size); number of excitation: 1; matrix size: 128 x 128; section thickness: 5 mm; intersection gap: none. DW sequences required a total of 96 s to scan on MR. Array spatial sensitivity encoding technique (ASSET) was used as parallel imaging technique.

Imaging findings were evaluated carefully, then, ADC values of different masses detected on conventional MRI sequences and DWI were measured through gray-scale ADC maps from each lesion at b 500, and b 1000 s/mm2 gradient values by using 3 circumferential region of interests (ROIs) and the average ADC values were recorded. ADCs were measured over the largest mass detected in patients with multiple liver lesions. Necrotic portions of solid lesions detected on contrast enhanced MRI were excluded.

Mean ADC values of benign lesion group (FNH and other benign liver masses) and malignant lesion group (HCC, cholangiocarcinoma and metastasis) at 2 different diffusion gradients were compared. Similarly, mean ADC values of each benign and malignant lesion at 2 different gradients were also recorded and compared in order to determine whether it would be possible to define the characteristic or type of individual benign and malignant lesion.

To validate our system ADC values of water was measured on phantoms one week before the ADC measurements. The ADC value for water was 2.21 $x10^{-3}$ mm^2/s, in agreement with other studies in the literature[16].

Statistical Analysis

Statistical analysis was carried out via Statistical package for so-

cial Science (SPSS) version 17 program on windows XP. Qualitative data were represented in the form of number and frequency, while quantitative data were represented in the form of mean ± standard deviation (mean±SD). Kolmogrov-smirnov test was used to test normality of quantitative data. Student's t test, Mann-Whitney U and Kruskal-Wallis Test were used to compare groups. Receiver operating characteristic (ROC) curve was computed to determine the cutoff value for the malignancy. All tests were considered significant if P value equals or less than 0.05.

Results

Mean size of all 60 focal hepatic lesions was 2.83±1.05 cm. Twenty six of the 60 lesions were benign and 34 were malignant. Benign lesions had a mean size of 2.96±0.77 cm (ranges 2–5 cm) whereas mean size of malignant lesions was 2.77±1.17 cm (ranges 1-6 cm) (table 1).

Mean ADC values of 26 benign lesions at b 500 and b 1000 gradients were $2.09\pm0.50x10^{-3}$, $1.55\pm0.43x10\text{-}3^{-3}$ mm^2/s, respectively. Mean ADC values of malignant lesions at b 500 and b 1000 gradients were $1.20\pm0.18 \times 10^{-3}$ and $0.85\pm0.18 \times 10^{-3}$ mm^2/s, respectively. Mean ADC values of benign lesions were higher than malignant lesions and these differences were statistically significant for the 2 diffusion gradients ($P< 0.0001$ & $P<0.0001$, respectively) (table 1).

Mean ADC values of all lesions at b 500 and b 1000 gradients and differentiation between subtypes of benign and malignant lesions are summarized in table 2.

An ADC cut-off value of $1.0x 10^{-3}$ mm^2/s at b 1000 diffusion gradient resulted in 90.3 % sensitivity, 78.6 % specificity and 86.7% accuracy for differentiation of benign and malignant focal hepatic lesion groups . The best result was obtained with the use of ADC cut off value of $1.5x10^{-3}$ mm^2/sec at b 500 and ADC cut off value of $1.0x10^{-3}$ mm^2/sec at b 1000, with 90.3% sensitivity, 92.86% specificity, and 91.1% accuracy. The results of ROC curve analyses, ADC cut off values for the differentiation between benign and malignant lesions at both

b500 and b1000 diffusion gradients are shown in table 3 and 4.

Seventeen of the 26 benign lesions show low SI and 9 benign lesions show intermediate SI on DWI. While 26 of the 34 malignant lesions show high SI and 8 malignant lesions show intermediate SI on DWI.

Cysts and hemangiomas showed the highest mean ADC values at b 500 and b 1000 gradients in benign lesion group (figure 1). Hepatocellular carcinomas showed highest mean ADC values at b 500 and b1000 in malignant lesion group (figure 2).

Comparison of ADC values revealed that mean ADC value of all benign hepatic focal lesions were significantly higher than all malignant focal lesions at b 500 and b 1000 gradients (P<0.0001) (figure 3 and 4).

Differentiation of benign and malignant subtype lesions from each other in their groups show some promising results. There was statistically significant difference between: the mean ADC values of cysts and adenoma at both b 500 and b1000 (P value = 0.002, < 0.001), the mean ADC values of cysts and FNH at b 500 (P value =0.001). However, there was no significant difference between mean ADC values of other benign focal lesions from each others.

At b 500 gradient, HCCs had significant high ADC value than metastases and cholangiocarcinoma (P value = 0.034, 0.014 respectively) with difficult differentiation between metastases and cholangiocarcinoma. At b 1000 gradient, there was no significant difference between HCCs, metastases and cholangiocarcinomas.

There was statistically significant difference between ADC values of solid benign (adenoma , FNH) and solid malignant subtype lesions, as there was significant difference between: mean ADC values of FNH and HCC at both b500 and b1000 (P value = <0.001, <0.001) (figure 5 and 6), mean ADC values of FNH and metastases at both b500 and b1000 (P value = <0.001, 0.004), mean ADC values of FNH and cholangiocarcinoma at both b500 and

b1000 (P value = 0.005 , 0.015) respectively. Also, significant difference found between: mean ADC values of adenoma and HCC at both b500 and b1000 (P value = <0.001, 0.008), mean ADC values of adenoma and metastases at both b500 and b1000 (P value = <0.001, 0.014), mean ADC values of adenoma and cholangiocarcinoma at both b500 and b1000 (P value = <0.001, 0.023) respectively.

Table (1): Mean size, mean ADC values of benign and malignant lesions at both b500 and b1000.

Report

Tumor		mean size	ADC b 500	ADC b 1000
Benign	Mean	2.9643	2.0929	1.5500
	N	26	26	26
	Std. Deviation	.77122	.50909	.43456
	Minimum	2.00	1.30	.80
	Maximum	5.00	2.90	2.00
	Range	3.00	1.60	1.20
	Median	3.0000	2.2000	1.8000
Malignant	Mean	2.7774	1.2065	.8587
	N	34	34	34
	Std. Deviation	1.17266	.18246	.18335
	Minimum	1.00	.90	.50
	Maximum	6.00	1.80	1.40
	Range	5.00	.90	.90
	Median	2.5000	1.2000	.8500
Total	Mean	2.8356	1.4822	1.0738
	N	60	60	60
	Std. Deviation	1.05876	.52105	.42833
	Minimum	1.00	.90	.50
	Maximum	6.00	2.90	2.00
	Range	5.00	2.00	1.50
	Median	2.5000	1.3000	.9000

Table 2: Mean size, mean ADC values & differentiation of different subtypes of benign and malignant lesions.

Report

Tumor Type		mean size	ADC b 500	ADC b 1000		P value ADC b 500	ADC b 1000
HCC	Mean	3.1250	1.2650	.8925	**HCC vs. cholangiocarcinoma**	**0.014**	**0.61**
	N	20	20	20	**HCC vs metastasis**	**0.034**	**0.27**
	Std. Deviation	1.22512	.17554	.18806	**Cholangiocarcima Vs metastasis**	**0.44**	**0.51**
	Minimum	1.50	1.00	.50			
	Maximum	6.00	1.80	1.40			
	Range	4.50	.80	.90			
	Median	2.8500	1.2000	.8900	**adenoma vs HCC**	**<0.001**	**0.008**
metastases	Mean	1.9000	1.1111	.8078	**adenoma vs metastases**	**<0.001**	**0.014**
	N	9	9	9	**adenoma vs cholangiocarcinoma**	**<0.001**	**0.023**
	Std. Deviation	.59372	.16159	.18089	**FNH vs HCC**	**<0.001**	**<0.001**
	Minimum	1.00	.90	.55	**FNH vs metastases**	**<0.001**	**0.004**
	Maximum	3.00	1.40	1.20	**FNH vs cholangiocarcinoma**	**0.005**	**0.015**
	Range	2.00	.50	.65			
	Median	2.0000	1.1000	.8000			
cholangiocarcinoma	Mean	3.2500	1.0500	.7500			
	N	5	5	5			
	Std. Deviation	.35355	.07071	.07071			
	Minimum	3.00	1.00	.70			
	Maximum	3.50	1.10	.80			
	Range	.50	.10	.10			
	Median	3.2500	1.0500	.7500			
FNH	Mean	3.2000	1.8600	1.4600	**FNH vs Hemangioma**	**0.16**	**0.27**
	N	5	5	5	**FNH vs adenoma**	**0.98**	**0.45**
	Std. Deviation	1.15109	.47223	.51284	**FNH vs cysts**	**0.001**	**0.05**
	Minimum	2.00	1.30	.90	**Hemangioma vs adenoma**	**0.22**	**0.06**
	Maximum	5.00	2.30	1.90	**Hemangioma vs cysts**	**0.15**	**0.47**
	Range	3.00	1.00	1.00	**adenoma vs cysts**	**0.002**	**<0.001**
	Median	3.0000	2.1000	1.8000			
Hemangioma	Mean	2.7500	2.3000	1.7500			
	N	8	8	8			
	Std. Deviation	.64550	.53541	.37859			
	Minimum	2.00	1.60	1.20			
	Maximum	3.50	2.90	2.00			
	Range	1.50	1.30	.80			
	Median	2.7500	2.3500	1.9000			
adenoma	Mean	3.0000	1.8667	1.2333			
	N	5	5	5			
	Std. Deviation	.50000	.49329	.37859			
	Minimum	2.50	1.30	.80			
	Maximum	3.50	2.20	1.50			
	Range	1.00	.90	.70			
	Median	3.0000	2.1000	1.4000			
cysts	Mean	2.7500	2.6000	1.8500			
	N	8	8	8			
	Std. Deviation	.35355	.14142	.07071			
	Minimum	2.50	2.50	1.80			
	Maximum	3.00	2.70	1.90			
	Range	.50	.20	.10			
	Median	2.7500	2.6000	1.8500			
Total	Mean	2.8356	1.4822	1.0738			
	N	60	60	60			
	Std. Deviation	1.05876	.52105	.42833			
	Minimum	1.00	.90	.50			
	Maximum	6.00	2.90	2.00			
	Range	5.00	2.00	1.50			
	Median	2.5000	1.3000	.9000			

Table (3): Mean ADC cut off value for the differentiation between benign and malignant liver lesions at b1000 diffusion gradient

Cutoff	AUC±SE	95%CI	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	PPV (95%CI)	NPV (95%CI)
ADC\1000 ≤ 1.0	0.895±0.059	0.767-0.967	90.32% (74.2– 98%)	78.57 (49.2-95.3)	86.7 (72 – 95.1)	90.3 (79.7- 96.5)	78.6 (55 – 92.2)

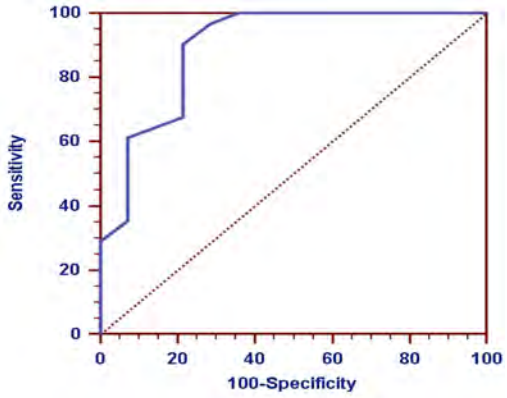
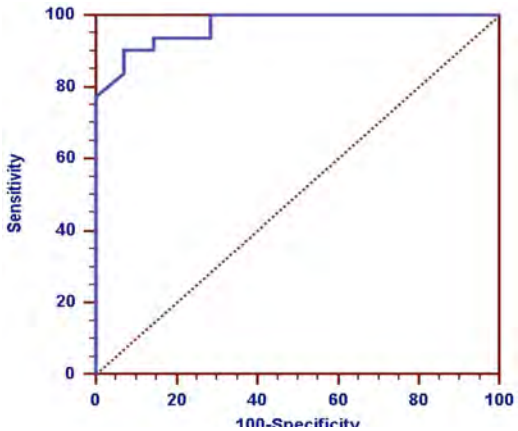


Table (4): Mean ADC cut off values for the differentiation between benign and malignant liver lesions at both b500 and b1000 diffusion gradients:

Cutoff	AUC±SE	95%CI	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	PPV (95%CI)	NPV (95%CI)
ADC 1000 ≤ 1.0 + ADC500 ≤ 1.5	0.97±0.02	0.87-0.998	90.32% (74.2– 98%)	92.86% (66.1– 99.8%)	91.1 (77.1 – 95.3	96.6 (85.7-99.8)	81.3 (61.5 – 87.2)



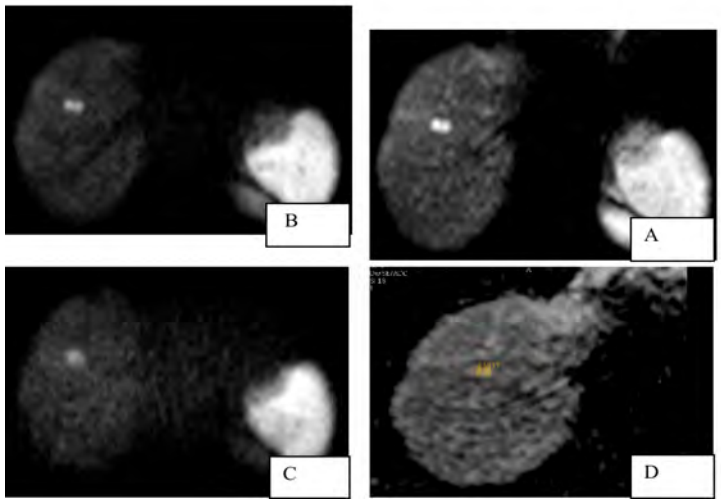


Fig. (1): Diffusion weighted MR images of female patient, aged 35 years with small focal hemangioma, DWI at b0 (A), b500 (B), b 1000 (C). ADC map at b1000 (D) shows relative high SI with high mean ADC value = 1.7 x 10^{-3} mm^2/s.

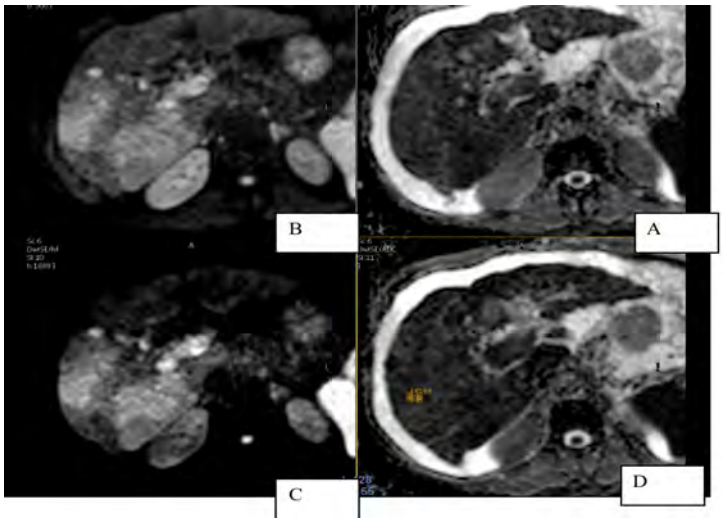


Fig. (2): Diffusion weighted MR images of 60 years old male patient with HCC , DWI at b0 (A) , DWI at b500 (B), DWI at b 1000 (C) and ADC map (D) show restricted diffusion with mean ADC value at b1000 = 0.85 x 10^{-3} mm^2/s.

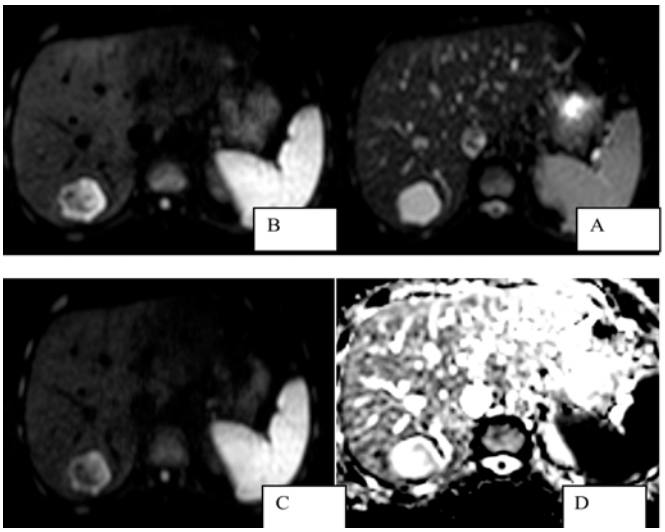


Fig. (3): Diffusion weighted MR images of 42 years old male patient with hemangioma, DWI at b0 (A), DWI at b500 (B), DWI at b 1000 (C) and ADC map (D) shows mixed high SI with high mean ADC value at b1000 = 2 x 10^{-3} mm^2/s.

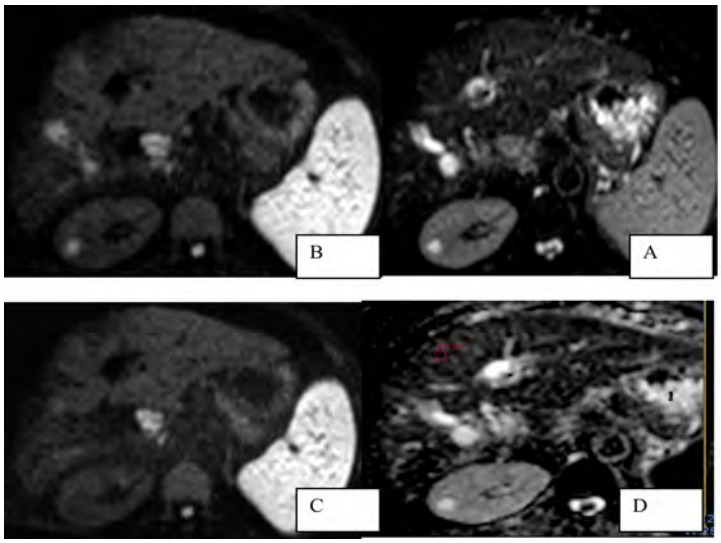


Fig. (4): Diffusion weighted MR images of 55 years old male patient with small HCC, DWI at b0 (A), DWI at b500 (B), DWI at b 1000 (C) and ADC map (D) shows isointense SI with restricted diffusion with mean ADC value at b1000 = 0.9 x 10^{-3} mm^2/s.

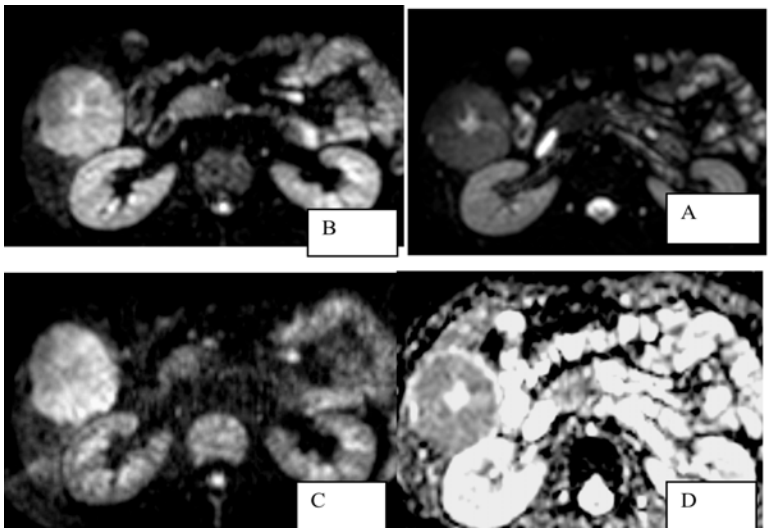


Fig. (5): Diffusion weighted MR images of 40 years old female patient with FNH, DWI at b0 (A), DWI at b500 (B), DWI at b1000 (C). ROI is located peripherally in the lesion since central part represents vascular scar tissue. Mean ADC value at b1000 (D) = 1.3 x 10^{-3} mm^2/s. Though lesion is hyperintense at DWMRI, it shows high ADC value.

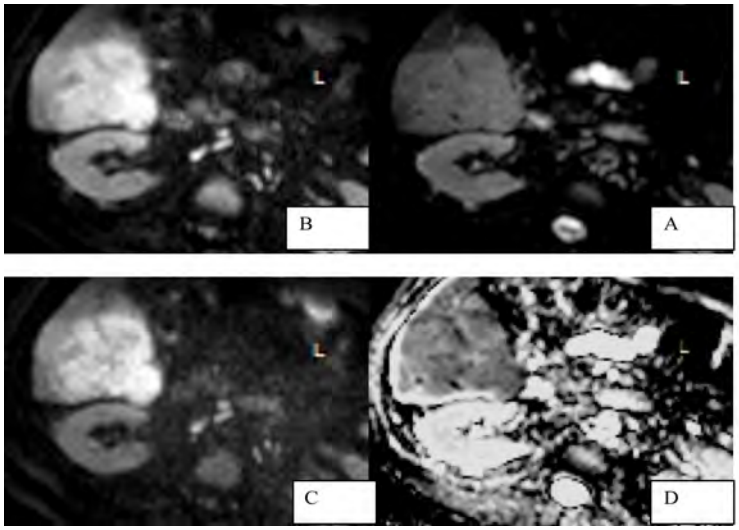


Fig. (6): Diffusion weighted MR images of 57 years old male patient with HCC, DWI at b0 (A), DWI at b500 (B), DWI at b 1000 (C). The lesion shows low SI on ADC map (D) with restricted diffusion and mean ADC value at b1000 = 0.8 x 10^{-3} mm^2/s.

Discussion

Reliable detection and characterization of focal liver lesions is critical for optimal patient management. Magnetic resonance imaging (MRI) has an established role in focal liver lesion detection and characterization and traditionally includes a combination of unenhanced T1 and T2-weighted sequences, in and out-of-phase T1-weighted sequences, and enhanced T1-weighted sequences after gadolinium administration or other liver-specific contrast agents. There is a good evidence that diffusion-weighted (DW) MRI has the potential to improve hepatic lesion detection rates and contribute to the differentiation of benign from malignant hepatic lesions[12].

Appearance of focal hepatic lesions on DWI especially at high b values was reported to be diagnostic in several studies due to restricted diffusion and increased signal intensity on DW images [8] and[17]. However this measurement was a qualitative assessment and represented a subjective interpretation. On the contrary, ADC value is a quantitative parameter of water diffusion. There are several studies in the literature emphasizing diagnostic utility of ADC measurement in the differentiation of benign and malignant focal hepatic lesions. According to these studies malignant lesions had lower ADC values compared to benign lesions which was attributed to high cellularity of malignant masses[7].

Chandarana and Taouli, 2010 [18], said that , there is no consensus in the scientific community about which b-values are optimal for liver imaging and when performing DW-MRI in the liver, it is advantageous to perform imaging with at least 3 b-values including both lower and higher b-values (e.g. using b = 0, b ≤ 100, and b ≥ 500 s/mm2). Goshima et al. 2008, [19] recommended DW-EPIs with low and high b values as supplementary sequences in the detection and characterization of benign and malignant hepatic lesions. In our study, DWI was done with 3 diffusion gradients at b0, b500 and b1000.

Most of the studies[1,20,10], indicated that a region of interest

(ROI) should be placed within the confines of the lesion in image analysis and put away from prominent vascular structures to avoid motion artifact. However, some authors[1,20] placed ROI that covered entire lesions without separating component with various signal intensities for analysis of heterogeneous lesions, whereas Gourtsoyianni et al, 2008[10] put the ROI in both sites in a lesion with different signal behavior in the periphery and center. In the current study, ROI was placed within the confines of the lesion and put away from vascular and necrotic portions of the lesion.

Kandpal et al, 2009[21] found that respiratory-triggered DWI was superior to breath-hold DWI for hepatic imaging because it provides higher SNR. In our study, DWI was done with respiratory triggered technique.

Most of the studies included hemangiomas and cysts in the benign lesion group[7,22,23]. Thus it was concluded that hypercellular benign lesions like FNH should also be studied in order to discriminate benign and malignant hepatic lesions more reliably[10]. While in the study of[11], solid (high cellular) benign liver lesions were included with exclusion of cystic benign lesions. In our study, cystic benign lesions as hemangiomas and cysts and solid benign lesions as FNH and adenoma were included in the benign lesion group.

Our results revealed that ADC measurements at b 500 and b 1000 diffusion gradients were useful in differential diagnosis of benign and malignant lesions and adding ADC cut off values at b 500 and b 1000 diffusion gradients increase sensitivity and specificity for differentiation.

In the present study, comparison of ADC values for individual benign and malignant lesions showed that there were statistically significant differences between lesions at different gradients. Mean ADC values at b 500 and b1000 of different benign lesions were as follow: FNH (1.86 and 1.46 x 10^{-3} mm^2/s), adenoma (1.86 and 1.23 x 10^{-3} mm^2/s), hemangioma (2.30 x 10^{-3} mm^2 and 1.75 x 10^{-3} mm^2/s) and cysts

(2.60 x 10^{-3} mm^2/s and 1.85 x 10^{-3} mm^2/s). While, mean ADC values at b 500 and b1000 of different malignant lesions were: HCC (1.26 and 0.89 x 10^{-3} mm^2), metastases (1.11 and 0.80 x 10^{-3} mm^2) and cholangiocarcinoma (1.05 and 0.75 x 10^{-3} mm^2).

In the study of Onur et al, 2012 [11], FNH was easily differentiated from malignant lesions except HCC at b 100 gradient. Other benign solid liver lesions also could be differentiated from metastases and cholangiocarcinomas at all gradients. They concluded that solid benign liver lesions did not show significant difference from HCCs at all gradients. Also,[8,9] said that The ADC values of these solid benign lesions were similar to ADC values of malignant lesions. Yet, in our study, FNH and other benign lesions could be differentiated from malignant lesions including HCC at both b500 and b1000 diffusion gradients.

Taouli et al, 2003[7], concluded that some overlap is present between metastatic lesions and FNHs, however, in our study, metastatic lesions had lower ADC values than FNH at both b500 and b1000 diffusion gradients. This matches with the results of Onur et al, 2012[11], who observed that ADC measurements were successful in differentiating these lesions . This may be due to exclusion of necrotic components of metastases at ADC measurement which may increased the ADC values of metastases.

In the current study, the differentiation between different subtypes of malignant lesions with mean ADC values showed some difficulty at both gradients. At b 500 gradient, HCCs had significant high ADC value than metastases and cholangiocarcinoma with difficult differentiation between metastases and cholangiocarcinoma. At b 1000 gradient, significant difference was only found between HCCs and cholangiocarcinomas. This is similar to the results of Gourtsoyianni et al, 2008, Kilickesmez et al, 2009 [10,24] and Onur et al, 2012[11], who concluded that the differentiation of malignant lesions with mean ADC values was difficult at all gradients and similarity of ADC values was found between malig-

nant hepatic lesions. Onur et al, 2012[11] said that, The reason for highest mean ADC values measured in HCCs among all malignant lesions may be due to relatively increased perfusion of HCCs than hypovascular metastases and cholangiocarcinomas and we agree with that explanation.

Results of our study showed that cysts and hemangioms have high ADC value than adenoma and FNH, however, (cysts and hemangiomas) and also (adenoma and FNH) couldn't be differentiated well from each other at both diffusion gradients, This is similar to the results of Onur et al, 2012 [11], as ADC measurements were not helpful in differential diagnosis of different types of solid benign lesions.

Sandrasegaran et al, 2009 and Miller et al, 2010[25] and[26] suggested that ADC values of solid benign lesions (FNH and adenoma) are similar to malignant lesions (metastasis and HCC) and DW imaging is not helpful in differentiating solid benign lesions from solid malignant lesions. However, in our study, there was significant difference between solid benign (adenoma, FNH) and solid malignant lesions. This is may be due to diminished perfusion effect due to high diffusion gradient as we used b 0, b 500 and b 1000 and previous studies used b 0, b 50 and b 500 as diffusion gradients.

Different studies reported variable ADC cut-off values for differentiation of benign and malignant focal hepatic lesions. Taouli et al, 2003[7] offered a threshold value as 1.5 x 10^{-3} at b 500 gradient with sensitivity of 84% and specificity of 89%. Parikh et al, 2008[8] reported an ADC value of 1.6 x 10^{-3} as a cut off value with sensitivity of 74.2% and specificity of 77.3% at b 500 gradient. Onur et al, 2012[11] concluded a cut-off value of 1.23 x 10^{-3} at b 1000 with sensitivity of 83% and specificity of 76% for differentiation between benign and malignant lesions. In our study, using 1.0 x 10^{-3} mm^2/s as an ADC cut-off value at b 1000 diffusion gradient resulted in 90.3% sensitivity, 78.6% specificity and 86.7% accuracy for differentiation of benign and malignant focal

hepatic lesion groups. The best result was obtained with the use of ADC cut off value (at b500) of 1.5 x $10^{-3}mm^2$/sec and ADC cut off value (at b1000) of 1.0 x $10^{-3}mm^2$/sec, with 90.3% sensitivity, 92.86% specificity, and 91.1% accuracy.

To best of our knowledge, only few studies in the literature measuring ADC values of different subtypes of either benign or malignant lesions. Our results showed that HCC had high ADC value than metastases and cholangiocarcinima in the malignant group liver masses. Cysts and hemangiomas had high ADC values than adenoma and FNH in the benign group liver masses.

Conclusion

So, adding DWI to routine abdominal MRI and ADC measurements at least at 2 different gradients is a useful tool in differential diagnosis of malignant from benign liver lesions and may be useful for differentiation of different subtypes of either benign and malignant lesions, further investigation in this point is recommended.

Limitation of the study:

There was small number of cases with FNHs and other benign liver masses and as well as cholangiocarcinoma. However these lesions were not seen as usually as metastases or HCC and most of these lesions (FNH, other benign liver masses and cholangiocarcinoma) were rarely compared with each other via ADC values in the literature.

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THE ROLE OF MR DIFFUSION IN DIFFERENTIATION OF MALIGNANT AND BENIGN HEPATIC FOCAL LESIONS

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DOPAMINE TRANSPORTER 3'UTR VNTR GENOTYPE AND WISCONSIN CARD SORTING TEST IN CHILDREN WITH ADHD AMONG EGYPTIAN POPULATION

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Abstract

Background: *ADHD is the most commonly diagnosed behavioral disorder of childhood, and that it occurs in 3 to 5 percent of school-age children, this means that it affects a great part of the Egyptian population. The etiology of ADHD is unknown. Therefore understanding the etiology and pathogenesis of ADHD is a key and important challenge in psychiatry.*

Method: *To investigate the relationship between the dopamine transporter gene (SLC6A3) 3'-UTR VNTR genotypes and Wisconsin Card Sorting Test in children with ADHD versus control, 50 children diagnosed with ADHD and 50 of control children were sequentially recruited, genotyped, and tested using neuropsychological tests .*

Results: *There were significant differences in Total Category First Completed (TCFC) and categories completed indices of WCST results between cases and control. No significant difference in genotype of DAT 3'UTR VNTR genotypes was found between cases and control. The most common genotype among both ADHD cases and control was 9/10 while the least genotype was 9/9 among both groups. No significant genetic correlation and WCST indices in ADHD children.*

Conclusion: *There is impairment of set shifting domain of executive function in ADHD children. No significant genetic correlation and WCST indices in ADHD children.*

Introduction

ADHD, one of the most prevalent conditions in child psychiatry, manifests as an unusually high and chronic level of inattention and/or impulsivity/hyperactivity. ADHD is estimated to occur in 8–12% of children worldwide[1].

According to DSM-IV-TR, the symptoms are categorized into either inattention or hyperactivity-impulsivity. The symptoms and impairment of functioning should be present in 2 or more settings. The symptoms could be in either categories, or in both, and so the following subtypes are identified; predominantly inattentive type, predominantly hyperactive-impulsive type, and combined type[2].

The etiology of ADHD is unknown. Therefore understanding the etiology and pathogenesis of ADHD is a key and important challenge in psychiatry. A number of recent studies, including family, twin and adoption studies, have provided various lines of evidences that genetic factors play a substantial role in the etiology of ADHD[3].

Dopamine transporter gene (DAT1) regulates dopamine (DA) transmission by removing DA from extracellular spaces and recycling it back into DA neurons. Several lines of evidence imply that dysregulation in DA transmission, particularly altered DAT1 function, is likely to be involved in the pathophysiology of ADHD: (1) Increased striatal DAT1 density has been found in imaging studies in both adults and in children with ADHD. (2) Stimulants are effective in the treatment of the core symptoms of ADHD. (3) Complete disruption of the DAT1 gene in mice alters DA tone and signaling [4].

The term executive functions (EF) refer to a wide range of central cognitive functions that play a critical role for all individuals as they manage multiple tasks of daily life. One model of EF includes the following six clusters of cognitive functions that tend to be impaired in individuals with ADHD (activation, focus, effort, emotion, memory, action)[5].

Finding an association between executive function dysregulation

and ADHD (with demonstration that dopamine neurotransmission is critical for normal executive functions) would highlight the plausibility of executive function as an endophenotype in ADHD. From a genetic standpoint, the DAT1 gene is known to be preferentially expressed in the basal ganglia and has been reported to influence caudate volume and aspects of executive functioning in normal subjects. Furthermore and in keeping with the above, the dopamine transporter has been argued to play a critical role in regulating cortical signal-to-noise ratio during working memory via a cortico- striato - thalamo - cortical pathway[6].

Aim of the Work

1- To assess of degree of impairment in WCST in ADHD children versus control.

2- Genetic correlation of WCST in ADHD children.

Methodology:

Assessment tools:

1- Clinical assessment:

A) Complete thorough physical and neurological examination.

B) Diagnostic assesement of the patient: using Mini International Neuropsychiatric Interview for children and adolescent (M.I.N.I KID)[7]. Arabic version of M.I.N.I KID was used in study[8].

2- Psychometric assessment, for all participants using the following tools:

a) Arabic version of Child behavior check list[9,10].

b) Arabic version of Conner's global index, parent version [11,12].

c) Arabic version of Wechsler Intelligence Scale for Children (WISC)[13,14].

d) Executive function assesment: By using Computerized Wesconsin card sorting tests[15].

3-Molecular genetics study:

- After consent, a blood sample will be taken from child with ADHD and will be investigated for polymorphism of gene (5p region).

- The blood samples were collected regularly in clinical pathology laboratory in Mansoura faculty of medicine.

- 3 untranslated region VNTR of dopamine transporter gene (DAT) was chosen.

- Blood samples were undergo-

ing the following steps:

1- DNA extraction.

2- PCR amplification (PCR).

3- Electrophoresis Gel.

4- Statistical Analysis:

(B)- Control gruop:

This group will include 50 children with the following criteria:

• No Psychiatric manifestations.

• Age and sex matched with the participants' sample.

• They will be subjected to the same scales and tools.

Results

Our results can be demonstrated in the following tables:

Table (1): showed that there were no significant differences among age, sex, education and residence. The mean age of ADHD cases was 8.18±1.48 and majority were males. In the control group, the mean age was 8.62±1.68 and the majority were males too.

Table (2): There were significant difference in Total Category First Completed (TCFC) and categories completed indices of WCST results between cases and control.

Table (3): The most common genotype among both ADHD cases and control was 9/10 while the least genotype was 9/9 among both groups. No significant difference in genotype of DAT 3'UTR (9/9,9/10,10/10) was found between cases and control .

Table (4): No significant genetic correlation among (9/9,9/10.10/10) genotypes and WCST indices in ADHD children.

Table (1) : Sociodemographic date among children with ADHD and control group:

Sociodemographic variables	Cases (N=50)	Control(N=50)	Significance
Age(Mean ±SD)	8.18±1.48	8.62±1.68	0.17
Sex			
Male	32(64%)	35(70%)	0.689
Female	18(36%)	15 (30 %)	
Education:(Mean ±SD)	2.49±1.29	3±1.4 8	0.07
Consanguinity: N (%)	4(8%)	4(8%)	
Residence N (%)			
Urban:	23 (46%)	23(46%)	
Rural:	27 (54%)	27(54%)	

Table (2): Wisconsin card sorting test among children with ADHD and control group:

Wisconsin Test	Cases (Mean ± SD)	Control (Mean ± SD)	Significance
Total errors	94.78±9.39	69.7±22.32	0.13
TCFC	79.8±51.72	56.74±49.07	0.034*
Categories completed	1.58±19.19	2.1±2.08	0.044*
Preservative error raw score	33.58±19.19	31.98±16.61	0.99
Non Preservative error raw score	41.56±23.64	38.34±21.28	0.53

Table (3): DAT 3' UTR gene polymorphism among children with ADHD and control group:

Genotypes of DAT 3´UTR	CASES		Control		P value
	NO	%	No	%	
9/9	5	10 %	9	18 %	0.25
9/10	24	48%	23	46.0%	0.84
10/10	21	42%	18	36 %	0.54

Table (4): Genetic correlation of WCST in ADHD cases:

Wisconsin card sorting test	9/9 Mean ±SD	9/10 Mean ±SD	10/10 Mean ±SD	Significance
Total errors	81.8±17.83	75.48±17.36	76.05±22.55	0.608
TCFC	101.4±38.42	87.7±49.4	64.5±55.27	0.198
Categories completed	5±1.2	1±1.44	2.4±4.08	0.390
Preservative error raw score	36.8±18.01	33.84±18.15	23.45±21.46	0.663
Non Preservative error raw score	45±20.4	42.5±23.43	39.5±25.51	0.856

Discussion

To our knowledge, this is the first study in Arab world to study the correlation of 3'UTR DAT polymorphism and WCST in ADHD children.

It is observed in our study that there is the lack of association of DAT 3'UTR gene polymorphism and ADHD. The most common genotype was (9/10) and the least genotype was (9/9). This is in agreement with[16,17,18,19] due to the relatively small frequency of the 9/9 genotype in the general population and to the consequently small sample sizes generally acquired for this genotype, 10/10 genotype groups are usually compared to a combined group of subjects with the 9/9 and 9/10 genotypes (as well as other very rare genotypes)[6].

The dopamine transporter gene (DAT1) has been reported to be associated with attention-deficit hyperactivity disorder (ADHD) in a number of studies[20,21,22,6].

That is finding in our study may be due to the following reasons. First of all, small sample size. The second one is the polymorphism that we studied in this sample is located in the 3' untranslated region of the SLC6A3 gene and thus does not change the structure of the DAT1 protein. Nonetheless, it has been suggested that it may affect the level of expression of the DAT1 gene, resulting in variable dopamine transporter phenotypes[6]. While this may be the case, the issue still remains controversial. Indeed, it has been reported that DAT binding availability is significantly lower for subjects who are homozygous for the SLC6A3 VNTR 10-repeat allele compared to carriers of at least one 9-repeat allele [23]while the opposite association [24])was reported in another study and yet no association [25]was found in a third. The third explanation may be related to the methodological differences. The Fourth one is ethnicity as this is first study to be done on Egyptian population.

In our study, there were a significant difference in TCFC and categories completed indices of WCST results between ADHD cases and control. On the other hand no significant differences in total errors, preservative errors and non-preservative error raw score.

Concerning Wisconsin Card Sorting Test, 17 out of 26 studies using the WCST found significant differences between ADHD and normal controls[26]. Children with ADHD complete fewer categories than normal controls[27], and showed more deficiency in flexibility and perseverative errors than high functioning autistic and control groups[28].

Limitation of the present study

First, the sample size might have limited our ability to detect more 3'UTR DAT gene polymorphism and its correlation to WCST in ADHD children. **Second,** assessment of executive functions using WCST which represent one

domain of executive function (set shifting). **Third,** the polymorphism that we studied in this sample is located in the 3' UTR DAT gene polymophysism that doesn't change structure of DAT protein.

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DOPAMINE TRANSPORTER 3'UTR VNTR GENOTYPE AND WISCONSIN CARD SORTING TEST IN CHILDREN WITH ADHD AMONG EGYPTIAN POPULATION

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EFFECT OF ORAL ANTICOAGULANT (WARFARIN SODIUM -COUMADIN) ON ARTERIAL BLOOD PRESSURE (EXPERIMENTAL STUDY)

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Abstract

Background: *Warfarin sodium (coumadin) is an anticoagulant that acts by inhibiting vitamin-K-dependent coagulation factors but there is no evidence that warfarin has a role in arterial blood pressure. People who suffer from high blood pressure and who also take coumadin for stroke prevention must always keep in mind that high blood pressure increases the risk of strokes, or strokes caused by bleeding in the brain.*

Aim of the work: *is to clarify the effect of warfarin sodium (coumadin) on arterial blood pressure in animals.*

Material & Methods: *In our laboratories in Mansoura University preliminary studies done to detect possible effect on blood pressure of dog and it was surprising that this anticoagulant has antihypertensive effect on the blood pressure, this directs our attention to study the possible site of action of warfarin sodium. Serial experiments were done on blood pressure of dog using different agonists and antagonists, also on isolated perfused hind limb of rat, also experiments were done on isolated perfused rabbit's heart.*

Results: *The study demonstrated that warfarin sodium has direct vascular smooth muscle relaxant effect with no effect on the heart rate or cardiac contractility.*

Conclusion: *warfarin sodium in need of further clinical studies to formulate a related compound with both anticoagulant and antihypertensive effect which is valuble in many diseases in which hypercoagulation and hypertension occur concurrently.*

Introduction

The oral anticoagulants are derived from coumarin, which is found in many plants. A prominent member of this class is warfarin(coumadin). It takes at least 48 to 72 hours for the anticoagulant effect to develop. This anticoagulants are used to treat patients with deep-vein thrombosis (DVT), pulmonary embolism (PE), atrial fibrillation (AF), and mechanical prosthetic heart valves[1].

Warfarin sodium was already established experimentally to have anticoagulant effect[2]. Coumadin (warfarin sodium) is an anticoagulant that acts by inhibiting vitamin K-dependent coagulation factors. Chemically, it is 3-(α-acetonylbenzyl)-4 hydroxycoumarin and is a racemic mixture of the R- and S-enantiomers. Crystalline warfarin sodium is an isopropanol clathrate. Its empirical formula is C19H15NaO4[3].

Coumadin is a vitamin K antagonist indicated for prophylaxis and treatment of venous thrombosis and its extension, pulmonary embolism. Prophylaxis and treatment of thromboembolic complications associated with atrial fibrillation and/or cardiac valve replacement, reduction in the risk of death, recurrent myocardial infarction, and thromboembolic events such as stroke or systemic embolization after myocardial infarction. Coumadin has no direct effect on an established thrombus, nor does it reverse ischemic tissue damage. Once a thrombus has occurred, however, the goals of anticoagulant treatment are to prevent further extension of the formed clot and to prevent secondary thromboembolic complications that may result in serious and possibly fatal sequelae[4].

As coumadin decreases the body's mechanisms which normally stop bleeding, people who take coumadin must always keep their blood pressure in check. In fact, a study has shown that even small reductions in systolic blood pressure (as low as 12 points) can decrease the risk of bleeding in the brain by almost 80%[5].

In humans, warfarin crosses the placenta, and concentrations in fetal plasma approach the maternal values[6].

Coumarins have shown some evidence of many biological activities, although they are approved for few medical uses as pharmaceuticals. The activity reported for coumarin and coumarins includes anti-HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-inflammatory, anti-osteoporosis, antiseptic, and analgesic (pain relief). It is also used in the treatment of asthma[4]. Coumarin has been used in the treatment of lymphedema[4].

Coumarins have acquired increasing significance as coumarin moiety is biologically and pharmacologically important it occurs in several natural products and especially in some antibiotics[4,8]. 3-substituted-4- hydroxyl coumarins are useful anticoagulants of low toxicity[4,7]. Dicoumarol or 3, 3- ethylene-bis (4-hydroxycoumarin) is an anticoagulant and responsible for haemorrhagic sweet clover disease of cattle[9]. Several other bis-compounds are similar to dicoumarol in its action[10]. Ethyl bis (-4-hydroxy-3 coumarin) acetate is physiologically very active and has certain advantages over dicoumarol as it is an anticoagulant of short duration. They are used instead of dicoumarol for reducing the prothrombin index of the blood and for its inhibitory effect on cholinesterase[11]. Cyclocoumarol is one of the most active anticoagulant among 106 synthetic compounds tested for their activity. In rabbits, dogs and healthy men cyclocoumarol induced and intense hypoprothrombinemia in minimal doses[12]. Same coumarin derivatives show antihypertensive effect[4].

Many diseases have been found to be associated with hypertension and hypercoagulation. Hypercoagulable state was found in patients with diabetes mellitus, pregnancy, induced hypertension and glomerular disease especially in those with uremia and nephritic syndrome[13]. Also erythrocyte flexibility was impaired in diabetic patients and was not influenced by the duration of diabetes, its type and treatment[7]. Risk factors as hypertension, arteriosclerosis and smoking have an aggravating effect.

The coagulation function were studied in patients with myocar-

dial infarction in relation to the presence of essential hypertension, hypercoagulation was founded to be more pronounced in cases of associated essential hypertension[14].

Also there is simultaneous tendency to the decrease of blood pressure and the inhibition of the aggregation capacity of platelets [15].

Aim of the Work

The present work is an experimental trial to obtain an effective drug for treatment of both hypertension and hypecoagulability as these disease are commonly present concurrently in the same patient and to detect its site of action as antihypertensive if possible.

Material and Methods

i) Chemicals used:

1- Warfarin sodium (Vial containing 5 mg lyophilized powder). Crystalline warfarin sodium occurs as a white, odorless, crystalline powder that is discolored by light.It is very soluble in water, freely soluble in alcohol, and very slightly soluble in chloroform and ether (Initial U.S. Approval: 1954).

2- Atropine powder obtained from Macfarian Smith Lt Edinburg.

3- Adrenaline powder obtained from G.H.Boehringer Sohn Company (Germany).

4- Norepinephrine powder obtained from Sigma Company.

5- Phenylephrine powder obtained from Sigma Company.

6- Acetylcholine was obtained from Sigma Company.

ii) Animals used and methods:

20 Mongerl dogs of both sex, weighing 15-20 kg, were used throughout this study they are prepared for recording blood pressure and assessment of drug effects on it according to method of Ghosh[16].

30 albino rats of either sex weighing 200-250 gm were used, they were prepared for assessment of the effect of the drug of the perfused hind limb of rat according to the method of Burn[1].

10 rabbits of either sex weighing 2 kg were used and prepared for recording the effect of the drug on the isolated rabbit's the heart

according to the method of Langendorff[17].

iii) The following experiments were done:

I. Effect of warfarin sodium on blood pressure of dogs:

1. Repeated doses of warfarin sodium were administered IV (every dose was dissolved in 100 ul distilled water starting from 5 ug/kg to 320 ug/kg according to the dose response cure to choose the effective dose.

2. Warfarin sodium was injected IV in a dose of 50 ug/kg and the same dose was repeated after 5 minutes followed by acetyl choline (1 ug/kg) then atropine (0.2 mg/kg) followed by acetyl choline (1 ug/kg) then warfarin sodium (50 ug/kg).

3. Warfarin sodium was administered IV in a dose of 50 ug/kg followed by phenylephrine (1ug/kg) then warfarin sodium (50 ug/kg) followed by phenylephrine (1ug/kg). The same steps were done with adrenaline (1ug/k).

4. Induction of hypertension was carried out according to the method of EL-Tahir et al.,[3] using norepinephrine (NE) infusion in anaesthized dog over a period of 60 minutes in a dose of 4 ug/minute. After initial elevation of blood pressure (1 minute) NE infusion then stopped and warfarin sodium (50 ug/kg) IV, then readministration of NE is continued[3].

II. Effect of warfarin sodium on perfused hind limb of rats: 30 albino rats of either sex weighing 200-250 gm were used, they were prepared for assessment of the effect of the drug of the perfused hind limb of rat according to the method of Burn [1]. Drugs were injected through rubber part just proximal to the canula. Control group composed of six rats were given 1 cc distilled water. The perfusate was counted for five minutes. Warfarin sodium was administered in different doses ranging from 50-400 ug/kg, each dose was administered in a separate preparation and the perfusate was collected every five minutes. The maximum effect was obtained after 1 hour and the mean was determined.

III. Effect of warfarin sodium on isolated perfused rabbit's heart: Isolated rabbit's heart was prepared according to the method of Langendorff[17]. Warfarin sodium was administered in different doses ranging from 10 ug to 320 ug and every dose was dissolved in 100 ul acetone.

The statistics were calculated by T-test[18] comparing the mean obtained by different dose of warfarin sodium compared to the mean of control saline treated group.

Results

1. Effect of warfarin sodium on blood pressure of dog (figures 1 & 2): Warfarin sodium has hypotensive action on blood pressure of dog.

2. Effect of atropine on the hypotensive action of warfarin sodium (Figure 3): Warfarin sodium is administered IV , in a dose of 50 ug/kg in the dog and the same dose was repeated after 5 minutes, it produced a hypotensive effect. Acetyl choline in a dose of 1 ug/kg IV produced hypotension. Atropine IV, in a dose of 0.2 mg/kg produced hypotension due to cutaneous vasodilatation. A test dose of acetyl choline 1 ug/kg produced no hypotensive effect denoting full atropinization and blockade of muscarinic receptors.

The same dose of warfarin sodium produced the same control hypotensive effect after blockade of muscarinic receptors denoting that this compound is not a muscarinic receptor agonist.

3. Effect of warfarin sodium on the hypertensive effect of adrenaline (figure 4): Warfarin sodium given IV to the dog in a dose of 50 ug/kg produced hypotension. Phenylephrine in a dose of 1 ug/kg given IV, produced hypertension indicating intact alpha receptors in the dog. Warfarin sodium given IV to the dog in a dose of 50 ug/kg given after phenylephrine produced hypotension. Phenylephrine in a dose of 1 ug/kg given IV, after warfarin sodium produced the same hypertensive effect denoting that warfarin sodium has no alpha blocking effect. Adrenaline in a dose of 1 ug/kg after warfarin sodium injection produced hypertensive effect due to alpha and beta agonist effects.

Warfarin sodium given IV to the dog in a dose of 50 ug/kg after adrenaline produced hypotension. Adrenaline in a dose of 1 ug/kg after warfarin sodium injection produced the same hypertensive effect in step before denoting that warfarin sodium is neither alpha nor beta blocker.

4. Effect of warfarin sodium on sustained hypertension induced by nor-adrenaline infusion (figure 5): Warfarin sodium produced antihypertensive effect on sustained hypertension induced by noradrenaline.

5. Effect of warfarin sodium on perfused hind limb of rats (table 1): Warfarin sodium produced a significant increase in the rate of perfusion of hind limb of rats.

6. Effect of warfarin sodium on isolated perfused rabbit's heart(figure 6): Warfarin sodium in different doses used has no effect on isolated rabbit's heart.

Table (1): Effect of different doses of warfarin sodium on isolated perfused hind limb of rats (number of drops in 5 minutes, mean ± standard error)

Group & drug dose (N=6)	Mean ±SE	P value
Control	401 ±29.95	
50 ug/kg warfarin sodium	580±44.8	P < 0.01
100ug/kg warfarin sodium	637.5±59.8	P1 < 0.01
200ug/kg warfarin sodium	645±53.8	P2 < 0.001
400ug/kg warfarin sodium	697±92.9	P3 < 0.001

P = test of significance between control mean values and 50 ug/kg warfarin sodium treated group.
P1= test of significance between control mean values and 100 ug/kg warfarin sodium treated group.
P2= test of significance between control mean values and 200 ug/kg warfarin sodium treated group.
P3= test of significance between control mean values and 400 ug/kg warfarin sodium treated group.

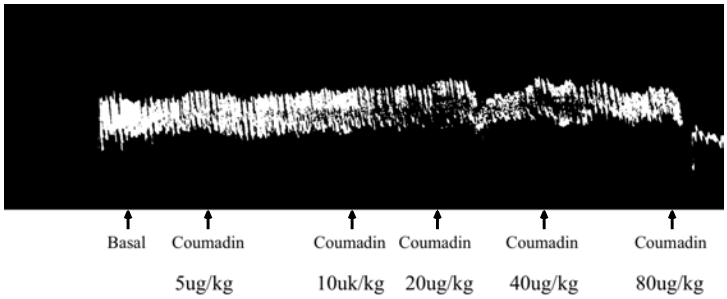

Fig. (1): Effect of different doses of warfarin sodium on blood pressure in dog.

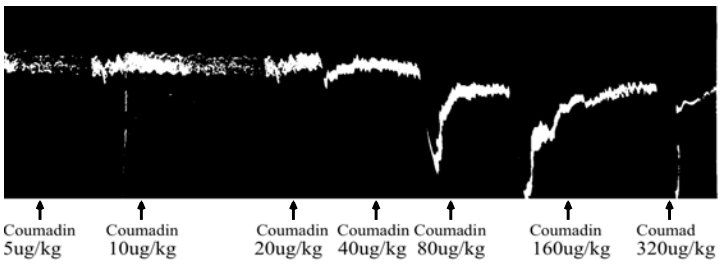

Fig. (2): Dose response curve of warfarin sodium showing effect on blood pressure in dog.

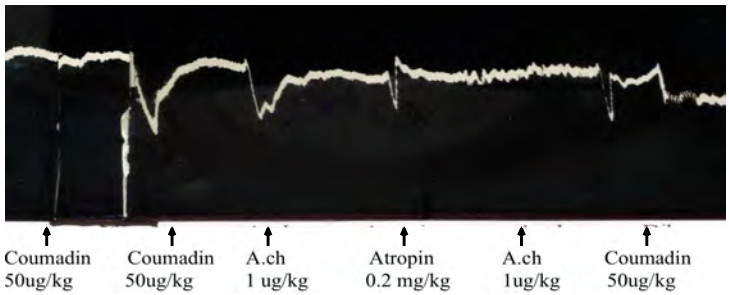

Fig. (3): Effect of of warfarin sodium on blood pressure in dog after atropine and acetyl choline.

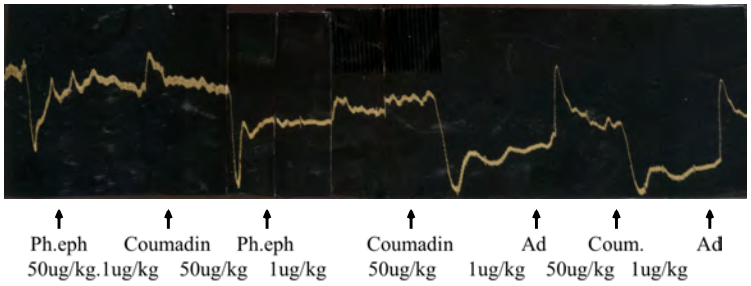

Fig. (4): Effect of phenylephrine & adrenaline on hypotensive effect of of warfarin sodium.

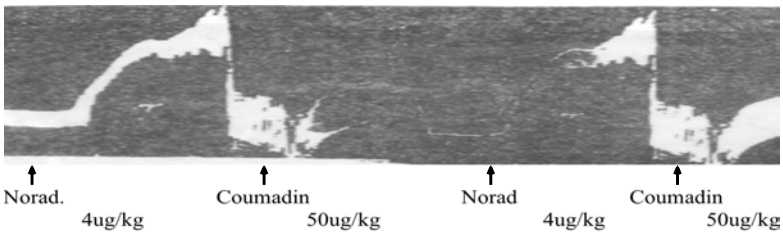

Fig. (5): Effect of of warfarin sodium on nor-adrenaline induced hypertension.

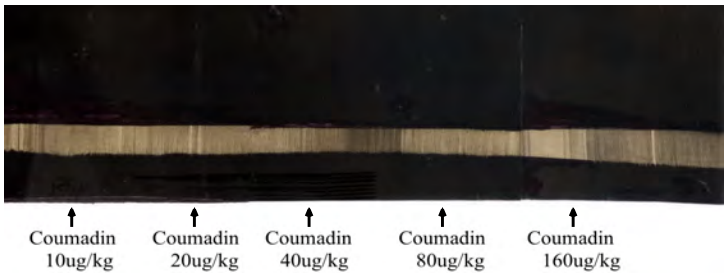

Fig. (6): Effect of different doses of warfarin sodium on isolated rabbit's heart.

Discussion

Coumadin, also known as warfarin, is a medication originally extracted from coumarin, a chemical found in some plants. It suppresses the body's ability to form blood clots, by blocking the function of vitamin K.[3].

Warfarin sodium was demonstrated to have anticoagulant effect already[4], and in our laboratories in Mansoura University, Faculty of Medicine an initial study on experimental animals was done (bood pressure of dog) and this initial experiments were promising and demonstrated that warfarin sodium has lowering effect on blood pressure of dog. This is in agreement with the results obtained by Ivanov et al.[6], they stated that some coumarine derivatives show antihypertensive effect. This preliminary studies forced us to do further experiments to demonstrate the site of action of warfarin sodium in a attempt to formulate it in corporation of drug companies to provide a new drug which is valuable in both hypertension and hypercoagulability as hypertension and hypercoagulation occurs concurrently in myocardial infarction, angina due to hypertension, glomrular disease especially in those with uremia and nephritic syndrome and in pregnant eclamptic patients also those females under contraceptive pills who are predisposed to hypertension and hypercoagulation[2,12]. Thus warfarin sodium will solve many problems need the use of a hypotensive compound which has anticoagulant effect. From these experiments, it was found that this compound has antihypertensive effect due to direct vascular smooth

muscle relaxant effect as demonstrated from its effect on blood pressure of dog (it has no muscarinic agonist effect and neither alpha adrenergic nor beta receptor blocking effect), and isolated perfused hind limb of rat (it produced significant increase in the perfusion of hind limb of rat). At the same time this compound ws found to have an effective antihypertensive effect on sustained hypertension induced by noradrenaline infusion which arise the beneficial use of it in essential hypertension and hypertensive emergencies. Also the absence of inhibitory effect on the heart is advantageous as it will provide us with antihypertensive compound without deleterious effect on the heart. Our finding not run with García-Beltrán at al.[19,20] who pooved that there is interaction between warfarin sodium and beta blockers (propranolol) and calcium channel blocker (verapamil) through CYP450 interactions putting in mind that this interaction prevent action of warfarin not antihypertensive effect. Lastly warfarin sodium is in need to further special clinical assessment trials.

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EFFECT OF ORAL ANTICOAGULANT (WARFARIN SODIUM -COUMADIN) ON ARTERIAL BLOOD PRESSURE (EXPERIMENTAL STUDY)

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BIOLOGICAL AND SEROLOGICAL STUDIES IN SCHOOL CHILDREN TO EVALUATE WIDAL TEST AS A SINGLE DIAGNOSTIC ONE IN TYPHOID FEVER

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Abstract

Background: *The value of the Widal test for the diagnosis of typhoid fever has been debated for as many years as it has been available.* ***The aim*** *of this study was to to spot light on the typhoid fever problems as endemic disease and to detect the base line of Widal test in school children in Banha region.* ***Materials and Methods:*** *This study was conducted on children in Benha region during the period from January 2013 to October 2013. The children were 250 Healthy child & 50 child with typhoid symptoms with and without positive blood culture & 50 child with fever (non typhoidal).* ***Results:*** *In our study 23.7% gave positive widal test, but 0% gave positive blood culture because of their antibiotics intake& we found that the control group gave (13.2%) positive widal test with titre (1/80) typhoidal group, (100%) gave positive widal test with titre ranges from (1/80: 1/320).*

Keywords: *Typhoid fever, Widal test, Agglutination test, Serologic tests.*

Introduction

Typhoid fever is widely recognized as a major public health problem in developing countries. It is a severe systemic infection caused by Salmonella typhi. The disease is endemic in the Indian sub-continent, South- East Asia, Africa, the Middle-East, South and Central America, where provision of pure water supplies and sewage control are inadequate (Gillespie

S. et al., (2003). It is less common under 3 years of age. Every system of our body bears the onslaught of typhoid fever. Typhoid fever is atypical in nature in preschool children (Dr. K Jagdish Kumar et al., (2010). Early symptoms include fever, general ill-feeling, and abdominal pain. A high (typically over 39.44 degrees) fever and severe diarrhea occur as the disease gets worse. Some people with typhoid fever develop a rash called "rose spots," which are small red spots on the abdomen and chest. Other symptoms that occur include Abdominal tenderness, Agitation, Bloody stools, Chills, Confusion, Difficulty paying attention (attention deficit), Delirium, Fluctuating mood, Hallucinations, Nosebleeds, Severe fatigue, Slow, sluggish, lethargic feeling, Weakness (Giannella Ra. Et al., (2010). The infection is transmitted by ingestion of food or water contaminated with faeces. Epidemiological data suggest that water borne transmission of S.Typhi usually involves small inocula, whereas food borne transmission is associated with large inocula (Ivanoff B, et al., 1960). The definitive diagnosis of typhoid fever depends on the isolation of S. Typhi from blood, bone marrow or a specific anatomical lesion. (Gasem MH, et al., 1995; Hoffman SL, et al., 1986; Soewandojo E, et al., 1998; Wain J, et al., 1998). The incubation period for Salmonella gastroenteritis in humans is usually 12 hours to 3 days. Enteric fever usually appears after 10 to 14 days. (Isaza R, et al., 2000). The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses. The definitive diagnosis of typhoid fever requires the isolation of Salmonella typhi or paratyphi from the patient. Bacteria can be isolated from blood in 73 to 97% of cases before antibiotic use (Pearson RD, et al., 2000). However, since patients often receive antibiotics prior to a medical diagnosis, bacteria can be isolated from the blood cultures in only 40 to 60% of the cases (Buke MG, et al., 1987; Kalayci C, et al., 1987; Willke ATH, et al., 1988). Table (1) Laboratory diagnosis of typhoid (Shirakawa T, et al., 2003).

Treatment of typhoid fever: The incidence of typhoid fever can be subsequently reduced by providing

clean water and proper hygienic conditions to the population. The traditional treatment for typhoid fever was obtained with Chloramphenicol, Ampicillin, Trimethoprim and Sulphamethoxazole, the so-called first line antibiotics. Prevention of typhoid feve: The major routes of transmission of typhoid fever are through drinking water or eating food contaminated with Salmonella typhi. Prevention is based on ensuring access to safe water and by promoting safe food handling practices. Health education is paramount to raise public awareness and induce behaviour change. (Acharya VI, et al., 1987).

Material and Methods

This study was conducted on children in Benha region during the period from January 2013 to October 2013. The children were:

- 250 Healthy child.
- 50 child with typhoid symptoms (high temperature, abdominal pain and headache) with and without positive blood culture.
- 50 child with fever (non typhoidal).

*** Inclusion criteria:**

1- Age from 5 years to 15 years.

2- School children.

3- The children were 179 males and 171 female.

*** Exclusion criteria:**

1- Patients less than 5 years or more than 15 years.

2- Children on antibiotics.

All patients were subjected to the following:

A- Medical history:

- Use of antibiotics.
- History about old infection.
- History of recurrent or prolonged hospitalization.

B- Clinical examination for looking signs of infection:

1-General look: pallor, profuse, headache, sweating.

2- Signs of respiratory distress (eg. Cough and feeling upset).

3-Abdominal examination: abdominal pain, diarrhea, hepatosplenomegaly and spots appear on the bottom of the chest and abdomen in the second weak.

4- High temperature.

C- Investigations:

1- Widal test.

2- Complete blood count (CBC).

3- Blood culture.

4- The erythrocyte sedimentation rate (ESR).

D- Methodology:

- Blood culture: used automatic monitoring blood culture system, Bactec 9050(in cases of blood samples).

Used BD BACTEC Mycosis- IC/F culture vials which are selective media for bacteria; and it is designed for use with BACTEC brand fluorescent series instruments.

*** Widal Test:**

- Used BioMed-Febrile Antigen Kit.

- INTENDED FOR USE:

Rapid Slide test for the qualitative and semi-quantitative determination of specific antibodies were presented in serum against Salmonellae, Rickettsiae and Brucellae pathogens.

- PRINCIPLE:

Febrile antigens were suitable for both the rapid slide and tube agglutination tests against human sera for the detection of these agglutinins.

- SPECIMEN COLLECTION:

- Clear fresh serum sample was required and not exposed to elevated temperature.

- The serum specimen was stored refrigerated. If testing is to be prolonged in excess of 24hours, serum was frozen.

- PROCEDURE:

1. All reagents were brought and serum samples to room temperature.
2. Pipette was used, added (20 µl) of the patient serum onto 4 cells of the glass slide.
3. Antigen vials were shaked gently, expelled contents of dropper and refilled, then placed one drop (50 µl) of each antigens suspension to respective cells of the glass slide.
4. Both were mixed together with the flat end of the dispensing pipettes.
5. The slide was rocked gently for two minute. A rotary shaker may also be used for rocking.
6. Results were observed at the end of one minute under high intensity light.

- FEBRILE ANTIGEN Kit is also suitable for titration purposes.

1:2, 1:4, 1:8, were prepared as needed dilutions of the specimen using physiological saline.

Qualitative procedures were carried-out on each dilution.

Final end point was determined by the highest dilution, which was positive.

Multiply the sensitivity of the test by the highest dilution with positive agglutination to calculate the titer of the sample.

- FEBRILE antigens are specifically designed for use in detecting febrile antibodies with increased sensitivity, specificity and overall readability. This new FEBRILE antigen series employs a unique system of dyes making the entire febrile profile user friendly.

- Statistical analysis:

The collected data were tabulated and analyzed using SPSS version 16 software (Spss Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Chi square test (X2), student 't' and ANOVA were used as tests of significance, when ANOVA is positive, it was followed by Benferroni test for multiple comparisons. The accepted level of significance in this work was stated at 0.05 ($P<0.05$ was considered significant).

Diagnostic test	Comments
Microbiological tests	
Blood culture	Widely regarded as the gold standard, but sensitivity may be low in endemic areas with high rates of antibiotic use—hence true specificity is difficult to estimate
Urine culture	Variable sensitivity
Stool culture	Sensitivity lower in developing countries and not used routinely for follow-up
Serological diagnosis	
Widal test (tube dilution and slide agglutination)	Classic and inexpensive. Despite mixed results in endemic areas, still performs well for screening large volumes. May need standardisation and quality assurance of reagents

Results

Table (1): Socio-demographic description of the studied sample.

Variable		No. (N=350)	% (100%)
The studied groups	Controls	250	71.4
	Typhoid cases	50	14.3
	Non typhoid cases	50	14.3
Gender	Male	179	51.1
	Female	171	48.9
Residence	Rural	284	81.1
	Urban	66	18.9
Age (years)	Mean ± SD	Minimum	Maximum
	10.1±2.6	5	15

Table (2): Blood culture results among the typhoid group.

Blood culture	Frequency	Percent
Negative	50	100.0
Positive	0	0.0

Table (3): comparing the studied groups regarding gender.

Gender		Group			Total
		controls	Typhoid cases	Non typhoid cases	
Male	Count	121	25	33	179
	% within group	48.4%	50.0%	66.0%	51.1%
Femae	Count	129	25	17	171
	% within group	51.6%	50.0%	34.0%	48.9%
Total	Count	250	50	50	350
	% within group	100.0%	100.0%	100.0%	100.0%

* X^2=5.2, P=0.07

Table (4): comparing the studied groups regarding residence.

Residence		Group			Total
		controls	Typhoid cases	Non typhoid cases	
Rural	Count	210	41	33	284
	% within group	84.0%	82.0%	66.0%	81.1%
urban	Count	40	9	17	66
	% within group	16.0%	18.0%	34.0%	18.9%
Total	Count	250	50	50	350
	% within group	100.0%	100.0%	100.0%	100.0%

* X^2=8.85, P=0.012

Table (5): comparing the studied groups regarding Widal results.

Residence			group			Total
			controls	Typhoid cases	Non typhoid cases	
Widal	positive	Count	33	50	0	83
		% within group	13.2%	100.0%	.0%	23.7%
	negative	Count	217	0	50	267
		% within group	86.8%	.0%	100.0%	76.3%
Total		Count	250	50	50	350
		% within group	100.0%	100.0%	100.0%	100.0%

*X^2=191.7, P<0.001

Table (6): comparing the studied groups regarding age.

Group	Age (ys)		ANOVA	P
	Mean	± SD		
controls	9.93	2.71318	3.6	0.026*
Typhoid cases	†11.0	2.45781		
Non typhoid cases	9.98	1.84601		

†→ significant with the controls.

Table (7): comparing the studied groups regarding WBCs count.

Group	WBCs ($x10^3$)		ANOVA	P
	Mean	± SD		
Controls	5.18	.72145	77.5	<0.001*
Typhoid cases	†‡4.00	.13242		
Non typhoid cases	5.34	.50030		

*†→ significant with the controls.
*‡→ significant with the non typhoidal.

Table (8): comparing the studied groups regarding platelets count.

Group	PLTs ($x10^3$)		ANOVA	P
	Mean	± SD		
controls	249.56	65.19885	89.3	<0.001*
Typhoid cases	††‡134.24	5.16882		
Non typhoid cases	262.64	46.03848		

*†→ significant with the controls.
*‡→ significant with the non typhoidal.

Table (9): comparing the studied groups regarding ESR.

Group	ESR		ANOVA	P
	Mean	± SD		
controls	13.7	6.01435	669.5	<0.001*
Typhoid cases	†‡45.5	6.07781		
Non typhoid cases	†38.3	9.21723		

*†→ significant with the controls.
*‡→ significant with the non typhoidal.

Discussion

The value of the Widal test for the diagnosis of typhoid fever has been debated for as many years as it has been available. The definitive diagnosis of typhoid fever requires the isolation of Salmonella enterica subspecies enterica serovar Typhi from the patient by cultures of blood. Our study was conducted on 350 children from 5 to 15 years in Benha region Qalubia during the period from January 2013 to October 2013. In our study 23.7% gave positive widal test, but 0% gave positive blood culture because of their antibiotics intake. We found that typhoid fever is more common in male (51.1%) than female (48.9%) which shows high resistance females in addition to the behavior of male students in exposure to injury-causing factors compared to females. In our study, we found that the control group gave (13.2%) positive widal test with titre (1/80) typhoidal group, (100%) gave positive widal test with titre ranges from (1/80: 1/320). In the present study, number of platelets and white blood cells decrease in typhoidal cases, while ESR values increase in typhoidal cases. The Widal test can be used as a complimentary serological diagnostic tool as and when it is required. However, the importance of the blood culture in the typhoid cases cannot be ignored, as the antibiotic susceptibility testing of the isolated strains is equally important).

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BIOLOGICAL AND SEROLOGICAL STUDIES IN SCHOOL CHILDREN TO EVALUATE WIDAL TEST AS A SINGLE DIAGNOSTIC ONE IN TYPHOID FEVER

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ACUTE APPENDICITIS IN THE OVER-FIFTY AGE GROUP

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Abstract

Abstract: *Acute appendicitis in elderly patients (ie. Patients above the age of 50 years) is rare but often associated with complications, increased morbidity and mortality.*

Objective: *The aim of our study is to review and analyze the diagnostic challenges, treatment and outcome of cases of acute appendicitis in elderly patients at Al Thowra Teaching hospital, El Beida, Libya between 1st January 2005 to 31st December 2009, over a period of 5 years.*

Method: *24 patients of acute appendicitis in elderly patients were reviewed in details about their presentation, treatment offered and outcome. Out of them 66.6% were males and 33.3% females. Majority of patients were in the age group of 50 to 70 years.*

Results: *Clinical presentation varied. Specific investigations including CBP, US Scan of abdomen, Plain X-Ray of the abdomen, were done for all cases. CT Scan was done in 2 cases of confused diagnosis. Early operation was performed in 29.16% cases and in 70.83% cases the surgery was delayed for more than 24 hours.*

The complications encountered in our study were Chest, Urinary tract & wound infection, prolonged ileus, MI. fecal fistula, wound dehiscence, septicemia and death.

Conclusion: *Acute appendicitis is less common among elderly people. Often elderly patients have associated co-morbid conditions. Complications are comparatively more. Early diagnosis and surgical intervention reduces the morbidity and mortality considerably.*

Keywords: *Appendicitis in elderly, complications, management.*

Introduction

Acute appendicitis occurs in 7%[1] of cases out of which 90% cases are seen among children and young adults, where as 10% cases seen in elderly patients[2,26]. The presentation in elderly may be atypical or confused, hence surgical treatment is often delayed[3,4]. Hence, complications of appendicitis specially perforation is more commonly observed; which may be due to delayed intervention, comorbid conditions, immunosuppression and poor defense mechanism[3]. Early diagnosis and prompt surgical management can reduce complications, morbidity and mortality rate[5,6,7,8].

The aim of this study was to review the diagnostic, therapeutic management and outcome of acute appendicitis in elderly patients.

Material and Methods

We have reviewed medical records of patients over 50 years age, who underwent appendectomy at El Thowra Teaching Hospital during 5 years period from 1st January 2004 to 31st December 2009. Total 24 cases were collected and analyzed retrospectively including demographic data, symptoms & signs, onset and duration of presentation, investigations, timing of surgery, surgical approach, hospital stay, morbidity and mortality.

Results

Out of 1160 appendectomies performed during the period 1st January 2005 to 31st December 2009; 24 cases were above the age of 50 years, which constitutes 2.07%. The mean age was 58.87 years of which 50% cases in 6th decade, 41.6%. In 7th decade, 8.3% in 8th decade. Male Female ratio was 2:1. (Table 1).

Clinical presentation was typical abdominal pain with shift to Rt.I.F. in 14(58.33%), atypical presentation in 41.66%, anorexia in 54.1%, nausea & vomiting in 50%, constipation in 25%, fever with chills & rigor in 33.33% of cases. Tenderness was elicited in 87.5%, rebound tenderness & guarding in 41.66, features of generalized peritonitis in 16.6%, mass

Rt.IF in 20.8% of cases.

The interval between the onset of symptoms and hospitalization varied from few hours to one week; where as 45.8% cases were admitted within 24 hours.

On investigation, leukocytosis (>11,000 cumm) was found in 50% cases. Chest X-Ray was done in all cases. Abdominal X-Ray was done in 41.66% cases. US Scan of abdomen was done in 66.6% cases but was informative only in 50% of them. CT Scan of abdomen was done in 8.3% cases, and the diagnosis was 100% accurate. (Table 2).

54.1% cases had associated co-morbid conditions like Diabetes mellitus in 29.1%, Hypertension in 16.6% and Cardiac disease in 12.5% of cases. Only 7(29.16%) cases were operated early. In rest, the surgery was delayed due to difficulty in diagnosis.

The morbidity rate was 37.5%. The complications encountered were appendicular mass and/or abscess in 33.33%, perforation in 29.16%, localized peritonitis in nearly 33.33%, generalized peritonitis in nearly 30% of cases. Normal appendix with mass in terminal ileum (Crohn's disease) was found in 1 case (4.16%); which was also operated.

Post operative complications encountered were wound infection in 4(16.6%), chest infection in 4 (1606%), urinary tract infection in 2(8.3%), prolonged paralytic ileus in 5(20.8%), MI in 1(4.16%), fecal fistula in 1(4.16%), wound dehiscence in 1(4.16%).

Hospital stay varied from 5-15 days. 37.5% of cases over stayed, more than one week. One patient (4.1%) died following septicemia, which got worsened due to associated co-morbid conditions.

Table (1): age and sex distribution.

Age – Interval(years)	Male	Female	Total	%
50-60	7	5	12	50%
61-70	7	3	10	41.66%
71-80	2	0	2	8.33%
>80	-	-	-	0%
%	16 (66.66%)	8 (33.33%)	24	100.00%

Table (2): Clinical and investigations finding.

	Finding	No of cases	%
Symptoms	Pain • typical • atypical	24 14 10	100% 57.33% 41.66%
	Anorexia	13	54.1%
	Nausea & Vomiting	12	50%
	Change in bowel habits [Constipation & diarrhea]	6	25%
	Urological symptoms [Dysuria and Hematuria,…]	2	8.3%
	Fever / chills / sweating	4	16.6%
Physical finding	Elevated temperatures	8	33.33%
	Abdominal tenderness: • Total • Guarding & Rebound tenderness • Rigidity	21 cases 10 (41.66%) 4 (16.6%)	87.5%
	Palpable mass	5	20.8%
Investigations	Elevated WBCs>11,000	12	50%
	CXR	24	100%
	AXR	10	41.66%
	USS-abdomen • informative • uninformative • NOT DONE	 8 8 8	 33.33% 33.33% 33.33%
	CT scan abdomen	2	8.3%

Table (3): Operative finding and Post operative complications:

	Finding	No of cases	%
Operative finding	Simple acute appendicitis	2	8.3%
	Phlegmnous (suppurative)	3	12.5%
	Gangrenous	3	12.5%
	Appendicular mass /abscess	8	33.33%
	Free perforation	7	29.16%
	Normal appendix with mass terminal ileum	1	4.16%
	Peritonitis: • General peritonitis • Localized peritonitis	 7 8	 29.16% 33.33%
	With Other pathology: • Ovarian cyst • Mass {fibroid uterus}	 1 1	 4.16% 4.16%
Post operative complications: (9 cases)	Infective complications: • Wound Infection • Chest infection • Urinary tract infection	 4 (16.6%) 4 (16.6%) 2 (8.3%)	41.6%
	Long Paralytic ileus	5	20.8%
	CVS complication (MI)	1	4.16%
	Fecal fistula	1	4.16%
	Wound dehiscence	1	4.16%
	Death	1	4.16%

Discussion

Acute appendicitis is the most common acute abdominal condition encountered[7]. The incidence of acute appendicitis is less common in elderly age group. Various factors like atypical & varied presentation, low immunity, poor defense mechanism, associated co-morbid conditions like diabetes, hypertension, malnutrition, dementia, more prone for malignant diseases; are responsible for delayed diagnosis, increased rate of complications, morbidity and mortality.

Abdominal pain is the most

common symptom of appendicitis [9]. Classical history of shifting of pain from umbilical region to Rt.IF was seen in 50% of cases[7] and 20% of elderly patients presented with anorexia, fever, right lower quadrant pain and leukocytosis[8]. Abdominal tenderness is less localized in case of elderly patients [3]. Elderly patients with acute appendicitis with peritonitis may not have classical findings of rebound tenderness and rigidity[5]. In elderly patients, the picture may be confusing and may not have typical signs, symptoms and leukocytosis in nearly 50% of cases[8]. Leukocytosis is also seen in 70% of other abdominal conditions[10], but serial repetition of leucocyte count may help and increase specificity. But there may be a initial fall of in leucocyte count in case of perforation[11]. Chest X-Ray and abdominal X-Ray may help to exclude other emergency conditions. US Scan and CT Scan specially Helical CT is more helpful to diagnose the confused and difficult cases. CT Scan can exclude other causes and more specific than US Scan of abdomen [4,12,13,14,15]. But routine use of CT Scan in all cases in developing countries is not cost effective, may further delay the management and thus increase the operative risk[16,17,18].

Laparoscopic appendectomy is associated with less pain, wound infection and rapid recovery [19,20], variable results seen with some surgeons[21,22]. Patients whose appendectomy was completed laparoscopically were younger and less likely to have perforation and abscess. The post operative hospitalization of these patients was shorter, reflecting the selection of less complicated cases for this subgroup[23]. Late presentation of patients to hospital, may be due to family negligence, psychological factors, taking native treatment initially, considering other common diseases like malignancy, pancreatitis, biliary diseases, inflammatory bowel diseases, bowel obstruction etc; may delay in surgical management[24].

Local & generalized peritonitis were seen in 2/3rd of our patients;

mainly following perforation and neglected appendicitis. The incidence of perforation in elderly patients is progressively higher with age and mainly due to delayed presentation, extreme age, abnormal location of appendix etc [1,24,25,26].

The morbidity rate in acute abdominal conditions increases with age, as was seen in 15% over the age of 50 years and more than 70% over the age of 80 years[27]. Similarly is also 5% higher in elderly patients, usually due to delayed diagnosis[1].

Conclusion

Acute appendicitis is less common in elderly patients but often associated with high morbidity and mortality. All efforts should be made for early and correct diagnosis, so that early surgical intervention shall improve the out come.

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ACUTE APPENDICITIS IN THE OVER-FIFTY AGE GROUP

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PHYSICAL INTIMATE PARTNER VIOLENCE: PREVALENCE, PREDICTORS, AND HEALTH IMPACT

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Abstract

Background: *Intimate partner violence (IPV) is an important public health issue with severe adverse consequences. In Egypt, it is difficult to make precise comparisons between the prevalence rates of IPV due to different data collections methods; sampling methods; and different approaches to asking the same questions.*

Aim: *to determine the prevalence of physical IPV against women in Mansoura centre and find out the predictors of exposure to violence and the impact violence on women health.*

Methods: *An observational community-based study was conducted using a structured questionnaire developed by the WHO. Simple random sample was selected form primary health centers and included 758 ever married women aged 15-49 years.*

Results: *Self-reported past-year and lifetime prevalence of physical violence was 28.8% and 34.3%, respectively. Female risk factors for physical IPV included low socioeconomic standard, low income, justifying wife beating, and exposure to IPV in childhood while husbands risk factors were exposure to IPV in childhood, low education, physical fighting with other people, alcohol and drug abuse. Physical IPV has poor physical ($p \leq 0.001$) and mental ($p \leq 0.001$) health oucome.*

Conclusion: *physical IPV is a common phenomenon in Mansoura district and it has poor consequences on women health and this requires immediate attention of policymakers.*

Keywords: *intimate partner violence, domestic violence, Mansoura, gender inequality.*

Introduction

A report of WHO highlights violence against women as a 'global health problem of epidemic proportions'. The study finds that intimate partner violence is the most common type of violence against women, affecting 30% of women worldwide[1]. The reality and threat of violence exists in women's everyday lives, affecting their ability to participate in their societies, affecting their potential to engage fully in the affairs of their communities. It limits women's choices directly by destroying women's health, disrupting their lives and constricting the scope of their activity, and indirectly by eroding their self confidence and self-esteem. The recognition of violence as a major risk factor for women's ill-mental health must be fully integrated into mental health policy. Resources must be allocated for preventing violence against women and mitigating its consequences in order for the mental health needs of women to be effectively addressed[2,3]. In Egypt, it is difficult to make precise comparisons between the prevalence rates reported in the studies conducted for a number of reasons. Depending on how survey questions are formulated, women may give different responses as to whether they have experienced physical assault perpetrated by their partners. Interpretations of physical or other forms of abuse may also differ across sociocultural contexts. There is considerable variation in how abuse is defined across these studies[4,5].

Subjects and Methods

Study locality: Mansoura center is one of 18 centers in Dakahlia governorate. The estimated population number in Mansoura in 2011 was 973152 according to information center in Dakahlia health administration. Mansoura center comprises Mansoura city and its suburbs which are 39 villages. Four urban primary health care centers were randomly selected, two were selected from East Mansoura district and two were selected from West Mansoura district. Eight rural family health centers were randomly selected. The selected centers of mansoura city serve attendants of different socioeconomic standards and provide different preventive and curative services.

Study design: A observational descriptive and analytic study was conducted using a standard questionnaire designed by WHO for studying violence against women (questionnaire version 10, 2003). Operational definitions of violence used in the WHO Multi-country Study on Women's Health and Domestic Violence against Women were used. All women in the child bearing period from 15 to 49 years attending the selected health centers for any reason were eligible except women who are too ill to participate. The females in the age group (15-49) represent about 26% of the population in Dakahlia governorate. So, the size of the population is (253019 females). Prevalence of physical IPV during the past 12 months was 20.4%. Sample size was calculated using EpiInfo verion6 and the minimum required sample is 691. Our sample included 785 women. Women were interviewed using the questionnaire after giving verbal consent to participate in the study. The interview was conducted with each woman separately to ensure privacy. The women decisions and choices was be respected. For socioeconomic standard assessment, social score modified after Fahmy & El-Sherbini, 1983 was used. Mental health was assessed by use of a self-reporting questionnaire of 20 questions (SRQ-20), developed by WHO to screen for emotional distress and validated in many settings (WHO, 1994). Chi square test and Fisher's Exact tests were used were used for comparing qualitative data. Statistically significant variables in the bivariate analyses were entered into the multivariate model, one at a time. Final models are displayed.

Results

Table (1) showed that the study included 758 ever married females, 47.9% of them were in urban residence and 21.5% were (illiterate, read and write) while 6.6% received basic education, 40.8% received secondary education, and 31.1% received higher education. The working females represented 41.6% of the sample and 29.7%, 54.7%, 15.6% of females had insufficient, sufficient income, and can save money respectively. The study found that (260) 34.3% and (218) 28.8% of women included in the

study, reported that they had been exposed to some form of physical IPV since marriage and during the past year respectively. The women who ever exposed to sever violence were (141) 18.6%. The prevalence of physical violence during pregnancy was 22.3%. The prevalence of specific form of this violence which is punching or kicking in abdomen while pregnant is 13.5%. The most prevalent forms of physical IPV was slapping (85%) followed by being pushing or shoving (66%). The severe acts of violence, such as being kicked, dragged (26%), choked, burned (11.2%), or threatened by a weapon (7.7%) were less common (data not shown in table).

We compared women exposed to current violence (216 women) with those not exposed to any type of IPV (242 women) to find predictors of violence. Table (2) shows Logistic regression analysis of women related predictors of physical IPV. On logistic regression, it was found that only socioeconomic standard, attitude toward wife beating, residence, income, and exposure to IPV in childhood are the most significant predictors of physical IPV. It was found that females with very low socioeconomic are 4.7 times more likely to be abused (P=0.001), Females with attitude justifying wife beating are 2.7 times more likely to be abused (P=0.001), also rural residence made females 2 times more liable to be abused (P=0.001). On the other hand, females who were not exposed to IPV in childhood and those with sufficient income are 0.15, 0.3 times less likely to be abused (P≤0.001). Table (3) shows Logistic regression analysis of husband related predictors of physical IPV. On logistic regression, it was found that only exposure to IPV in childhood, education, history of physical fighting, and alcohol and drug abuse are the most significant predictors of physical IPV. It was found that females whose husbands were not exposed to IPV in childhood, with higher education, and those with no history of physical fighting with other people are 0.1 times less likely to be abused (P≤0.001), and females whose husbands were not alcohol or drug abuser are 0.2 times less likely to be abused (P=0.001).

Table (4) illustrated self-reported physical health status of women exposed to current physical IPV and non abused women. Poor health was reported by 29.8% of abused women while this was reported by 14.5% of non abused women (p≤0.001). Acute pain was reported by 23.9% of abused women while 11.6% of non abused women reported acute pain (p≤0.001). Among abused group, 29.4% of women reported many problems that may prevent them from performing usual activities while 11.6% of non abused women reported so (p≤0.001). Dizziness was reported by 54.6% of abused women in the last 4 months compared to 45.5% of non abused women (p=.05). Vaginal discharge was reported by 48.2% of abused women in the last 4 weeks compared to 30.6% of non abused women (p≤0.001). About two thirds of abused women said they had medical consultation in past 4 weeks compared to 56.2% of non abused women (p=.031). Among abused group, 16.1% of women were hospitalized for at least one night compared to 6.6% of non abused group (p=.001). There was more emotional distress in abused group compared to the other group and this was estimated using self reported questionnaire (SRQ20). In the abused group 45.4% of women reported score (0-10), 54.6% reported score (11-20), while in the non abused group 77.3% of women reported score (0-10), and 22.8% of women reported score (11-20) (p≤0.001). Among abused group, 24.3% of women reported many problems with memory while 15.7% of non abused women reported many problems (p≤0.001). Among abused group, 10.6% of women had thought of committing suicide in the previous 4 weeks while only .4% of non abused women reported so (p≤0.001) and 12.8% of women in abused group ever attempted suicide compared to only .4% of non abused women (p≤ 0.001).

Table (1): Sociodemographic characteristics of studied subjects (N=758).

Sociodemographic characteristic		Number	(%)
Site	Urban	363	47.9
	Rural	395	52.1
Age	15 -	134	17.7
	25 -	342	45.1
	35 -	187	24.7
	45 - 49	95	12.5
Education	None (illiterate, read and write)	163	21.5
	Basic	50	6.6
	Secondary	309	40.8
	Higher	236	31.1
Employment	Not working for cash	443	58.4
	Working for cash	315	41.6
Income	Insufficient	225	29.7
	Sufficient	415	54.7
	Save money	118	15.6

Table (2): Multiple logistic regression analysis of women related predictors of physical IPV.

Predictor		β	p	AOR (95% CI)
Residence	Urban	-		1(r)
	Rural	0.73	0.009	2 (1.2-3.6)
Socioeconomic standard	High	-		1(r)
	Middle	1.2	≤ 0.001	3.3 (1.7-6.3)
	low	0.9	0.21	2.5 (1.2-5.6)
	Very low	1.6	=0.001	4.7 (1.8-12)
Income	Insufficient	-		1(r)
	Sufficient	-1.3	≤ 0.001	0.3 (0.1-0.5)
	Save	-1.4	0.003	0.3 (0.1-0.6)
Attitude towards wife beating	Not accepted at all	-		1(r)
	Accepted some times	0.9	0.001	2.7 (1.5-4.8)
Mother exposure to IPV	Yes	-		1(r)
	No	-1.9	≤ 0.001	0.15 (0.09-0.3)
	Not live together	2	0.065	7.8 (0.9-68.4)
	Not know	-1.2	0.032	0.3 (0.09-0.9)
Constant		0.1		
Model x^2		250, p ≤ 0.001		
% correctly predicted		81.3		

(r= reference group, AOR= Adjusted odds ratio, CI= confidence interval)

Table (3): Multiple logistic regression analysis of husband related predictors of physical IPV.

Predictor		β	p	AOR (95% CI)
Mother exposure to IPV	Yes	-		**1 (r)**
	No	**-2.5**	**≤ 0.001**	**0.1 (0.04-0.2)**
	Not live together	**-1.2**	**0.009**	**0.3 (0.1-0.7)**
	Not know	**-0.8**	**0.061**	**0.5 (0.2-1)**
Education	Illiterate, read and write	-		**1(r)**
	Basic	**- 0.07**	**0.896**	**0.9 (0.3-2.8)**
	Secondary	**-0.2**	**0.637**	**0.8 (0.4-1.7)**
	higher	**-2.1**	**≤ 0.001**	**0.1 (0.05-0.3)**
Physical fighting	Yes	-		**1(r)**
	No and not know	**-2**	**≤ 0.001**	**0.1 (0.08-0.3)**
Alcohol and drug abuse	Yes	-		**1(r)**
	No or not know	**-1.8**	**=0.001**	**0.2 (0.1-0.5)**
Constant	**4.668**			
Model x^2	**320.5, p≤ 0.001**			
% correctly predicted	**85.7**			

(r=reference group, AOR=Adjusted odds ratio, CI=confidence interval)

Table (4): I. Self-reported physical health status of women currently exposed to physical IPV and non abused women. (WHO Multi-Country Study questionnaire, Version 10, 2003).

Physical health status		Physical IPV Present N = 218		Violence Absent N = 242		Significance test	
		No.	%	No.	%	χ^2	P value
Assessment of own health	Good	37	17	92	38	31.287	< 0.01
	Fair	116	53.2	115	47.5		
	Poor	65	29.8	35	14.5		
Problems within the past four weeks							
- Pain or discomfort	No	29	13.3	80	33.1	30.620	< 0.01
	Mild, moderate	137	62.8	135	55.8		
	Acute, sever	52	23.9	27	11.2		
-Performing usual activities	No, few problems	49	22.5	119	49.2	42.617	< 0.01
	Some problems	105	48.2	95	39.3		
	Many problems, not able	64	29.4	28	11.6		
- Dizziness	**Yes**	119	54.6	110	45.5	3.826	0.50
- Vaginal discharge	**Yes**	105	48.2	74	30.6	14.922	<0.01
Medical consultation in past 4 weeks		144	66.1	136	56.2	4.678	0.31
Hospitalization in past 12 months		35	16.1	16	6.6	10.375	0.01
Mental health status							
Emotional distress in past four weeks (SRQ20)	0-5	26	11.9	77	31.8	60.014	< 0.01
	6-10	73	33.5	110	45.5		
	11-15	90	41.3	50	20.7		
	16-20	29	13.3	5	2.1		
Memory or concentration	No, few problems	66	30.3	131	54.1	26.670	< 0.01
	Some problems	99	45.4	73	30.2		
	Many problems, not able	53	24.3	38	15.7		
Ever thought of committing suicide last 4 weeks		23	10.6	1	.4	23.833	< 0.01
Ever attempted to commit suicide		28	12.8	1	.4	30.002	< 0.01

Discussion

EDHS-95 indicated that 34% of women in this sample have ever been beaten by their current husband[6] while EDHS (2005) found also that life time physical IPV prevalence was 33.2%. both figure are very proximate to our prevalence. Our prevalence is higher than that found by other studies[7,8,9] which are only (11.1%), (29.9%) and (20.5%) respectively for a lifetime prevalence of physical abuse. Also our prevalence is higher than that found by another study[10] which found that physical IPV was (40%) but the cause may be using different questionnaire and the sample that was taken from Health Insurance Clinics in Alexandria that may over estimate the problem. EDHS (2005) found that the prevalence of physical violence during pregnancy was 6.6% and this figure is much lower than that obtained by our result. Our figures are higher than results of population-based studies from Canada, Chile and Nicaragua that have found that 6-15% of ever-partnered women have been physically abused during pregnancy, usually by their partners[11,12]. Regarding the forms of violence, our results are consistent with WHO multicountry study which showed that the most commonly experienced acts of physical aggression in most countries include being slapped, having arms twisted, or hair pulled. The more severe acts of violence were less common[13].

The findings of this research as well as many other studies support the view that poverty and its associated stress are key contributors to intimate partner violence. High levels of female empowerment seem to be protective against intimate partner violence, but power can be derived from many sources such as education, income, and community roles and not all of these convey equal protection or do so in a direct manner[14]. Rural women represented the larger part of the women abused by any type of violence. This could be explained through community-level gender inequality (operationalized as women's autonomy, women's status, male patriarchal control, and intimate partner violence). Community-level gender inequality is associated with women's own experience of IPV[15]. Women's attitude to male dominance affects

the level of their acceptance or tolerance of violence from their husbands. Nearly all studies that have included a variable on witnessing interparental violence have found this experience to be a risk factor for women experiencing violence and men perpetrating physical IPV[16,17,18,20].

Husbands of abused women were less educated. These finding is in line with other studies whichfound that men with a higher education were less likely to abuse their wives physically[21,22]. Alcohol consumption has been positively associated with domestic violence in several communities[19,23,24,25]. Findings from the WHO multicountry study on women's health and domestic violence showed also women whose partners were involved in a fight with another man in the past year experienced higher levels of IPV than those with partners who did not fight [25]. A history of antisocial personality and alcohol abuse among men, may account for all or part of this association[26].

Vives-Cases et al. found that physical IPV is associated with a greater likelihood of poor self-perceived health[27]. These results are in agreement with the results of WHO multicountry study. For most specific health problems, in most places, significant associations existed between reported violence and self-reported specific health problems as women with lifetime experiences of physical IPV were significantly more likely to report poor health, and that within the past 4 weeks they had experienced difficulties with daily activities, pain, memory loss, dizziness, and vaginal discharge[2].

The current study showed significant association between IPV and poor mental health. The study showed that women who experienced current IPV were more likely to report emotional distress in the past 4 weeks than non abused women and this was measured by SRQ20. Also IPV was significantly associated with memory problems, suicidal thoughts in the past 4 weeks, and suicidal attempts. Physical IPV was associated with a greater likelihood of psychological distress[27]. Another study found that 10.4% of the physically abused women had attempted suicide at least

once[19]. Intimate partner violence was among the most consistent risk factors for suicide attempts among women who participated in WHO multicountry study[3].

Conclusion and Recommendations

Physical IPV is a common hidden problem. Those having the least resources are most affected. This situation requires rapid attention at all levels of societal organization, by policymakers, political stakeholders, and professionals. Policy initiatives are needed, as are legal actions, to criminalize men's violence against women. Basic education needs to be made available for both girls and boys, with special attention placed on female education. Gender equality teaching and training should be included at different levels in the school curriculum. Mass media involvement is necessary to create a debate on such gender discrimination practices and to encourage women's empowerment in society.

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INDUCTION OF CANCER STEM CELL IN HEPATOCELLULAR CARINOMA: THIOACETAMIDE MODEL

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Abstract

Introduction: *Hepatocellular carcinoma (HCC) is one of the most common tumours worldwide, and about 600,000 patients suffer from HCC yearly, beside it is the third leading cause of cancer-related death worldwide and now it represents the leading cause of death among most of the cirrhotic patients. The term cancer stem cell (CSC) is used to define cancer cells that possess the same characters as normal stem cells, especially the ability of differentiation to all cell types found in a particular tumor, which are thought to be associated with chemo-resistance and radio-resistance that complicate the traditional therapy and leads to its failure. Identification criteria of CSC can be a valuable tool in early detection of the disease.*

Aim: *To establish a model for induction of CSC in albino rat.*

Material and Methods: *Thioacetamide (TAA) was used to induce HCC. Immunoflourscent staining of CSC markers: CD133, CD90, CD44 were performed in control rats and in cancer stage to detect their progression.*

Result: *CSCs were successfully induced in this model; three markers CD133, CD90, CD44, were used to identify CSCs, which were found to increase in number and percentage in the cancer stage.*

Conclusion: *HCC induced by TAA is a good model to study CSC.*

Introduction

Liver tumours represent about 11.75% of gastrointestinal malignancies and about 1.68% of total malignancies. Liver tumors include HCC represent about 70.48%, hepatoblastoma 10.24%, non-Hodgkin's lymphoma 4.21% while unspecified adenocarcinoma constitute 9.03% according to

cancer institute pathology registry [1].

HCC is prevalent more between rural resident and farmers especially those infected with hepatitis C virus. The rate of liver cancer in men is typically two to four times higher than in women, but is equal with women after menopause[2,3].

The combined effects of hepatitis B and C virus infections account for more than 80% of liver cancer cases worldwide[3].

The concept of tumor cells and the stem cell origin of tumors are closely linked. If tumors develop from undifferentiated stem cells, then the cancer stem cells are probably the direct descendant of the initiated population. It was hypothesized that tumors may arise from embryonic cells that stay dormant throughout the whole life, embryonic and neoplastic tissue shared similar morphologic and functional characteristics, and a certain relationship may exist between both[4].

Various researches investigated variations of the stem cell origin hypothesis, however, no histological evidence was found for "Embryonic rests". With the technological advances in immunophenotypic characterization of cell lineages and the tracking of transplanted cells ultimately enabled the discovery of stem cells within nearly every adult tissue, which reopened possibility of a stem cell origin of tumors[5].

1.1. Cancer Stem Cell:

Cancer stem cells (CSCs) are defined as cancer cells that possess the same characters as normal stem cells, especially the ability of differentiation to all cell types found in a particular tumor. CSCs are thought to be associated with chemo-resistance and radio-resistance that complicate the traditional therapy and leads to its failure[6].

The cancer stem cell hypothesis states that the origin of cancer cells could be mutated normal stem cells. Also there are subsets of cancer cells which posses stem cell criteria, and can give rise to a cell linage having highly proliferative tumor cells, leading to tumor

initiation, progression, and recurrence[7].

1.2. Criteria Of Cancer Stem cell:

Normal stem cell (SC) and cancer stem cell share common criteria that are targeted by the researchers; one of these criteria is their mode of proliferation. In stem cell, scientists focus is on certain type of cell proliferation which is self-renewal type that characterizes the stem cells. Self-renewal indicated that the cells have the ability to give rise to daughter cells with the same developmental potential. They observed that there is a link between carcinogenesis and genetic deregulation of cancer stem cells as some of genes that regulate self-renewal are oncogenes and on the other hand some of the genes, which inhibit self-renewal are tumor suppressor genes[8,9].

These observations led to the hypothesis that some cancers can originate from cells that have intrinsic self-renewal activity (i.e. stem cells) or in non-stem cells, which acquire self-renewal criteria by genetic mutations[10].

Progressive genetic instability and/or environmental factors are believed to result in sequential mutations that lead to the malignant tumors. Since the early 1990s, and scientists are trying to find the link between stem cell and CSCs; many clinical observations and genetic studies were performed on variety of tumors leading to evolution of a hypothesis that six genetic mutations are essential to convert a normal somatic cell into a cancer cell, which are (a) self-sufficiency for growth signals, (b) insensitivity to antigrowth signals, (c) evasion of apoptosis, (d) limitless ability to replicate, (e) sustained angiogenesis, and (f) tissue invasion and metastasis[11].

1.3. Potential Markers Of Hepatocellular Carcinoma:

1.3.1. CD133:

CD133 was first recognized as a human hematopoietic stem cell marker, and has since been shown to distinguish stem cells and tumors from several tissues [5]. Investigation of liver cancer cell lines denoted that they contained a subpopulation of cells that expressed CD133[12].

1.3.2.CD90:

CD90 has emerged as a very hopeful marker for isolation of human liver cancer stem cells. CD90 positive cells isolated from liver cancer easily established tumors in immunocompromised mice; CD90 positive cells isolated from these tumors were serially transplantable into secondary and tertiary recipients[5].

1.3.3.CD44:

CD44 is an adhesion molecule that was proved to contribute to tumor invasiveness, was also shown to be greatly expressed by the CD90 positive cells. Blockage of this CD44 by neutralizing antibody meaningfully induced apoptosis of the CD90 positive cells in vitro. The most interesting aspect of these studies was the discovery that the blood of hepatocellular carcinoma patients contained a population of CD44 negative, CD90 positive cells[5].

This powerfully suggests that CD90 positive cells may be blamable for establishment of metastasis, and the metastatic potential of these cells, which is partially dependent on functional CD44. Taken these data together, it is considered that CD90 may potentially be used clinically as a prognostic marker, and CD44 as a therapeutic target[5].

Understanding the criteria of CSC will help to develop novel therapies through targeting of cancer stem cells in tumor bulk which can be a promising cure for the resistant and recurrent tumors.

Material and methods

2.1. Animal Used:

Twenty male albino rats were used in this study, weighting 200-250 grams, they were housed in stainless steel mesh cages under temperature control (23 C ± 2), and fixed 12:12-hours light/ dark cycle. All the experiments were carried out according to the rules and regulations of Mansoura University.

2.2. Induction of Hepatocellular carcinoma:

HCC was induced using Thioacetamide (TAA) (Sigma -Aldrich, St Louis, MO Lot # 163678) by being added to drinking water at concentration of 300 mg/L for 20 weeks.

Animals were divided into two groups; negative control, ten male rats were maintained on standard laboratory diet and tape water then sacrificed after 16-20 weeks, cancer group ten rats were sacrificed 16-20 weeks after administration of TAA. Specimens were collected at 8,12,16,20 weeks. HCC was confirmed with by histopathological evaluation by Heamtoxylin and Eosin stain and biochemical evaluation by high level of Alfa Feto protein (AFP) if > 400ng/ml.

At the above assigned times, 5 rats from each group were weighted, SGOT and SGPT levels were measured and groups were compared together for biochemichal confirmation. Each rat was anesthetized with ether, the thoracic cage was opened, the heart was exposed and the right atrium was incised followed immediately by intracardiac perfusion with 200 ml freshly prepared solution of 4% formaldehyde in phosphate-buffered saline (PBS, PH 7.4). The liver were removed and weighted and sections were collected from each lobe and preserved in 10% formalin.

The specimens were processed for paraffin sectioning by gradual dehydration using graded concentrations of alcohol, cleared in xylene and embedded in soft then hard paraffin wax. Sections were cut using microtome at thickness 3-5 µm and stained with Hematoxylin and Eosin to confirm the pathology and immunoflourescent to detect CSC.

2.2.Immunoflourescent staining:

For immunoflourescent staining deparafanization was done, antigen retrieval using 10mmol soduim citrate in microwave on high for 5 min.

Reagents: Goat serum (Abcam -Cambridge, MA), CD133 Fitc conjugated (green), rat anti-rat monoclonal antibody (Bioss inc - Woburn, MA).CD90 Fitc conjugated (green) human anti-rat monoclonal antibody (Milteny Biotec - Germany). CD44 PE-Cy5 conjugated (red), rat anti-rat monoclonal antibody (Bioss inc Woburn, MA).Mounting media with DAPI (blue) as a counter stain (Abcam, Cambridge, MA).

Slides were marked with hydro-

phobic PAP-pen then incubated with 1% goat serum in 1x buffered PBS, PH=7.4 at room temperature then incubated with the conjugated antibodies either CD (133+44) or CD (90+44) 1:100 for 4 hours in room temperature in dark. T slides were washed for 3 times 5 min each on shaker in room temperature in dark and left to dry before adding the mounting media with DAPI then slides were kept in dark. Imaging was done in Mansoura Urology center, using Olymbus microscope installed with cellSens (Ver.1.3) imaging software. Image analysis was done using Image -J software and according to the NIH user guide manual.

Results

3.1. General and biochemical findings:

3.1.1. Body weight:

The animals showed general weakness, cachexia, limited activity and a significant decrease in the body weight. There was a significant decrease in body weight between the control and the cancer group.

3.1.2. Biochemical assessment:

There was a significant increase in the level of Serum glutamic oxaloacetate transe aminase (SGOT) and serum glutamic Pyruvate transe aminase (SGPT) in the cancer group comparing to the control group.

3.2. Histopathological findings:

3.2.1. Hematoylin and Eosin stain:

Sections from rat liver stained with Hematoxylin and Eosin stain showed intact liver architecture with preservation of its lobular pattern. The lobules shows normal portal triads at the periphery and a central veins in the middle, while the sections in HCC showed loss of normal hepatic architecture with malignant transformation in the hepatocyte pattern in which they are arranged in sinusoidal pattern with thick cord formation more than 2 cell thickness (Figure 1).

3.2.2.Immunoflourescent staining:

3.2.2.1. Single marker analysis:

All Sections stained with the three markers, two markers in each set; 90/44 and 133/44. The

control group showed almost no reaction to the three markers, while the HCC group showed a highly significant increase in the color intensity of cancer stem cell in comparison to control (Figure 2).

3.2.2.2. Double marker analysis:

The percentage of cancer stem cell was calculated in both groups CD90/CD44, and CD133/CD44 by manual counting in 20X and 40X field. The control group showed very small percentage to the three markers, while the HCC group showed a highly significant increase in the percentage of cancer stem cell in comparison to control (Figure 3).

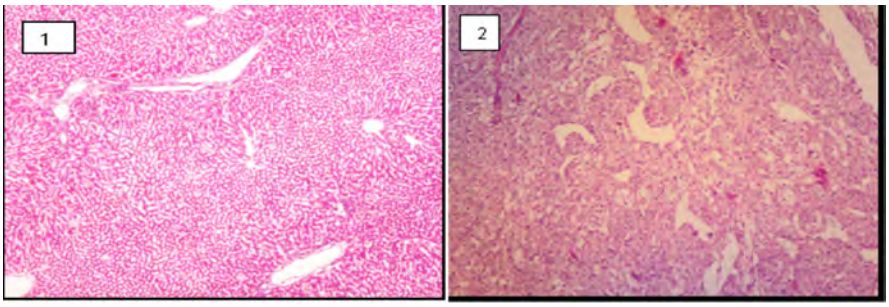

Fig. 1: Sections in rat liver stained with Hematoxylin and Eosin 4X magnification:
1- Control rat shows normal architecture with portal triad at the periphery and central vein in the middle.
2- HCC shows loss of normal hepatic architecture and malignant transformation of hepatocytes.

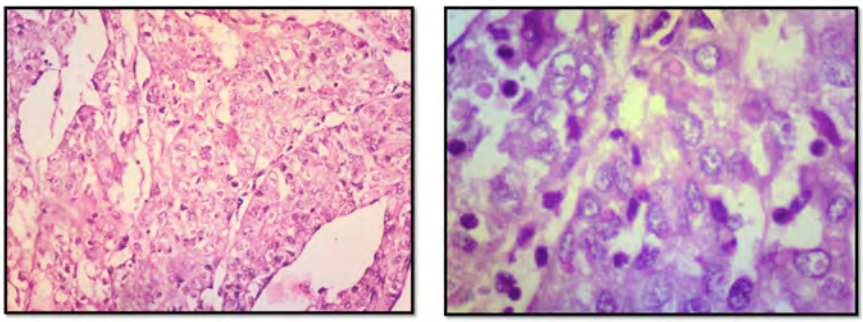

Fig. 2: Left: 10X magnification section showing malignant hepatocytes showing pleomorphic cells with increased nuclear cytoplasmic ratio, the nucleus is large and vesicular with eosinophilic nucleoli and abundant eosinophilic cytoplasm.
Right: 400X magnification section showing malignant hepatocytes with multiple nuclei with prominent nucleoli.

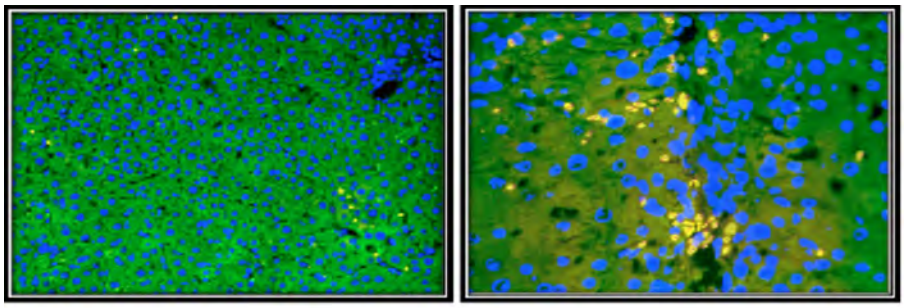

Fig. 3: Representative images for Immunoflourescnet staining of sections in rat liver comparing for the percentage of CSC double marked with CD90/CD44, in control (left) and cancer (right), yellow cells are the CSC.

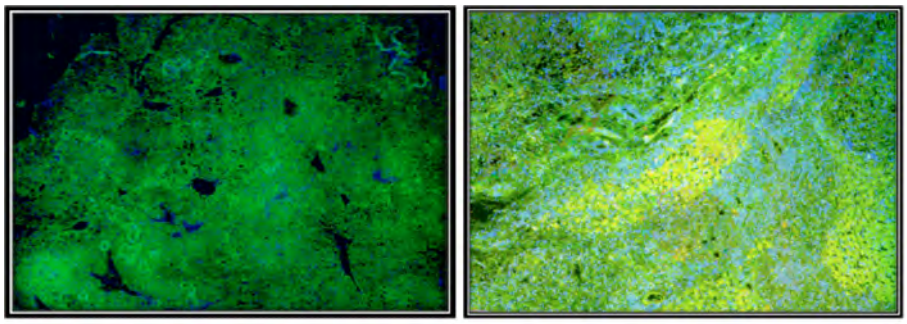

Fig. 4: Immunoflourscent staining in sections of rat liver comparing between control (left) and cancer (right), sectioned is double marked with CD133/CD44 the yellow color indicates the intensity of the reaction 4X magnification.

Discussion

The main finding of this study was that there was a significant increase in the number and the distribution of CSC was a dramatic increase in the number of the CSC in the hepatocellular carcinoma stage.

Thioacetamide was added to drinking water in the dose of 300mg/L as mentioned[13]. TAA at this dose causes cirrhosis by induction of chronic inflammation through increase the oxidative stress and up regulate the radioactive oxygen species (ROS) which in turn recruit large number of macrophages and Kuffer cells leading to fibrosis then cirrhosis[14].

The relation between inflammation and recruitment of CSC was previously investigated[15], through increasing the (ROS) which in-

creased in response to the inflammatory reactions mediated by cytokines and growth factors.

Based on the above mention information TAA was found to be a perfect model to start the cirrhosis and ending by cancer which allowed us to track the progression of CSC in the HCC stage which was the main aim of the study.

The selection of these specific surface markers for identifying CSC was based on number of some studies such[16,17,18].

The cell surface marker CD133 is now accepted as a cancer stem cell marker for various solid tumors, but its function in cancer stem cell biology is not yet fully understood, several studies stated that its important role specifically in the HCC, CD133-positive cells in liver tumors were found to have extensive proliferative and self-renewal abilities and was identified as CSCs in many HCC cell lines, and was proven to contribute to the initiation and growth of HCC supporting the CSC hypothesis. Besides re-expression of CD133 was reported to be found extensively in regenerating rat liver indicating that CD133 positive cells are associated with liver cell proliferation, and could be a possible link to hepatocellular carcinoma[19].

$CD133^{+}CD44^{+}$ was shown to play a key role in hematogenous metastasis of liver cancers, in which CD133 is blamable for tumor growth and CD44 is responsible for tumor invasion, two important factors in tumor metastasis [20]. Their result suggested that $CD133^{+}$ cells act as tumor initiating cells (TIC) as they showed higher abilities in colony formation and tumorigenicity.

The CD44/CD90 positive cells were reported to be highly invasive, although both CD133 and CD90 were suggested as markers of liver progenitor cells, they might represent different populations. For example, CD90 and CD133 mark for mesenchymal and hematopoietic origin, respectively[20].

Chronic inflammatory conditions may affect both carcinogenesis and normal stem cell function, and it is possible that the conver-

gence of these behavior contributes to the formation or regulation or both of CSCs. Increased intracellular production of ROS produced by immune response or inflammatory cytokines are a common feature of chronically inflamed tissues and may have multiple effects on CSCs[21].

Our finding agrees with the above literature and can explain the significant increase in the number of the CSC in the active stage of the cirrhosis where there is a lot of inflammation and replacement by regenerating nodules and increase in the cell division.

In the late stage of the cirrhosis the sections showed decrease in the number of mononuclear cells in filtrate which indicates that there is no active inflammation, and this explains the decrease in the number of the CSC and that it is strongly correlated to the inflammation.

Findings made in the CSC field will feed back into other zones of stem cell research because many marker gene products found in CSCs also share with the normal stem cell population. It is also predictable that a better understanding of the processes that control autonomous growth, differentiation and cell migration will contribute to novel regenerative-medicine-based treatments that will transform therapeutic strategies and bring renewed hope to cancer patients.

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EVALUATION OF DIFFERENT SCORING SYSTEMS PREDICTING NONSENTINEL LYMPH NODE STATUS IN BREAST CANCER PATIENTS

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Abstract

Background: *Axillary lymph node dissection (ALND) performed after a positive sentinel lymph node biopsy (SLNB) in breast cancer patients, often results in no additional positive nodes. Scoring systems have been published to aid in the prediction of non sentinel lymph node (NSLN) metastasis. Our aim was to assess the validity of these scoring systems in our patients.*

Methods: *This prospective study conducted on 48 patients who underwent ALND after a positive SLNB in Mansoura University Hospital from March 2009 to December 2012. The accuracy of the Memorial Sloan-Kettering Cancer Center (MSKCC) nomogram, the MD Anderson scoring system and the Tenon scoring system were tested for the prediction of NSLN status. Receiver operating characteristics (ROC) curves were drawn, and the areas under the curves were calculated to assess the discriminative power of each system. Univariate analysis was performed to assess the predictability of individual patient and tumor characteristics.*

Results: *Nonsentinel lymph nodes were positive in 38 (79%) patients. The areas under the ROC curves were 0.84, 0.90, and 0.91, respectively. The pathological tumor size was the only individual predictors of nonsentinel node metastasis.*

Conclusions: *Scoring systems provide additional information regarding the likelihood of metastasis in nonsentinel nodes, but their predictability remains less than optimal. The use of scoring systems must be applied with caution until future studies provide a more accurate assessment of risk for patients with a positive SLNB.*

Key Words: *Scoring systems - Sentinel lymph node - Nonsentinel lymph node - Breast cancer.*

Introduction

The introduction of SLNB for nodal staging has revolutionized the surgical approach for early breast cancer. The goal of SLNB is to reduce the morbidity of breast cancer surgery by avoiding unnecessary ALND in patients with negative SLN. However, if a positive SLN is found, it is currently recommended to continue with ALND. In 40 - 70% of patients the SLN is the only involved axillary node, implying that these patients undergo ALND unnecessarily [1,2,3].

Several studies have investigated clinicopathologic factors that may predict which patients have a higher risk of non sentinel lymph node involvement, none of which are sufficiently predictive when used alone[4,5].

Scoring systems have been developed using a combination of several factors, such as tumor size, histology, hormone receptors, presence of lymphovascular invasion, the number of sentinel nodes removed, as a guide to determine which patients may forego ALND, if they had a positive SLN[6,7].

Three scoring systems were identified in the existing medical literature using a Medline-based search engine. The first scoring system (MSK) is a nomogram from Memorial Sloan-Kettering Cancer Center (MSKCC) in New York, USA, that includes eight characteristics (nuclear grade, lymphovascular invasion, multifocality, estrogen receptor status, number of positive and negative sentinel nodes, tumor size, and method of detection of sentinel node metastasis) that ultimately generates a total point value, which then corresponds to a percentage of risk [8,9,10].

The second scoring system [M.D. Anderson (MDA) score] was developed at the M.D. Anderson Cancer Center in Houston Texas, USA and is based on four characteristics (tumor size, number of sentinel nodes, size of metastasis, and lymphovascular invasion), where a or □ coefficient was determined for each, and the sum of rounded coefficients results in a score ranging from -2 to 4[5,6,7].

The third scoring system (Tenon score) was derived at the Hos-

pital Tenon in Paris, France, and includes three characteristics (size of metastasis, tumor size, and proportion of sentinel nodes involved) which are assigned point values that, when added, result in a score between 0 and 7[11,12].

The aim of our study is to evaluate available three scoring systems for accurate prediction of metastasis in nonsentinel lymph nodes in breast cancer patients with a positive sentinel lymph nodes biopsy.

Patients and Methods

This prospective study was done on all patients with invasive breast cancer admitted to Mansoura University Hospital, from March 2009 to December 2012, where sentinel lymph node biopsy will be chosen as the procedure to deal with the axillary lymph node status.

This study was carried on 58 patients with primary invasive breast cancer with clinically negative axilla operated upon with modified radical mastectomy in 53 cases (91%) or wide local excision and axillary clearance in 5 cases (9%).

Technique of SLNB:

Sentinel lymph nodes were mapped in the first 6 cases using a subdermal injection of 0.5 mCi of filtered ^{99m}Tc Sulfur Colloid in 5 ml of saline 2 hour before surgery. In the next 52 cases sentinel lymph nodes were mapped using peritumeral injection of 5 ml of a blue dye (Methylene blue in 50 cases and patent blue in 2 cases) 15 minutes before axillary dissection.

SLNs localization was undertaken in the radioisotope group using a hand held gamma probe to determine radioactivity levels in the axilla, looking for 'hot' nodes (nodes with counts at least tenfold those of the background), and in the blue dye group as the most proximal node the pathway of a blue-stained lymphatic, or any blue nodes.

After identification of SLNs, complete axillary dissection was done and all axillary lymph nodes were taken as a separate specimen, with the breast tissue as the third specimen and were fixed in 10% formalin saline. The three specimens were sent for histo-

pathological examination.

Pathological examination:

Each primary tumor was evaluated for size of the invasive component, histological type, nuclear grade, estrogen receptor status, multifocality of the tumor, and presence of lymphovascular invasion.

Pathological SLN examination includes routine hematoxylin and eosin (HE), serial HE, or immunohistochemistry (IHC). Each half-SN was sliced at 3-mm intervals. All 58 cases were examined by routine (HE) we found 38 cases positive, (H&E) negative sections (20 cases) were examined by serial HE we found 10 cases positive, and(serial H&E) negative sections (10 cases) were examined by IHC we found all 10 cases negative.

NSLN obtained from axillary dissection specimen were analyzed by HE staining only. The total number and number of positive NSLN were recorded.

All clinical data recorded in the MSKCC, MD Anderson and Tenon studies were prospectively collected as part of our clinical study and then retrospectively reviewed.

All data were collected in a performed sheet then entered into an electronic spreadsheet (Microsoft Excel) and transferred into SPSS (Statistical Package for Social Sciences).

Statistical Analysis

Statistical analyses were performed by using the SPSS (Statistical Package for Social Sciences) version 15, 2006. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data was tested for normality by Kolmogrov-Smirnov test. Normally distributed data was presented as means ± standard deviations (SD). Student t-test was used to compare between two or more groups.

The discriminatory accuracies of the three scoring systems were compared by constructing receiver operating characteristics (ROC) curves and measuring areas under these curves (AUC). A model with a ROC of 0.5 is equal to the toss of a coin. A model with a ROC

of 0.7- 0.8 is considered good, whereas a ROC of 0.81 - 0.9 has excellent discrimination. Categorical data are reported as percentages with 95% confidence intervals (95% CI).

Results

This prospective study compromised of 58 patients underwent SLNB, 10 (18%) of which had a negative nodes, were excluded from our study, leaving 48 (82%) patients with positive SLNB, and this group therefore comprised our study sample.

All patients in the study were female, with a mean age of 49 years (range, 30-74) years. The mean pathological tumor size was 3.3 cm (range, 2-7 cm).

The mean pathological tumor size was 3.35 cm (range, 2-7 cm). There were no cases with tumor size less than 2cm due to absence of screening programs in Mansoura University Hospital. There was a statistical significance between tumor size and NSLN status.

The mean total number of SLN was 2.35 (range 1-4), the mean number of positive SLN was 1.9 (range 1 - 4), and a median of 11 (range 5 - 24) nodes were dissected during ALND. Non sentinel lymph nodes were positive in 38 (79.2%) patients and negative in 10 (20.8%) patients. There was no statistical significance between total number of SLN, number of positive SLN and NSLN status.

The MSKCC nomogram in our patients shows all patients (4) with score less than 20% have negative NSLN (negative predictive value=100%), and all patients (22) with score more than 60% have posative NSLN (posative predictive value = 100%), so we can predict the NSLN status in 55%of our patients. The areas under the curve of MSKCC was 0.84 (exellent).

The MD Anderson score in our patients shows all patients (6) with score less than 0 have negative NSLN (negative predictive value = 100%), and all patients (21) with score more than 2 have posative NSLN (posative predictive value = 100%), so we can predict the NSLN status in 57 % of our patients. The areas under the curve of the MD Anderson score

was 0.90 (exellent).

The Tenon score in our patients shows 11 patients with score □ 3.5, seven patients have negative NSLN (negative predictive value = 90%), and four patients have posative NSLN (posative predictive value = 30%). This is due the lowest score in our study is 1.5 (the smallest tumor size =2cm). All patients (26) with score more than 6 have posative NSLN (posative predictive value = 100%), so we can predict the NSLN status in 55%of our patients.. The areas under the curve of Tenon score was 0.91 (exellent).

Table (1): Univariate analysis of the tumor size and the NSLN metastasis.

Tumor size		NSLN		
		Negative	Positive	Total
≤ 2 cm	Count	7	10	17
	% of Total	14.6%	20.8%	35.4%
2.1 –3cm	Count	1	13	14
	% of Total	2.1%	27.1%	29.2%
3.1 –5cm	Count	2	10	12
	% of Total	4.2%	20.8%	25%
> 5cm	Count	0	5	5
	% of Total	0%	10.4%	10.4%
Total	Count	10	38	48
	% of Total	20.8%	79.2%	100%

P-value = 0.044

Table (2): Univariate analysis of tumor characteristics and non sentinel lymph node metastasis.

Tumor characteristics		NSLN			P-value
		Negative	Positive	Total	
LVI	Negative	6	4	10	0.001
	Positive	4	34	38	
M F	Negative	9	33	42	0.788
	Positive	1	5	6	
E R	Negative	6	6	12	0.004
	Positive	4	32	36	
MM<2mm	Negative	0	30	30	0.001
	Positive	10	8	18	

LVI = Lymphovascular Invasion, MF =Multifocality, ER =Estrogen Receptor, MM = Micro Metastasis.

Table (3): Results of series validating the MSKCC nomogram

Author	Year	Country	Num patients	AUC
Van Zee et al [8]	2003	USA	373	0.77
Kocsis et al [19]	2004	Hungary	140	0.84
Soni et al [20]	2005	Australia	149	0.75
Hunt et al [21]	2005	USA	200	0.74
Smidt et al [22]	2005	Netherlands	222	0.78
Degnim et al [23]	2005	USA	89	0.86
Specht et al [15]	2005	USA	33	0.72
Lambert et al [9]	2006	USA	200	0.71
Cripe et al [24]	2006	USA	92	0.82
Ponzone et al [6]	2007	Italy	186	0.71
Dauphine et al [7]	2007	USA	51	0.63
Zgajnar et al [25]	2007	Slovenia	276	0.72
Alran et al [10]	2007	France	588	0.72
Cserni [26]	2007	Hungary	140	0.73
Pal et al [27]	2008	England	118	0.68
Kohert et al [28]	2008	USA	171	0.77
Cho et al [18]	2008	Korea	82	0.78
Gur et al [16]	2009	USA	319	0.70
D'Eredita et al [17]	2010	Italy	95	0.72
Fougo et al [29]	2011	Portugal	98	0.67
Hidar et al [30]	2011	Tunisia	87	0.73
van la Parra et al [31]	2012	Netherlands	222	0.67
Sasada [32]	2012	Japan	116	0.73

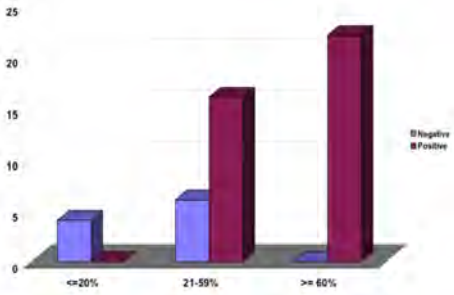

Fig. 1: The relation between **MSKCC** score and **NSLN** metastasis.

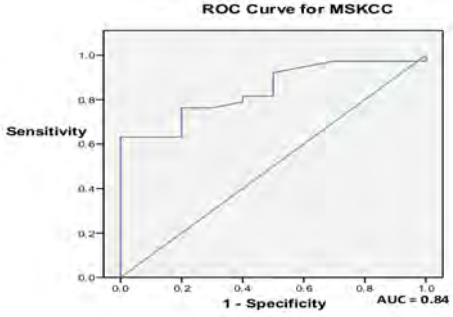

Fig. 2: Receiver operating characteristics curve for the MSKCC nomogram.

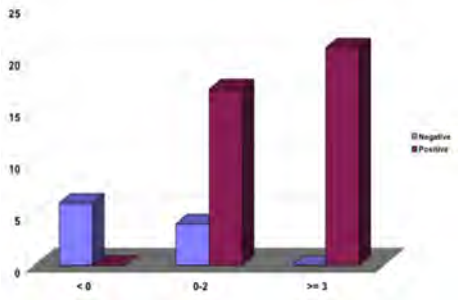

Fig. 3: The relation between **MD Anderson** score and **NSLN** metastasis.

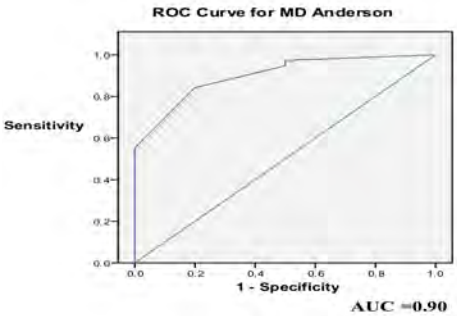

Fig. 4: Receiver operating characteristics curve for the MD Anderson.

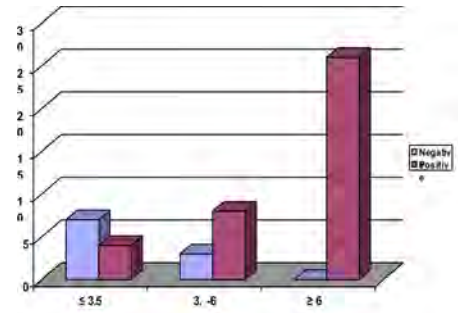

Fig. 5: The relation between Tenon score and **NSLN** metastasis.

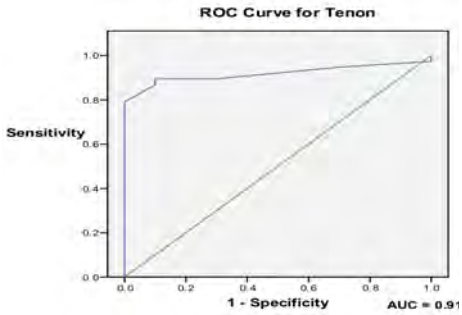

Fig. 6: Receiver operating characteristics curve for the **Tenon** score.

Discussion

The introduction of SLNB has decreased the need for axillary adenectomy in nearly two-thirds of patients with a clinically negative axilla. With accurate risk stratification, over half of the remaining third, can also be spared ALND. It is becoming apparent that performing complete axillary dissection routinely on patients with a positive SLNB is not likely to positively impact outcomes and may add to morbidity[13].

Meanwhile, we suggest that treatment of the axilla may not be significantly different from the treatment of breast cancer at the primary site. Just as a patient receives counseling about her options for breast conservation, she may now have the option for "axillary conservation" and limit morbidity resulting from an axillary dissection that is often unnecessary[11].

In a majority of cases, the re-

mainder of nodes in the axilla on ALND after a positive SLNB shows no further metastatic disease (negative NSLN). Veronesi et al[14] found 40% (32 from 81) of patients with negative NSLN, Hwang et al[5] found 60% (78 from 131) of patients with negative NSLN, Dauphine et al[7] found 41% (16 from 39) of patients with negative NSLN, and in our study we found 20.8% (10 from 48) of patients with negative NSLN.

We observed positive non sentinel nodes in 79% of our patients with a positive SLNB, higher than the 30-50% reported in the literature [7,11,12]. There are two likely explanations for this finding: First, as mentioned earlier, micrometastasis is under represented in our study sample. Secondly, the average tumor size in our patients is larger than that reported by others. For example, in Alran et al[10] study the mean pathological tumor size was 1.55cm (range 0.2-6 cm), associated with a 35% rate of non sentinel node metastasis, whereas the average tumor size in our study was 3.35 cm (range, 2-7 cm).

In this study the mean age of our Egyptian patients was 49 years (ranging from 30 to74 years). There were 22 of our patients (45.8 %) older than 50 years of age. There were 38 (80%) patients with positive NSLN with a mean age of 48 years, and 10 (20%) patients with negative NSLN with a mean age of54 years. In Coutant et al[12] study the mean age of French patients was 57 years (ranging from 32 to78 years). In Dauphine et al[7] study the mean age of American patients was 53 years (ranging from 45 to 62 years).

In our study the mean pathological tumor size was 3.35 cm (range, 2-7 cm). There were no cases with tumor size less than 2cm. In Dauphine et al[11] study the mean pathological tumor size was 2.5cm (range 1.7 - 4 cm). In Alran et al[10] study the mean pathological tumor size was 1.55cm (range 0.2-6 cm). In Barranger et al[11] study the mean pathological tumor size was 1.44cm (range 0.1-11.6 cm). In our study there was a statistical significance between tumor size and NSLN status with P-value = 0.044.

Evaluation of the MSKCC nomogram:

The MSKCC nomogram has been validated by 23 studies worldwide (table 3), and other validation series are certain to follow. The nomogram had proved robust despite differences in patient demographics, clinical characteristics, surgical technique, and pathologic processing. The AUC values range from 0.63 to 0.86 and as one might expect, the highest (0.86) and lowest (0.63) values come from studies with fewer than 100 patients.

In our study done on 48 patients in Mansoura, Egypt, the AUC of MSKCC nomogram is 0.84. It was an excellent prediction for additional NSLN metastasis in SLN positive patients. The MSKCC nomogram in our patients shows all patients[4] with score less than 20% have negative NSLN (negative predictive value = 100%), and all patients[22] with score more than 60% have posative NSLN (posative predictive value = 100%), so we can predict the NSLN status in 55%of our patients.

Evaluation of the MD Anderson score

Hwang et al[5] found 9 patients with score 4, all of them have positive NSLN (positive predictive value was 100%), and 21 patients with score less than zero, all of them have negative NSLN (negative predictive value was 100%).

Ponzone et al[6] found the majority of the patients (85%) had a score between 0 and 3. A score of -2 was associated with no additional positive NSLNs identified at ALND, but this group included only 2 patients, whereas patients with a score of 4 had about a 50% chance of carrying other positive node in the axilla. As a consequence, sensitivity, specificity, and positive and negative predictive values associated with each possible score were all well below those reported by Hwang et al[5].

In Dauphine et al[7] study the AUC of the M.D Anderson score was 0.70 (good prediction), and he found the proportion of sentinel nodes that were positive and the total number of sentinel nodes retrieved were the only individual predictors of non sentinel node metastasis.

The MD Anderson score in our

patients shows all patients[6] with score less than 0 have negative NSLN (negative predictive value = 100%), and all patients[21] with score more than 3 have posative NSLN (posative predictive value = 100%), and only 45% of our patients had a score between 0 and 3. The areas under the curve 0.90 (exellent).

Evaluation of the Tenon score:

In Brranger et al[11] study all patients with a score of 3.5 or less had a 97.3% chance of being free of NSLN involvement. The main limitation of this score is that is does not take into account extracapsular extension of SN metastasis, which is a powerful predictor of non-SN metastasis.

In Coutant et al[12] study 120 (53.1%) of the 226 patients had a Tenon score of 3.5 or less. Among these patients, five had at least one positive NSLN (negative predictive value was 95.8% and the false-negative rate was 4.2%), with an AUC of 0.82.

In Gur et al[16] study 115 (36.0%) of the 319 patients had a score □3.5 according to the Tenon model, and in this group, 20 had at least 1 positive NSLN. With a score cut-off of 3.5, the negative predictive value was 72% and the false negative rate was 17.3%, with an AUC of 0.69.

In Hidar et al[30] study, 26 (30%) of the 87 cases were reported with a Tenon score □3.5, and in this subset of patients, 15 were associated with a negative NSLN (negative predictive value was 57% and the false-negative rate was 43%), with an AUC of 0.75.

In our patients the Tenon score shows 11 patients with score □ 3.5, seven patients have negative NSLN (negative predictive value = 90%), and four patients have posative NSLN (posative predictive value = 30%). This is due the lowest score in our study is 1.5 (the smallest tumor size =2cm).All patients[26] with score □ 6 have posative NSLN (posative predictive value = 100%), so we can predict the NSLN status in 55%of our patients.. The areas under the curve of Tenon score was 0.91 (exellent).

Conclusion

To our knowledge, the current

study is the first to report, to compare and to validate NSLN metastases predictive tools in Egyptian population. The three scoring systems (MSK, MD Anderson, Tenon) were comparable, as determined by their AUC, 0.84, 0.90, 0.91 respectively with sufficient predictability to be called valid.

The MSKCC nomogram was outperformed in our study population because it presents as graphic nomogram and as digital available at Web site, www.mskcc.org/nomograms, and appears to provide a useful quantitative estimation on NSLN involvement when discussing the indication to completion ALND with the patient.

The use of scoring systems must be applied with caution until future studies provide a more accurate assessment of the risk NSLN metastases for patients with a positive SLNB.

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EVALUATION OF DIFFERENT SCORING SYSTEMS PREDICTING NONSENTINEL LYMPH NODE STATUS IN BREAST CANCER PATIENTS

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ANEMIA, PHYSICAL PERFORMANCE RELATIONSHIP AMONG ELDERY PATIENTS ATTENDING GERIATRIC OUTPATIENT CLINICS IN MANSOURA CITY

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Abstract

Background: *There is limited insight into the effect of anemia on functional status of elderly person.* ***Aim of work:*** *To assess prevalence of anemia and identify the effect of anemia on the physical performance of the elderly patients attending the geriatric outpatient clinics in Mansoura hospitals.* ***Method:*** *an observational study carried on 200 elderly patient's ageing≥ 60 years attending geriatric outpatient clinics at the specialized medical hospital and the general hospital in Mansoura city. Patients were interviewed individually by the researcher to collect data.* ***Results:*** *The prevalence of anemia at baseline was 30% according to WHO criteria (Hb<13 g/dl in males and <12 g/dl in females). Anemic elderly person had poorer performance and more disability in activities of daily living, instrumental activity of daily living, short physical performance battery and International Physical Activity than person without anemia.* ***Conclusion:*** *Anemia in elderly persons appears to be associated with disability and poorer physical performance.* ***Recommendation:*** *Health education programs to elders about the disease process and In- service training programs to all nurses and health care providers in hospitals and outpatient clinics to update their knowledge, increase their ability to care for elderly patients with anemia.*

Introduction

Anemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs (WHO, 2011). Normal range of hemoglobin levels is defined by the World Health Organization as a hemoglobin concentration lower than 13

g/dL in elderly men and lowers than 12 g/dL in women[3,10].

Anemia is a common problem among elderly and it's causes are divided into three broad groups: nutrient-deficiency anemia, most often iron deficiency anemia; and/or anemia of chronic disease, perhaps better termed as anemia of chronic inflammation; and unexplained anemia[13,33].

Anemia has been associated with loss of physical function; independent of underlying disease status[9,23] but the exact pathway through which anemia may negatively affect physical function has not been studied extensively. It could be hypothesized that anemia results in fatigue and diminished muscular oxygenation, which may affect muscle strength, quality and subsequently physical performance. In addition, because older persons with anemia have been shown to have higher serum levels of C-reactive protein (CRP), a state of underlying chronic inflammation may have caused greater physical decline[9].

In Egypt generally and Dakahlia particularly there is lacking in studies assessing the prevalence of anemia among elderly and its impact on their physical performance.

Aim of the Study

This study was carried out to assess the prevalence of anemia and find the relationship between presence of anemia and the physical performance of the elderly patients attending the outpatient clinics hospital in Mansoura city.

Subjects and Method

Study design: It is an observational study.

Settings: Carried out at geriatric outpatient clinics at the specialized medical hospital and the general hospital in Mansoura city after taking the consent of the managers.

Time of study: from March 2012 to September 2013.

Subjects:

Using DDS research .com soft ware, for sample size calculation and using percentage of anemia among males 66.4% in 1986 and 77.4% in 2000 and Alfa error 5%, Beta error 20%. The sample size is

105+20% =126.

This study included 200 elderly attendants of both sexes from the geriatric outpatients clinics in the above mentioned hospitals during the time of study fulfilling the following criteria: aged 60-95 years, able to communicate, willing to participate in the study and available at the time of data collection. Excluded elderly those who refuse blood sample, who had blood transfusions within12 weeks prior to the beginning of the study, active bleeding, severe cognitive impairment, or subjects were over 95 years, elderly with neurological disease and other neurodegenerative diseases or severe organ insufficiency (that limit the patient's autonomy) and elderly with terminal illness.

Tools: six tools were used:

Tool I: A questionnaire about Socio demographic and medical data :

It was developed by the researcher after literature review and it included:-

1: Socio-demographic characteristics of the patients such as age, sex, residence and co-inhabitance marital status, level of education, occupation before retirement and income.

2: Dietary habits, feeding pattern, daily fluid intake and appetite changes.

3: Risk behaviors in the life style such as smoking and caffeine consumption

4: Medical history of diseases (especially GIT, hepatic, renal, cardiac).

5: Medications and drugs used & nutrient supplements,

6: Previous hospitalization and Surgery conduction.

7: Medical history of anemia (signs, symptoms and complications of anemia)

8: Family history of anemia.

Tool II: Mini Nutritional Assessment short form scale (MNA):

The MNA was developed by Vellas et al (2006)[31] for elderly; the total score is 14 points which categorized into three levels; normal nutritional status (12 to 14 points), at risk of malnutrition (8 to 11 points), and malnourished (Less than 7 points).

Tool III:

Katz and Akpom scale (1976)

[20] was used to assess activities of daily living. The total score of the scale is 6-18. According to Katz and Akpom scale elderly were classified into three categories:

• Totally dependence: those who scored 13 to 18.

• Partially dependence: score 7 to 12 points.

• Independence: those with score of 6 points.

Tool IV:

Lawton and Brady scale of instrumental activities of daily living (1969)[21]. The scale includes eight items: ability to use the telephone, go shopping, food preparation, housekeeping, laundry, transportation, responsibility for own medication and ability to handle finances. The answers were given a score:

Able (2) Unable (1)

The maximum score was 16 for females and 10 for males. Six points from the maximum score were subtracted for males for gender-specific questions. The score achieved by the elder was calculated as a percentage. The degree of the elder's performance of IADL was categorized as follows: totally dependent (0-<25%), partially dependent (25-<75%), independent (≥75%) (Translated into Arabic and tested for its validity and reliability by Fatma Hallaj 2007 (Hallaj, 2007)[18].

Tool V: The short physical performance battery:

It is developed by Guralnik, et al (1999)[17] to assess walking speed, standing balance, and ability to rise from a chair. Walking speed was defined as the best performance (time in seconds) of two 4-m walks along a corridor. For standing balance, participants were asked to stand in three progressively more-difficult positions for 10 seconds each: a position with the feet side by side, a semi-tandem position, and a full-tandem position. For the chair-stand test, participants were asked to stand up from and sit down in a chair five times without using hands; the performance was timed. Each physical performance test was categorized into a five-level score, with 0 representing inability to do the test and 4 representing the highest level of performance.

Tool VI: International Physical Activity Questionnaire (IPAQ)

short form:

It is developed by Craig et al (2003)[11]. It was used to assess the level of physical activity of the elderly patients during the last week. The tool include 7-items that measure three specific types of activity, namely walking such as " How much time in total did you usually spend walking on one of those days?, moderate intensity activity such as " How much time in total did you usually spend on one of those days doing moderate physical activities, and vigorous-intensity activity such as " How much time in total did you usually spend on one of those days doing vigorous physical activities?

Method:

1. Consent of the managers was taken. Based on the schedule of the outpatient clinics at specialized medical hospital and general hospital. The researcher visited each clinic twice/ week.

2. A verbal consent of every elder included in the study was obtained after explanation of the purpose of the study. Patient's privacy & confidentiality was maintained.

3. For every elderly the questionnaires were fulfilled and the six tools of the study were completed (by the researcher) then physically examined, weight and height were measured to calculate the body mass index (BMI) and blood sample was obtained for hemoglobin estimation.

4. A pilot study was carried out on 20 (10%) of elderly patients at the specialized medical hospital before starting the data collection to test the feasibility of the tools and to make the necessary modifications.

5. Data obtained from this study were analyzed using PC with statistical package for social science (SPSS) version 13. The mean and percentage were used for descriptive statistics While for Analytical statistics Chi square ($\chi 2$), Fisher Exact Test probability (FET),and student t-test were used. The 0.05 level was used as the cut off value for statistical significance.

Results

Data analysis of the socio-demographic characteristics of the

studied subjects revealed that, the age of the studied subjects ranged from 60 to 85 years, (the mean ±SD = 65.74±5.57 years). Females constituted 51.5% of the elders, while 48.5% were males.

In this study the prevalence of anemia among elderly persons who attended the geriatric outpatient's clinics of Mansoura hospitals was 30% (Fig.I). As regard severity of anemia 20.5% had mild anemia with hemoglobin level between 10-12mg/dl while 9.5% had moderate anemia with hemoglobin level between 7-9mg/dl and no one had severe anemia (Fig. II).

Comparison between anemic and non anemic elderly as regard personal Socio-demographic characteristic, **table(I)** showed that the mean age of non anemic elderly was 64.8±4.8 years and it was 67.9±6.6 among anemic (t=3.3, P=0.001). Concerning occupation of elders, it was observed that there was a statistically significance difference between anemic and non anemic elderly (Chi square (χ2) = 123.6, P=0.018).

On studying medical history and use of medications. **Table (II)** showed a statistical significant difference between the number of chronic diseases affecting elders and occurrence of anemia (P=0.002). Also a significant relation was found between hypertension and diabetes mellitus to occurrence of anemia (P=0.035) and (P=0.028) respectively. Moreover, using antihypertensive drugs and anti diabetic drugs affected significantly anemia (P=0.010) and (P=0.024) respectively. Not only that but there were also a significant difference between both groups concerning smoking and caffeine consumption (Chi square (χ2)=8.3, p=0.015) and (χ2=4.4, p=0.036) respectively.

Table III: Shows the relation between the number of meals consumed by the elderly and occurrence of anemia. Differences between groups of anemia was statistically significance (Fisher Exact Test probability (FET) =42.333, p=0.000). Concerning the type of food consumed by the elderly, the differences between groups regarding consumption of enough milk (FET=32.737, P=0.000), enough meat, poultry or

Fish (FET=96.801, P=0.000), enough fruits or vegetables, (FET=28.315, P=0.000). And in relation to fluid intake (FET=13.363, P=0.000).

Table V: It appears from the table that there was significant difference between anemic and non anemic elders as regard past history of anemia ($\chi 2$=37.8, P=0.000), family history of anemia ($\chi 2$=33.7, P=0.000), previous hospitalization ($\chi 2$=6.585, P=0.010), previous surgery ($\chi 2$=8.747, p=0.003), manifestation of easy fatigability and palpitation (P=0.039 and P=0.024) respectively and periodic follow up examination($\chi 2$= 5.5, p=0.019).

Results of using the mini nutritional assessment tool (MNA) are shown in **Table VI:** There was a significant difference in health status of anemic and non anemic elders ($\chi 2$= 101.2, p=0.0008). Also There was statistically significant difference between both groups concerning the (IPAQ) ($\chi 2$=86.8, p=0.000), ADLS (FEP, p=0.000), IADLS ($\chi 2$=41.8, p=0.000) and PPB ($\chi 2$=114.5, p=0.000).

After regression analysis **Table VII:** it appears that MNA score, IADL score, physical function, age and income were the most important risk factors associated with anemia. Regarding MNA score (Odds Ratio=0.11;95% Confidence interval:0.0-0.51),considering income, those with insufficient income had 12 times more risk for having anemia than those with sufficient income, for age (Odds Ratio=0.70; 95% Confidence interval: 0.5-0.90), Considering physical function (Odds Ratio= 0.15; 95% Confidenceinterval: 0.0-0.42) and IADL score (Odds Ratio =0.70; 95% CI: 0.5-1.0).

Table (I): Socio-demographic data of anemic and non anemic elders.

Socio demographic data		Anemia				X^2	P
		No		Yes			
		No	%	No	%		
Age	60-	78	75.7	25	24.3	16.4	0.003*
	65-	35	79.5	9	20.5		
	70-	21	58.3	15	41.7		
	75-	5	41.7	7	58.3		
	80+	1	20.0	4	80.0		
Mean ± SD		64.8 ± 4.8		67.9 ± 6.6		t=3.3	0.001*
Gender	Male	67	69.1	30	30.9	0.08	0.781
	Female	73	70.9	30	29.1		
Marital status	Single	1	25.0	3	75.0	4.4	0.225
	Married	103	71.0	42	29.0		
	Widowed	35	70.0	15	30.0		
	Divorced	1	100.0	0	0.0		
Education	Illiterate	82	66.1	42	33.9	8.7	0.068
	R & R	28	65.1	15	34.9		
	Middle education	21	87.5	3	12.5		
	Secondary education	5	100.0	0	0.0		
	University education	4	100.0	0	0.0		
Occupation before retirement	Employee	19	86.4	3	13.6	123.6	0.018*
	Farmer	26	56.5	20	43.5		
	Housewife	71	71.0	29	29.0		
	Trader	14	93.3	1	6.7		
	Handcraft	4	44.4	5	55.6		
	Others	6	75.0	2	25.0		
Co-habitate	Alone	5	45.5	6	54.5	5.4	0.068
	With family	102	68.9	46	31.1		
	Sons	33	80.5	8	19.5		
Residence	Rural	98	69.5	43	30.5	0.06	0.813
	Urban	42	71.2	17	28.8		
Income	Sufficient	136	71.2	55	28.8	2.9	0.087
	Insufficient	4	44.4	5	55.6		
Source	Retirement	118	68.6	54	31.4	1.4	0.710
	Social affaire	1	100.0	0	0.0		
	Sons ids	3	75.0	1	25.0		
	Still working	18	78.3	5	21.7		

Table (II): Medical history, use of medication and risk behavior of the studied elders.

Medical history	Anemia				P. Value
	No		Yes		
	No. 140	%	No. 60	%	
No. of Diseases	22	15.7	5	8.4	0.002*
▪ No disease					
▪ One disease	81	57.9	14	23.3	
▪ Two diseases	31	22.1	15	25.0	
▪ Three or more diseases	6	4.3	26	43.3	
$ Type of Diseases					
▪ Respiratory	6	4.3	5	8.3	0.558
▪ Cardiac	7	5.0	8	13.3	0.874
▪ HTN	65	46.4	31	51.7	0.035*
▪ Renal	7	5.0	7	11.7	1.000
▪ DM	52	37.1	26	43.3	0.028*
▪ GIT	15	10.7	15	25.0	1.000
▪ Musculoskeletal	7	5.0	7	11.7	1.000
▪ Cancer	1	0.7	0	0.0	0.714
$Drugs					
▪ Analgesic	19	12.9	13	21.7	0.063
▪ Anti-coagulant	7	5.0	9	15.0	0.428
▪ Antacid	2	1.4	1	1.7	0.053
▪ Vitamins	21	15.0%	20	33.3	0.865
▪ Corticosteroids	8	5.7	7	11.7	0.668
▪ HTN	63	45.0	29	48.3	0.010*
▪ Neurological	1	0.7	0.0	0.0	0.775
▪ DM	49	35.0	26	43.3.	0.024*
▪ Diuretics	1	0.7	1	1.7	1.000
▪ Antibiotic	1	0.7	2	3.3	0.417
▪ Rheumatic	0	0.0	1	1.7	0.635
▪ Hepatic	15	10.7	16	26.7	0.758
▪ Hypercholesterolemia	1	0.7	1	1. 7	1.000
▪ Cancer drugs	1	0.7	0	0.0	1.000
Smoking	27	79.4	7	20.6	0.015*
Having caffeine	127	72.6	48	27.4	0.036*

$: More than one response.

Table (III): Dietary habits of elderly as a predictors for anemia and degree of anemia.

Dietary habits		Non anemic		Mild Anemia		Moderate Anemia		FET P
		N140	%	N41	%	N19	%	
Number of Meals consumed/Day	1	0	0.0	2	4.9	3	15.8	42.333 0.000
	2	15	10.7	12	29.3	8	42.1	
	3	106	75.7	27	65.9	8	42.1	
	4	19	13.6	0	0.0	0	0.0	
Milk OR Milk Products / day	Adequate	115	82.1	29	70.7	4	21.1	32.737 0.000
	In adequate	25	17.9	12	29.3	15	78.9	
Cereals / Day OR One Egg / Week	Adequate	138	98.6	40	97.6	18	94.7	1.306 0.521
	In adequate	2	1.4	1	2.4	1	5.3	
Meat OR Poultry OR Fish / Day	Adequate	128	91.4	12	29.3	2	10.5	96.801 0.000
	In adequate	12	8.6	29	70.7	17	89.5	
Fruits OR Vegetables / Day	1 Serving	33	23.9	16	42.1	12	80.0	28.315 0.000
	2 Servings	44	31.9	15	39.5	3	20.0	
	3 Servings	39	28.3	6	15.8	0	0.0	
	4 Servings	22	15.9	1	2.6	0	0.0	
Fluids Intake / Day	<1000	56	40.0	25	61.0	14	73.7	13.363 0.000
	1000-2000	68	48.6	15	36.6	3	15.8	
	>2000	***16***	***11.4***	***1***	***2.4***	***2***	***10.5***	

Table (V): Family history of anemia, previous surgery or hospitalization, periodic check up, previous history of anemia and manifestation of anemia among studied elders

History	Anemia				X^2	P
	No		Yes			
	No	%	No	%		
Previous anemia	2	12.5	14	87.5	37.8	0.000*
Family history of anemia	26	41.9	36	58.1	33.7	0.000*
Previous hospitalization	12	48.0	13	52.0	6.585	0.010*
Previous surgery	6	37.5	10	62.5	8.747	0.003*
Manifestations						
Easy Fatigability (Tired)	3	2.1	32	53.3	FET	0.039*
Palpitation	115	82.1	2	3.3	FET	0.024*
Periodic check up	52	61.2	33	38.8	5.5	0.019*

Table (VI): Nutritional state and physical function of anemic and non anemic elders

Predictor	Anemia				X^2	P
	No		Yes			
	No	%	No	%		
Mini nutritional assessment tool					101.2	0.0008
▪ Malnutrition	0	0.0	7	11.7		
▪ At risk	0	0.0	42	70.0		
▪ Normal nutrition	140	100.0	11	18.3		
International Physical Activity Questionnaire (IPAQ) short form					86.8	0.000*
▪ Inactive	13	9.3	41	68.3		
▪ Minimally active	40	28.6	17	28.3		
▪ HEPA active	87	62.1	2	3.3		
Basic activities of daily living (ADLs)					*FEP*	0.000*
▪ Need help	0	0.0	6	10.0		
▪ Independent	14	100.0	54	90.0		
Instrumental activities of daily living (IADLs)					41.8	0.000*
▪ Need help	1	0.7	18	30.0		
▪ Independent	139	99.3	42	70.0		
Physical Performance Battery (PPB)					114.5	0.000*
▪ Very low	0	0.0	7	11.7		
▪ Low	0	0.0	28	46.7		
▪ Moderate	25	17.9	23	38.3		
▪ High	115	82.1	2	3.3		

Table (VII): The most risk factors affecting anemia in the study

Risk factor	Regression Coefficient	Standard Error	P Value	Odds Ratio	95.0% C.I for OR	
					Lower	Upper
Mini nutritional assessment tool	-2.6	1.0	0.008*	0.11	0.0	0.51
Instrumental activities of daily living (IADLs)	-0.3	0.1	0.025*	0.70	0.5	1.0
Physical function	-2.0	0.6	0.000*	0.15	0.0	0.42
Age	-0.4	0.2	0.009*	0.70	0.5	0.90
Insufficient income	2.5	1.5	0.049*	12.8	1.2	78.9
Constant	75.2	21.3	0.000*			
P value for the model	0.000*					
Classification accuracy	92.8%					

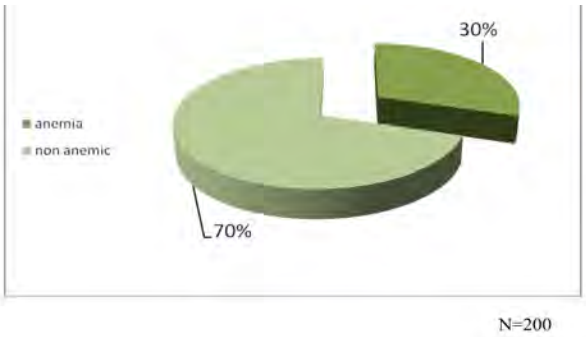

Fig. I: The numbers of the elderly with anemia who attending the geriatric outpatient clinics hospital at mansoura city.

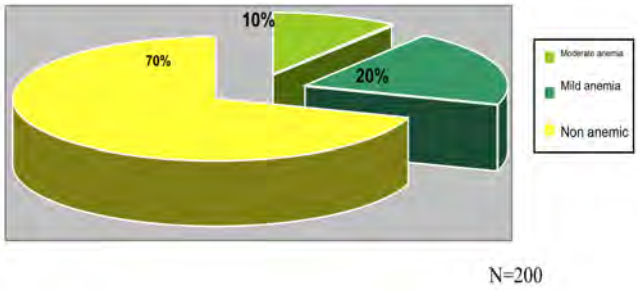

Fig. II: The distribution of anemic elderly according to level of hemoglobin.

Discussion

Anemia is a very common problem and is often overlooked in older persons despite considerable evidence that low hemoglobin levels indicate physiologic decline in these patients. Multiple studies demonstrated that anemia is an independent risk factor for increased morbidity and mortality, and decreased quality of life in community-dwelling older persons [5,27].

The present study revealed that the prevalence rate of anemia in elderly who attended outpatient's clinics was 30% (based on the WHO criteria for defining anemia). This result is slightly higher than the result of another study conducted in geriatric clubs in Egypt by Mortagy et al., (2008)[22], which was 24%.The explanation of this difference may be related to the setting in which the study was conducted, is the site of the study as the first one is a hospital based while the second is community based. In Brazil the prevalence

rate of anemia was 25% in the outpatient clinics and 21% in the community-based cohort[4]. In developed countries this rate is much lower; it was 10.6% in USA [16], 11% in Italy[2,14].

On classifying anemia according to degrees or severity in this study 20.5% of elderly patients had mild anemia (Hb=10-12mg/dl) and 9.5% had moderate anemia (Hb=7-9mg/dl), while no participant had severe anemia (Hb <7mg/dl). The rate of mild anemia is higher than that reported by Tettamanti et al, (2010)[29] in Italy which was11.8%.

The present study revealed that the rate of anemia is significantly increases with age advancing. This result agreed with a study done by Brenda et al, (2004)[9] in USA reported also the same result was given by Gaskell et al, (2008) [15] that is explained by increased association of co-morbidity.

The mean age of anemic elderly in the present study was 67.9±6.6 years (ranged from 60 to 85 years). Terekeci et al (2009)[28] in Turkey demonstrated that the mean age of subjects was 71.5± 5.1 years (range, 65-91).

Considering income, this study revealed that it has a significant effect on anemia as those with insufficient income had 12 times more risk for having anemia than those with sufficient income keeping all other factors constant. As this low income insufficient to bay a funky diets not the high biological value diet. A study done by Bodnar et al, (2002)[7] in USA shows that low socioeconomic state is risk factor for iron deficiency anemia. WHO (2004)[32] estimates that, iron deficiency anemia (IDA) resulted in 273000 deaths with 97% occurring in low- and middle-income countries.

The present study showed that there was a significant difference between both groups as regard positive family history of anemia, it was 41.9% in non anemic and 58.1% in anemic elders (P=0.000). This result may be related to sharing the same diet with family in Oriental and Arab countries.

As regard past history of anemia, it was positive in 87.5% of

anemic compared to 12.5% of non anemic elders in the study. The difference was significant (P= 0.000). This can be attributed to the monotonous starchy diet upon which the poor elders depend and unable to change it. This agree with study done in Egypt by Mortagy et al., (2008)[22] which revealed that there is statistical significance difference between anemia and past history of anemia (P=0.001).

As regard manifestation of anemia the study revealed that there were statistically significant difference between two group as regard easy fatigability and palpitation (P=0.039 and P=0.024) respectively. It agree with a study done by Beghe et al, (2004)[6] in USA demonstrated that anemia is associated with symptoms ranging from weakness and fatigue to increased falls and depression, and in severe cases can lead to congestive heart failure.

Concerning risk behavior and periodic check up this study revealed that there were statistically significant reverse relations between smoking caffeine consumption (p=0.036), adherence to periodic check up (p=0.019) and occurrence of anemia. As 19.3% of non anemic elders were smokers compared to 11.6% smokers in anemic elders (p=0.015). The rate of caffeine consumption among non anemic was 90.7% and it was 80% in anemic elders (p=0.036). On the contrary, a study done by Nelson and Poulter (2004)[23] in UK reported that tea drinking limits the absorption of non haem iron.

The present study revealed that there is a statistical significant inverse relation between dietary habits and anemia regarding number of meals (P=0.000), enough consumption of: milk and milk products (P=0.000), meat, poultry or Fish (P=0.000), and fruits or vegetables (P=0.000). Regarding consumption of cereals the relation was statistically insignificant (P=0.521). This result disagree with the study done by Mortagy et al., (2008)[22] in Egypt which revealed that there is a statistically significance difference between anemia and legume intake with (P-value=0.01).

Concerning medical history,

the present study showed that 43.3% of anemic elders were complaining of three or more chronic illness compared to 4.3% of non anemic (P=0.002). As regard the relation between type of chronic disease and anemia occurrence, this work revealed that the significant effect was detected only in diabetes and hypertension. As 43.3% of anemic elders have diabetes compared to 37.1% of non-anemic (p=0.028). This agrees with a study done by Anand et al, (2005) in USA[1] on 5000 participants and found that 34% of anemic patients were diabetics. Also using of medications by elders in this study revealed significant effect on occurrence of anemia only when using hypoglycemic agents (P=0.024) and antihypertensive drug (P=0.010) There is good evidence that many drugs used to treat diabetes may exacerbate anemia associated with diabetes. Study done by Bolen et al, (2007) [8] in USA revealed that five distinct oral drug classes are now available for the treatment of type 2 diabetes and most of these agents lower hemoglobin levels approximately 1% to 2%. Also 51.7% of anemic elders in this work had hypertension compared to 46.4% of non-anemic (p=0.035). The explanation of this is that erythropoietin and anemia have important interactions with blood pressure control in both health and disease. In patients with essential hypertension, endogenous erythropoietin levels are positively correlated with blood pressure levels and total peripheral resistance, independent of hemoglobin levels. It is conceivable that a reduction in erythropoietin synthesis may act to partly counterbalance blood pressure elevation associated with fluid retention in diabetes and Chronic Kidney Disease[30].

The relation between anemia and previous surgery is evident in this work as 62.5% of those who were anemic compared to 27.2% of non anemic elderly conducted previous surgery and the difference was significant (p=0.003) This is in-agreement with a study done by Jeong et al (2012)[19] in Korea. Also previous hospitalization was positive in 52.0% of those who were anemic compared to 48.0% of non anemic elderly (P=0.010). This is agree with a study done by Riva et al (2009)[26]

in Italy and reported that the risk of hospitalization was higher among the mildly anemic non anemic elderly subjects.

Concerning the relation between anemia and nutritional status of the elders, in this work using Mini Nutritional Assessment Scale revealed that 11.7% of anemic elders were mal-nourished and 70% at risk of malnutrition while all the non anemic were well-nourished (P=0.000) After regression analysis regarding MNA score, the increase in the score by one degree was associated with decreased risk for anemia by about 89% (Odds Ratio=0.11; 95% Confidence Interval: 0.0-0.51).

Increasing functional deterioration is associated with decreasing hemoglobin concentration in an inverse and linear manner. It is important to note, that even low normal hemoglobin levels may be a marker for declining function [10,25].

This study revealed that there were significant relations between anemia and ADLS result's where 10% of anemic elders need help (P=0.000), IADLS result's revealed that 30% of anemic elders need help (P=0.000), Physical Performance Battery (PPB) scale showed that only 3.3% of them had high performance (P=0.000) and for International Physical Activity Questionnaire (IPAQ) scale it appeared that 68.3% were inactive (P=0.000). After regression analysis considering physical function, a 85.0% lower risk for anemia was recorded for increased physical function (Odds Ratio=0.15; 95% Confidence Interval: 0.0-0.42). the increase in IADL score by one more degree was associated with decreased risk for anemia by about 30% (OR=0.70; 95% CI: 0.5-1.0) .

This is agree with another study done by Penninx et al, (2004)[25] in Italy which revealed that anemic persons had poorer performance (8.8 vs. 9.6, P=.003) than persons without anemia. Another study done by Den Elzen et al, (2009)[12] in Canada was similar to the present study which demonstrated that Participants with anemia had a greater increase in disability in basic activities in daily living compared with participants who did not have

anemia during follow-up (difference in annual change in Groningen Activity Restriction Scale score 1.7, 95% CI 0.8 to 2.7, p<0.01).

Conclusion

Based on findings of the present study, it can be concluded that anemia is a common problem among elderly and it's prevalence increase with advancing age. The most important predictor for anemia in the present study was age, income and nutritional habits. Anemia has several adverse consequences in the elderly; it increases the incidence of fatigue, and has a negative impact on cognitive and physical function.

The present study revealed that the increase in IADL score by one more degree was associated with decreased risk for anemia by about 30% (OR=0.70; 95% CI: 0.5-1.0). Considering physical function, a 85.0% lower risk for anemia was recorded for increased physical function (OR=0.15; 95% CI: 0.0-0.42). Functional deterioration is associated with decreasing hemoglobin concentration in an inverse and linear manner. Impaired physical function or disability lead to anemia as the elder person become unable to do shopping, preparing food or even feed himself , this lead to anemia that increases his disability.

Recommendations

Based on the results of this study, the following recommendations are suggested:

• Health education programs to elders about the disease process and the importance of adoption healthy lifestyle.

• Early identifying causes and high risk behaviors conducive to anemia by the caregivers is important in order to motivate elders to modify them. It can be achieved through comprehensive assessment of elders which will provide the nurse with baseline data, help to identify high risk groups, plan and implement appropriate nursing intervention.

• In- service training programs to all nurses and health care providers in hospitals and outpatient clinics to update their knowledge increase their ability to care for elderly patients with anemia, and teach patients to modify their un-

healthy lifestyle behaviors.

• The evaluation of newly diagnosed anemia should not be delayed in patients with a life expectancy of 1 year or longer, because anemia may be the first sign of serious underlying pathology.

• Follow up visits either to the clinic or through home visits is important in order to evaluate the progress of patient's condition and motivate them to adhere with preventive measures.

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EFFECT OF ANTICOAGULANT (WARFARIN) AND L-CARNITINE ON HAEMOSTATIC FUNCTION AND OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Background and Aim of Work: *Diabetes mellitus (DM) is a complex progressive disease characterized by hyperglycemia and a high risk of atherothrombotic disorders affecting the coronary, cerebral and peripheral arterial trees. Oxidative stress is reported in diabetic patients. We investigated the haemostatic functions and oxidative stress in streptozotocin (STZ)-induced diabetic rats and the effects of anticoagulant (warfarin) and L-carnitine on those parameters.*

Materials and Methods: *Forty male Sprague-Dawley rats were divided into four groups; control, DM, DM received warfarin or L-carnitine. In all rats, blood glucose, insulin, haemoglobin A1c (HBA1c), fibrinogen, factor VII (FVII), plasminogen activator inhibitor-1 (PAI-1), fibrin degradation products (FDP), protein C, malondialdehydes (MDA), and anti-oxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione) were measured. Also, prothrombin time (PT), activated partial thromboplastin time (aPTT) and platelet aggregation were evaluated.*

Results: *In STZ-induced diabetic rats, plasma glucose, HBA1c, MDA, fibrinogen, FVII, PAI-1 and platelet aggregation increased while insulin, PT, aPTT, FDP, protein C and anti-oxidants decreased. Warfarin administration to diabetic rats decreased fibrinogen and FVII and increased PT and aPTT with no effect on MDA, anti-oxidants, PAI-1, protein C, FDP and platelet aggregation. On the other hand L-carnitine decreased fibrinogen, FVII, PAI-1, MDA and platelet aggregation and increased PT, aPTT, protein C, FDP and anti-oxidants in diabetic rats.*

Conclusion: *Hyperglycemia plays an important role in hypercoagulation state and oxidative stress in STZ-induced DM. L-carnitine improves oxida-*

tive stress and decreases the hypercoagulation state in DM. On the other hand, warfarin normalize the hypercoagulation state in DM with no effect on oxidative stress.

Key Words: *diabetes mellitus, coagulation, L-carnitine, warfarin, oxidative stress.*

Introduction

According to the International Diabetes Federation, percent of diabetes in Egypt was 11.4% in the year 2010 and this likely to increase to 13.7 % by the year 2030 [1]. Diabetes mellitus (DM) is a complex progressive disease, which is accompanied by multiple complications. It has been recognized as the sole independent risk factor for the development of cardiovascular disease[2]. Administration of streptozotocin (STZ) causes pancreatic beta cell destruction that leads to the development of hyperglycemia, dyslipidemia and renal dysfunction in rats[3]. The STZ animal model develops characteristic symptoms of diabetes such as hyperglycemia, hyperlipidemia and increased water and food intake without body weight gain. Reactive oxygen species (ROS), which cause cellular damage by the oxidation ability, have been implicated in the pathogenesis of diabetes mellitus[4]. During diabetes, persistent hyperglycemia increases the production of ROS through glucose autoxidation[5]. In addition, oxidative stress in diabetes mellitus results from reduction in capacities of the antioxidant defense system including scavenging enzymes such as superoxide dismutase and glutathione reductase, and deficiencies of antioxidants such as vitamin C and E[6]. The oxidative stress has also been associated with diabetic states in animals and humans[7]. A study using STZ-induced diabetic rats showed that levels of lipid peroxidation had increased, as indicated by thiobarbituric acid reactive substances (TBARS), which is one of oxidative stress markers suggesting the occurrence of oxidative stress[8]. Moreover, diabetic patients have significant defects in antioxidant defense elements, and enhanced ROS generation is one of the major determinants of diabetic complications[9]. Administration of antioxidants as vitamins C and E has been reported

to reduce the complications in DM by arresting free radical damage [10].

Besides hyperglycemia, diabetic patients also suffer from dyslipidemia[11], which can lead to increased atherogenesis and heart disease[12]. In addition, clinical and epidemiological observations have led to the concept of a procoagulant state in type 2 diabetes. Thrombophilia in diabetic patients is a well recognized phenomenon which contributes an additional risk of coronary heart disease (CHD). In a series of 1980 type 2 diabetic patients, both male and female diabetic patients showed significantly shorter activated partial thromboplastin time (aPTT) [13]. Acang and Jalil[14] have reported significantly high fibrinogen and short prothrombin time (PT) and aPTT in diabetic patients, especially those who suffered from diabetes for a long time. Increased concentration of fibrinogen in uncontrolled non insulin dependent diabetes mellitus (NIDDM) patients is implicated in vascular damage induction[15]. Yurekli et al [16] have concluded a subtle activation of extrinsic pathway with a concomitant decrement in intrinsic pathway of the coagulation cascade in type 2 diabetes. In addition, Bae et al[17] observed that PT and aPTT did not differ significantly between diabetic patients and controls; however a significant decrease was observed in fibrinogen levels in diabetic patients. Short aPTT and PT have been associated with increased risk for thromboembolism in renal transplant patients[18]. In a series of 35 type 1 diabetic patients, there was no significant difference in coagulation parameters and none of the patients developed thrombosis or vascular disease in a two year follow up[19].

L-carnitine is a naturally occurring compound and an antiradical widely distributed in the body, and decreases lipid peroxidation [20]. L-carnitine prevents TBARS formation and increases the antioxidants in aged rats' brain[20]. Although these previous studies suggest that L-carnitine possesses antioxidative activity, it remains unknown whether this compound is able to affect coagulation associated factors. It is hypothesized that L-carnitine could attenuate

diabetes associated haemostatic disorder, at least via diminishing oxidative stress. In addition, many physiologic and pathologic conditions affect blood, tissue and urinary concentrations of carnitine in both animal and human subjects. In acute or chronic STZ-induced diabetic rats, the pancreatic content of carnitine was found to be significantly lower than in nondiabetic control rats[21]. Furthermore, studies on the antioxidant effect of carnitine on diabetes mellitus are few. Therefore, we investigated the effect of L-carnitine on the diabetogenic action of STZ in rats.

Warfarin (Coumadin) is an anticoagulant normally used in the prevention of thrombosis and thromboembolism[22]. Warfarin inhibits the vitamin K-dependent synthesis of biologically active forms of the calcium-dependent clotting factors II, VII, IX and X, as well as the regulatory factors protein C, protein S, and protein Z [23]. As there are few studies considering the anticoagulant effect of warfarin and L-carnitine on diabetes mellitus, therefore, the aim of the present work was to investigate the haemostatic functions and oxidative stress in streptozotocin (STZ)-induced diabetic rats, a well-characterized animal model of type 1 diabetes, and the effects of anticoagulant (warfarin) and L-carnitine on those parameters.

Materials and Methods

Chemicals: STZ (streptozotocin), warfarin [4-Hydroxy-3-(3-oxo-1-phenylbutyl)coumarin] and L-carnitine were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Animals: Forty male Sprague Dawley rats, weighing 220-240 g, were used in the present study. They were purchased from Vaccine and Immunization Authority (Helwan, Cairo, Egypt) and housed (Animal House, Medical Physiology department, Faculty of Medicine, Mansoura University, Egypt) under controlled conditions (temperature 23±1°C, and a 12:12 light/dark cycle).The animals were allowed free access to food and tap water. All animal procedures were performed in accordance with protocols approved by the Medical Research Ethics Committee of Man-

soura University, Egypt and in compliance with standards and regulations for the care and use of laboratory rats set by the National Institutes of Health.

Experimental design:

The animals were randomly divided into four groups of ten rats each. First group (GI) consisted of untreated control (normal) animals. The second group (GII) served as untreated diabetic group. The third group (GIII) included the diabetic rats treated with warfarin. The forth group (GIV) included the diabetic rats treated with L-carnitine. Diabetes was induced in rats of second, third and forth groups by single intra-peritoneal (i.p) injection of 70 mg\kg streptozotocin[24] (dissolved in 0.1 M sodium-citrate buffer, pH 4.5). The control rats received an equivalent amount of the sodium-citrate buffer. In order to confirm diabetes, three days after streptozotocin injection, blood glucose was measured using glucometer instrument (Accu-check-active, ROCHE, Germany) and animals with blood glucose over 200 mg\ dL were considered as diabetics [25]. The rats with warfarin treatment (GIII) were administered 0.25 mg/liter warfarin potassium (26) in drinking water from the day of STZ injection for 21 consecutive days. Because diabetic rats drink much more water, the dosage of warfarin was reduced to 0.06 mg/liter[26] from 2 days after injection of STZ. Group IV rats were injected intraperitoneally with L-carnitine a dose of 500 mg/kg/day for 21 consecutive days after STZ injection[27].

Sampling protocol:

Blood samples: At the end of experimental period, blood samples were collected by cardiac puncture into tubes containing EDTA, mixed well and divided into two tubes: the first one was used for determination of hemoglobin A1c (HBA1c), and the second tube was centrifuged at 3000 r/min (1000g) for 10 min to obtain plasma samples which were used for measurement of glucose, insulin, malondialdehyde (MDA), and antioxidants (reduced glutathione, glutathione peroxidase, superoxide dismutase, and catalase) levels.

Biochemical investigations:

Plasma glucose was quantitat-

ed by glucose oxidase-peroxidase method using the kit supplied by SPINREACT, Spain (Ref:1001190). Plasma insulin was determined using Ultra Sensitive Rat Insulin ELISA Kit (Cat.No. 90060, Crystal Chem INC, Spain) following manufacturer's instructions. In addition, hemoglobin A1c (HBA1c) was measured by a commercial kit (Crystal Chem's rat HbA1c Kit, Catalog. No. 80300).

Malondialdehyde (MDA) was analyzed by measuring the production of thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust[28] using TBARS assay kit (Cat. No. 10009055, Cayman, USA). Reduced glutathione (GSH) (Cayman kit, Cayman, USA, Cat. No. 703002), glutathione peroxidase (GSH-Px) (Northwest Life Science Specialties [NWLSS^{TM}] kit, Canada, Cat. No. NWK-GPX01), superoxide dismutase (SOD) (Cayman kit, Cayman, USA, Cat. No. 706002), and catalase (CAT) (Northwest Life Science Specialties [NWLSS^{TM}] kit, Canada, Cat. No. NWK-CAT01) were measured according to the manufacturer's instructions.

Haemostatic functions:

Assessment of prothrombin time (PT) and activated partial thromboplastin time (aPTT):

PT and APTT were measured using calcium rabbit brain thromboplastin and kaolin platelet substitute techniques (DIAGEN DIAGNOSTIC REAGENT LTD, OXON, U.K). Briefly, PT was assayed with two hundred microlitre of calcium rabbit brain thromboplastin reagent placed in a clotting tube and incubated in a water bath at 37°C for 2 minutes. Hundred microlitre of plasma is then added and a stop watch started. The tube is gently tilted at regular interval and the watch was stopped when the clot formation was observed. For aPTT, two hundred microlitre of kaolin platelet substitute mixture was placed in a clotting tube and incubated in a water bath at 37°C for 2 minutes. One hundred microliter of plasma was then added and the tube was gently tilted at interval for exactly two minutes. One hundred microliter of calcium chloride (pre- incubated at 37°C) was then added and a stop watch started. The tube was tilted at intervals and the time for clot formation was recorded.

Measurement of coagulation and anticoagulation factors:

Coagulation factors, FI (fibrinogen) and FVII; anticoagulation factors, AT-III (antithrombin III) and protein C; plasminogen activator inhibitor-1 (PAI-1) and fibrin degradation products (FDP) were measured. Blood samples were anticoagulated using sodium citrate according to the protocols provided by the manufacturers. Fibrinogen level was measured using TEClot FIB kit (TECO, GmbH, Germany). In addition, FVII activity was determined by a commercial kit (CoasetQR FVII, Chromogenix, Lexington, Mass., U.S.A.). The activity of AT-III and protein C in plasma was measured by commercial AT-III and protein C kits (Sigma Chemical Co.), respectively. The activity of AT-III and protein C was expressed as a percentage related to the activity of standard plasma. PAI-1 activity (pg/ml) was assayed by a commercial kit (Trinity Biotech plc., Bray, C. Wicklow, Ireland). Fibrin degradation products (FDP) is measured by Cusabio rat FDP ELISA assay kit (Catalog No. CSB-E07942r).

Assessment of Platelet Aggregation:

Platelet rich plasma (PRP) preparation: To obtain PRP, the blood was centrifuged at about 250 g at 4°c for 10 min. Platelet count was made by coulter LH 750 analyzer, (Beckman Coulter Inc., USA) for PRP and for whole blood.

Platelet Aggregation: The platelet aggregation profile was evaluated by the method of Born [29] by measuring turbidity with a Chronolog optical aggregometer (AGGRO/LINK® Model 810-CA software) using ADP at a concentration of 5 and 10 µmol/l as an agonist. The results were expressed as percentage of aggregation.

Statistical analysis: The data were expressed as mean ± standard deviation of mean (Mean±SDM). Data were processed and analyzed using the Statistical Package of Social Science version 10.0 (SPSS, version 10.0). One way ANOVA was done followed by Tukey's post hoc test. A minimum level of significance is considered if P is ≤0.05.

Results

Table 1 shows the results of insulin, glucose, and hemoglobin

A1c (HbA1c). Plasma glucose and HbA1c levels significantly increased while insulin decreased in STZ-induced diabetic rats as compared to the control group ($p<0.05$). Moreover, diabetic rats receiving warfarin or L-carnitine showed no changes in FBG, HbA1c or plasma insulin levels (Table 1).

The changes in platelet count, fibrinogen, plasminogen activator inhibitor 1 (PAI-1) and fibrin degradation products (FDP) concentrations, and prothrombin time (PT) and activated partial thromboplastin time (aPTT) are shown in Table (2). Streptozocin-induced diabetic rats showed a significant increase in fibrinogen and PAI-1 and decrease in FDP, PT and aPTT in comparison with control rats ($p<0.05$). No significant change in platelet count in the whole blood or PRP was observed in STZ-induced diabetic rats as compared to control group ($p>0.05$). Warfarin administration to diabetic rats significantly increased PT and aPTT ($p<0.05$) and decreased fibrinogen concentration ($p<0.05$) with no significant effect on FDP, PAI-1 and platelet count ($p>0.05$) compared with untreated diabetic rats. Fibrinogen concentration, PT and aPTT in diabetic rats treated with warfarin showed a normal values similar to those of control group ($p>0.05$). On the other hand, L-carnitine significantly increased FDP, PT and aPTT ($p<0.05$) and decreased fibrinogen concentration and PAI-1 activity ($p<0.05$) in diabetic group compared with untreated diabetic rats, but their values were significantly different from those of control and warfarin treated diabetic groups ($p<0.05$). In addition, no significant effect on platelet count was detected in diabetic rats with L-carnitine treatment ($p>0.05$) (Table 2).

The changes in factor VII, ATIII and protein C are demonstrated in figures 1, 2 and 3 respectively. While ATIII, protein C significantly decreased in STZ-induced diabetic rats when compared with control group ($p<0.05$), factor VII increased. Warfarin significantly decreased factor VII (Figure 1) ($p<0.05$) but had no effect on ATIII (Figure 2) and protein C (Figure 3) ($p>0.05$), in diabetic rats. On the other hand, L-carnitine significantly decreased factor VII (Figure 1) and increased ATIII (Figure 2)

and protein C (Figure 3) activities in diabetic rats but their values were significantly different from those of control and warfarin treated diabetic groups ($p<0.05$).

Table 3 shows the effects of warfarin and L-carnitine on ADP-induced platelet aggregation in control and STZ-induced diabetic rats. ADP-induced platelet aggregation was significantly increased in STZ-induced diabetic rats when compared with the control group ($p<0.05$). Warfarin had no significant effect on ADP-induced platelet aggregation in diabetic rats ($p>0.05$). On the other hand, L-carnitine significantly decreased ADP-induced platelet aggregation in diabetic rats when compared with control and warfarin treated diabetic groups ($p<0.05$).

The changes in GSH-Px, CAT, and SOD activities and MDA and GSH levels in the plasma of control and STZ-induced diabetic rats are shown in Tables 4. GSH-Px, CAT, and SOD activities and MDA and GSH levels in the plasma were affected by diabetes mellitus. While GSH-Px, CAT, and SOD activities and GSH levels in the plasma were significantly decreased ($p<0.05$) in the STZ-induced diabetic group compared with the control group, MDA levels increased ($p < 0.05$). L-carnitine significantly increased GSH levels and GSH-Px, CAT, and SOD activities and decreased MDA levels in the plasma of diabetic rats compared with the control group ($p<0.05$). On the other hand, warfarin had no significant effect on GSH-Px, CAT, and SOD activities and MDA and GSH levels in the plasma of diabetic rats ($p>0.05$).

Table (1): Effect of warfarin and L-carnitine on plasma glucose, insulin and hemoglobin A1c (HBA1c) levels in control and streptozotocin (STZ)-induced diabetic rats.

	Control rats (n=10)	Diabetic rats (STZ) (n=10)	Diabetic rats (STZ)+warfarin (n=10)	Diabetic rats (STZ)+L-carnitine (n=10)
Fasting blood glucose (mg/dl)	141±6	389±21[a]	403±22[a]	396±24[a]
Insulin (nM)	0.29±0.03	0.12±0.005[a]	0.11+0.004[a]	0.13+0.004[a]
HbA1c (%)	4.8±0.1	13.1±0.5[a]	13.7±0.3[a]	13.6±0.2[a]

The values were expressed as mean ± SDM
a: significant ($p<0.05$) compared with control group
b: significant ($p<0.05$) compared with diabetic group
c: significant ($p<0.05$) compared with diabetes+warfarin group

Table (2): Effect of warfarin and L-carnitine on haemostatic functions in control and streptozotocin (STZ)-induced diabetic rats.

	Control rats (n=10)	Diabetic rats (STZ) (n=10)	Diabetic rats (STZ)+warfarin (n=10)	Diabetic rats (STZ)+L-carnitine (n=10)
Platelet count ($x10^3$/ul) in whole blood	770±61	852±56	863±51	856±62
Platelet count ($x10^3$/ul) in PRP	995±65	1044±61	1059±64	1053±55
Fibrinogen (mg/dl)	181.6±7.5	232.4±8.2a	186.6±7.1b	210.4±6.5abc
PT (sec)	15.6±0.3	10.1±0.4a	15.3±0.2b	13.3±0.2abc
aPTT (sec)	20.7± 0.3	14.6 ± 0.5a	20.1 ± 0.4b	16.7 ± 0.3abc
PAI-1 (pg/ml)	0.85±0.02	1.33±0.04a	1.29±0.03a	1.11±0.03abc
FDP (ng/ml)	15.6±1.2	8.2±0.5a	8.9±0.7a	11.6±0.6abc

The values were expressed as mean ± SDM
a: significant ($p<0.05$) compared with control group
b: significant ($p<0.05$) compared with diabetic group
c: significant ($p<0.05$) compared with diabetes+warfarin group

Table (3): Effects of warfarin and L-carnitine on ADP-induced platelet aggregation in control and STZ-induced diabetic rats.

groups	agonist	
	ADP 5 uM	ADP 10 uM
Control	31.4 ± 3.1	39.8 ± 3.7
DM	55.8 ± 3.5a	65.0 ± 4.4a
DM+warfarin	58.5 ± 3.7a	69.0 ± 4.1a
DM+L-carnitine	40.8 ± 2.8abc	49.8 ± 3.1abc

The results are expressed as percentage of aggregation (mean ± SD). DM: diabetes mellitus.
a: significant ($p<0.05$) compared with control group
b: significant ($p<0.05$) compared with diabetic (DM) group
c: significant ($p<0.05$) compared with DM+warfarin group

Table (4): Effects of warfarin and L-carnitine on oxidative stress parameters in control and STZ-induced diabetic rats.

	Control rats (n=10)	Diabetic rats (STZ) (n=10)	Diabetic rats (STZ)+warfarin (n=10)	Diabetic rats (STZ)+L-carnitine (n=10)
MDA (nmol/ml)	3.55±0.32	5.94±0.31a	5.88±0.28a	4.11±0.35bc
GSH (mol/l)	91.5±5.6	30.7±6.4 a	33.8±6.8 a	87.3±6.1 bc
GSH-Px (mol/ml)	0.42±0.05	0.21±0.07a	0.22±0.06a	0.4±0.03bc
SOD (U/ml)	22.5±1.5	10.5±1.3a	11.7±1.6a	20.2±1.7bc
CAT(mmol H_2O_2 decomposed/ml)	6.3±0.2	2.9±0.1a	2.8±0.1a	5.9±0.3bc

The values were expressed as mean ± SDM
a: significant ($p<0.05$) compared with control group
b: significant ($p<0.05$) compared with diabetic group
c: significant ($p<0.05$) compared with diabetes+warfarin group

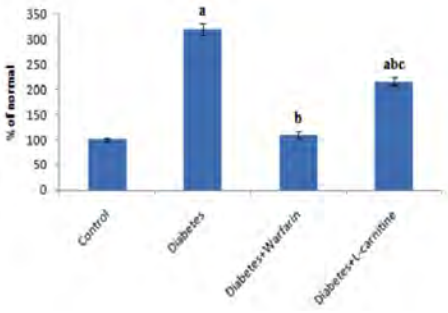

Fig. (1): Factor VII (% of normal). DM: diabetes mellitus.
a: significant (p<0.05) compared with control group
b: significant (p<0.05) compared with diabetic group
c: significant (p<0.05) compared with diabetes+warfarin group

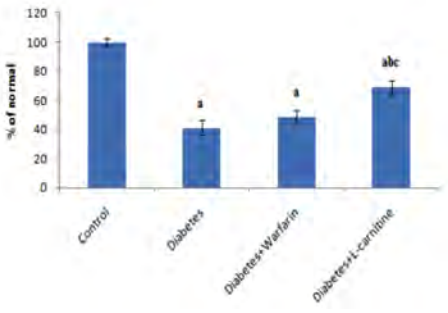

Fig. (2): Antithrombin III (ATIII) (% of normal). DM: diabetes mellitus.
a: significant (p<0.05) compared with control group
b: significant (p<0.05) compared with diabetic group
c: significant (p<0.05) compared with diabetes +warfarin group

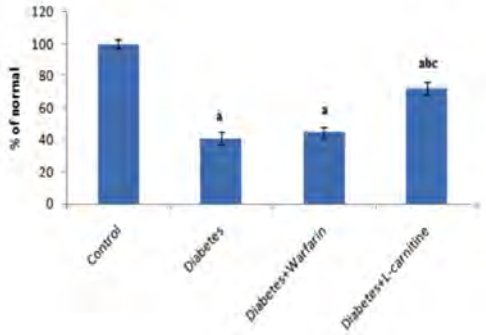

Fig. (1): Protein C (% of normal). DM: diabetes mellitus.
a: significant (p<0.05) compared with control group
b: significant (p<0.05) compared with diabetic group
c: significant (p<0.05) compared with diabetes+warfarin group

Discussion

The pathological characteristics of diabetes include hyperglycemia, cytokine imbalance, and coagulation predomination[30]. Coagulatory disorder often occurs in diabetic patients with poor glycemic control[31]. Some of the established effects of hyperglycemia on oxidative stress, endothelial cell dysfunction, extracellular matrix formation and apoptosis are considered to play an important role in diabetes-related vascular damage. Hyperglycemia per se has been implicated in the develop-

ment of prothrombotic changes in clamp studies where under conditions of euinsulinaemic hyperglycemia, twofold increases in thrombin–antithrombin complexes and circulating soluble tissue factor were documented[32]. Interestingly, hyperinsulinemia in the presence of euglycemia led to increases in PAI-1, and the authors made the observation that glucose modulated thrombotic processes whilst insulin regulated fibrinolysis[32]. In addition, non-enzymatic glycation is capable of altering the functionality of a molecule. This mechanism has been demonstrated in vitro for ATIII[33]. Glucose probably occupies the lysine residue which binds the ATIII to its natural co-factor, heparin, making the molecule less active[33]. The glycation mechanism seems to intervene at other levels. Fibrin may be glycated, thus becoming less sensitive to the processes of fibrinolysis [34]. Non-enzymatic glycation may also involve the platelet membrane proteins, reducing their fluidity and making them more sensitive to the thrombin pro-aggregant stimulus[35]. Furthermore, the advanced end-products of glycation, may also be implicated in diabetic thrombophilia. It has been demonstrated in vitro that cultured endothelial cells exposed to these products express a greater procoagulant activity[36]. Finally, in vitro, glycated albumin has been reported to increase tissue factor expression in both monocytes [37] and umbilical vein endothelial cells[38] to indicate a mechanism by which glycation might initiate coagulation processes. In the present study, our results showed a significant increase in fasting plasma glucose, HBA1c (Table 1), FVII (Figure 1), fibrinogen and PAI-1 (Table 2) and decreased insulin, FDP (Table 1), ATIII (Figure 2) and protein C (Figure 3) levels in STZ-induced diabetic rats suggesting the development of a hypercoagulability state. Clinical studies revealed that the increase of coagulation factors such as factor VII (FVII), and decrease of anticoagulation factors such as antithrombin-III (AT-III) in circulation of diabetic patients led to hypercoagulability, which promoted the development of diabetic complications, especially vascular diseases [39]. Lemkes et al.[40] indicated that enhanced oxidative stress

from hyperglycemia disturbed the balance between coagulation and anticoagulation, which facilitated the occurrence of arterial and venous thrombotic events. It is reported that excessive production of reactive oxygen species (ROS), acting as signaling molecules in thrombogenic cycle, favors the progression of thrombosis[41]. In addition, on the basis of oxidative stress, ATIII is rapidly inactivated during induced hyperglycemia [42] due to the release of free radicals. Furthermore, reduced levels of vitamin E are reported in the platelets of diabetic subjects, accompanied by hyperaggregation[43]. The results of the current work showed a significant increase in MDA with decreased anti-oxidants (GSH, GSH-Px, SOD, and CAT) in plasma of STZ-induced diabetic rats suggesting the development of oxidative stress (Table 4). Another possible mechanism for the development of thrombophilia in diabetes is linked with the reduced synthesis of heparan sulphate [44]. Thus, hemostatic imbalance warrants attention to avoid diabetic vascular diseases. Therefore, any agent with anticoagulatory activity may potentially prevent or delay the development of diabetic hemostatic disorders.

Animal models of diabetes mellitus can be produced by use of chemicals such as streptozotocin. Streptozotocin is an agent widely employed to induce experimental diabetes due to its ability to selectively targets and destroys insulin producing pancreatic islet β-cells [45]. It has a diabetogenic action as it causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP ribose) synthetase and thus depletes the intracellular NAD and NADP levels. NAD depletion by STZ inhibits proinsulin synthesis and thus induces diabetes[46]. In the present study, administration of STZ caused a significant increase in plasma glucose and HBA1c and decrease in insulin levels (Table 1), therefore inducing experimental type 1 diabetes mellitus. On the other hand, reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen, have been implicated to play important roles in diabetes. Also in the case of diabetic models induced by STZ, ROS were pro-

posed to be formed and involved in the death of β-cells[47]. Superoxide radicals are converted to hydrogen peroxide by superoxide dismutase (SOD) and hydrogen peroxide is converted to water by glutathione peroxidase (GPx) and/or catalase[48]. Hydrogen peroxide can react with transition metals in Fenton reactions, thereby being converted into hydroxyl radicals. Hydrogen peroxide can cross all membranes and lead to hydroxyl radical formation at more distant sites. The hydroxyl radical can cause lipid peroxidation, which in turn leads to damage of cellular organels and membranes, thus causing cell death[48]. With respect to oxidative stress, an increased free radical generation was reported in diabetic plasma and tissues. In diabetes, oxidative stress seems mainly due to both increased free radical concentrations and sharp reduction of antioxidative defenses[8]. In consistent with these studies, the current work showed a significant increase in MDA level and decrease in GSH level and GSH-Px, CAT and SOD activities (Table 4) in STZ-induced diabetic rats indicating the development of oxidative stress.

In the laboratory, measurement of PT, APTT, and fibrinogen concentration are the most commonly employed laboratory tests in patients with a suspected coagulopathy[49]. Prothrombin time is a laboratory screening test used to detect disorders involving the activity of the factors I, II, V, VII, and X of the extrinsic and common pathways[50]. Activated partial thromboplastin time is used to screen for abnormalities of the intrinsic and common clotting systems and to monitor the anticoagulant effect of circulating heparin. It measures the activities of factors I, II, V, VIII, IX–XI, and XII of the intrinsic and common pathways[51]. Changes in these proteins favour the development of hypercoagulable and prothrombotic state, which may in turn enhance cardiovascular risk by increasing the possibility of developing an occlusive thrombus within a coronary/cerebral artery [52]. PT and APTT can therefore be used to assess the risk of clotting complications in patients with diabetes mellitus although modern coagulation diagnostic test are becoming more sophisticated, PT and APTT are still important basic

examinations in clinical laboratories[53]. In the current study, PT and aPTT were evaluated in control and STZ-induced diabetic rats. Prothrombin time and aPTT were significantly decreased in STZ-induced diabetic rats in comparison with control group (Table 2) suggesting the development of prothrombotic state in diabetic rats. In addition, administration of L-carnitine to STZ-induced diabetic rats significantly increased PT and aPTT when compared with control rats but their values were significantly different from those of control and warfarin treated diabetic groups (Table 2). On the other hand, warfarin , a coumarin derivative,normalized the values of PT and aPTT (Table 2) in STZ-induced diabetic rats.

Plasminogen activator inhibitor-1 (PAI-1) is the primary physiologic inhibitor of fibrinolysis[54]. Moreover, FVII is the first enzyme in blood coagulation system for triggering the clotting cascade[55]. Epidemiological studies have reported that high levels of factor VII are associated with a high mortality rate for cardiovascular events [56]. In addition, fibrinogen, the most interesting coagulation factor, is recognized as having an important predictive value as a marker of cardiovascular risk[57]. Thus, the elevated FVII (Figure 1) and PAI-1 (Table 2) activities and fibrinogen level (Table 2) as we observed in STZ-induced diabetic rats reflected a disruption in the balance of factors regulating coagulation and fibrinolysis, and obviously promoted the progression of thrombosis. However, we found that L-carnitine administration lowered FVII (Figure 1) and PAI-1 (Table 2) activities and fibrinogen level (Table 2) level which contributed to attenuation of coagulatory disorder. In addition, warfarin decreased fibrinogen (Table 2) level and FVII (Figure 1) activity to normal control value with no effect on PAI-1 (Table 2), thus reducing the risk of developing a thrombus. Warfarin produces an anticoagulant effect by inhibiting the vitamin K-dependent synthesis of biologically active forms of the calcium-dependent clotting factors II, VII, IX and X, as well as the regulatory factors protein C, protein S, and protein Z.[23]. The precursors of these factors require carboxylation of their glutamic

acid residues to -y-carboxyglutamates (Gla) on the N-terminal regions to allow the coagulation factors to bind to phospholipid surfaces inside blood vessels on the vascular endothelium[58]. The enzyme that carries out the carboxylation of glutamic acid is gamma-glutamyl carboxylase. The carboxylation reaction will proceed only if the carboxylase enzyme is able to convert a reduced form of vitamin K (vitamin K hydroquinone) to vitamin K epoxide at the same time. The vitamin K epoxide is in turn recycled back to vitamin K and vitamin K hydroquinone by another enzyme, the vitamin K epoxide reductase (VKOR). Warfarin inhibits epoxide reductase (specifically the VKORC1 subunit), thereby diminishing available vitamin K and vitamin K hydroquinone in the tissues, which inhibits the carboxylation activity of the glutamyl carboxylase[59]. When this occurs, the coagulation factors are no longer carboxylated at certain glutamic acid residues and are incapable of binding to the endothelial surface of blood vessels, and are thus biologically inactive. As the body's stores of previously produced active factors degrade and are replaced by inactive factors, the anticoagulation effect becomes apparent. The coagulation factors are produced, but have decreased functionality due to undercarboxylation[60].

The pathogenesis of the vascular complications in diabetes is complex and has several potential contributors including alterations in platelet morphology and function[61]. In fact, growing evidence suggests that platelets of diabetic patients are larger and hyperreactive, and consequently present deregulation of several signaling pathways leading to an increase of adhesion, activation, and aggregation[61]. Moreover, it has been demonstrated that the platelets of these patients are more prone to spontaneous aggregation and are highly hypersensitive to agonists such as thrombin, collagen, and ADP[62]. Extracellular nucleotides such as ATP, ADP, and their nucleoside derivative, adenosine, have become clearly recognized for their important role in modulating processes linked to vascular inflammation and thrombosis[63]. In the vascular system,

the nucleotide ADP acts upon platelets promoting their aggregation and modifying their shape [64], while ATP has been postulated to be a competitive inhibitor of ADP-induced platelet aggregation [65]. In support for these observations, the results of the present study showed that ADP-induced platelet aggregation was significantly increased in STZ-induced diabetic rats as compared to control rats (Table 3). In addition, adenosine, produced by the nucleotide catabolism, is recognized as an important modulator of vascular tone and a potent inhibitor of platelet aggregation[66]. Schmatz et al[67] reported that despite the increase of ATP, ADP, and AMP hydrolysis contributing to an increase of adenosine production in STZ-induced diabetic rats, an elevation of the adenosine deaminase (ADA) activity was found in the platelets of those diabetic rats. Adenosine deaminase (ADA) is an important enzyme that degrades adenosine into inosine, tightly regulating local extracellular concentrations of adenosine[68]. Therefore, a rise in the activity of this enzyme may lead to increased adenosine deamination, causing a reduction of the levels of this nucleoside in the circulation. Consequently, this situation may produce a favorable scenario for the development of vascular diseases in diabetic state, since adenosine has an important role in the prevention of platelet aggregation and atherothrombotic complications [66].

On the other hand, there is accumulating evidence that the hyperglycemia contributes to greater reactivity and aggregability of platelets, particularly through the generation of reactive oxygen species (ROS) and by the glycation of platelet membrane proteins[61]. In this study, we found an increase in the glucose levels in STZ-induced diabetic rats, accompanied by an elevation in the glycated hemoglobin levels (Table 1), which may be an indicative that the platelet proteins and vascular wall protein can also be suffering non-enzymatic glycosylation. In addition, our results revealed increased MDA level and decreased GSH level, GSH-Px, CAT and SOD activities in plasma of STZ-induced diabetic rats indicating the development of oxidative

stress in those rats (Table 4).

In fact, it has been found that polyphenols can inhibit the glycation and autoxidation of glucose, preventing the initiation and propagation of protein modification[69]. Furthermore, it is well known that the main polyphenols present in red wine (RW) and grape juice (GJ) are powerful antioxidants, protecting many tissues and cells, including platelets, of damages caused by oxidative stress[69,70]. In addition, it has been shown that the resveratrol exerted a potent antioxidant effect on the generation of different ROS in activated platelets[70]. Based on these findings, the authors suggest that the strong antioxidant properties of RW and GJ may contribute to the prevention of an increase in the platelet aggregation found in diabetic rats treated with both beverages derived from grape. In agreement with these findings, the results of the current work showed that administration of L-carnitine, an anti-oxidant that decreased MDA and increased GSH level, GSH-Px, CAT and SOD activities in plasma of STZ-induced diabetic rats (Table 4), caused a significant decrease in ADP-induced platelet aggregation in the diabetic group (Table 3). On the other hand, warfarin treatment of diabetic rats caused no change in ADP-induced platelet aggregation (Table 3).

Hyperglycemia has been accepted as an essential factor in the development of diabetic complications[71]. Oxidative stress plays an important role in the development of diabetes complications, both microvascular and cardiovascular[72]. It is known that hyperglycemia induced oxidative stress causes endothelial dysfunction, and enhances the release of PAI-1 and vWF[73]. vWF is involved in platelet adhesion and aggregation[73]. Furthermore, ROS could stimulate platelet hyperactivity, and facilitate coagulation [74]. In agreement with these studies, ADP-induced platelet aggregation (Table 3), and PAI-1 (table 2) significantly increased in STZ-induced diabetic rats. Thus, lowering ROS production could benefit hemostatic balance. The antioxidative property of L-carnitine under diabetic condition has been observed[75], and those research-

ers reported that this compound could decrease ROS generation and improve redox imbalance. In accord with these studies, the results of the current work revealed that the intake of this compound effectively reduced MDA as well as increased GSH level and GSH-Px, SOD and CAT activities in the plasma (Table 4). These findings agreed that L-carnitine via its antioxidative activity alleviated redox disorder in circulation of diabetic rats. Thus, it is possible that L-carnitine diminished oxidative stress, which consequently reversed the imbalance of hemostatic factors, and abated hypercoagulatory risk in the diabetic rats. In support for this suggestion, the present work showed that L-carnitine significantly decreased ADP-induced platelet aggregation (Table 3), fibrinogen level and PAI-1 (Table 2) and FVII (Figure 1) activities in STZ-induced diabetic rats. In addition, AT-III and protein C are anticoagulation factors because AT-III inhibits the activity of several proteases in the coagulation cascade, and protein C inactivates coagulation factors such as FVII[39]. The decreased biological activity of ATIII, due to hyperglycemia, results in a reduced thrombin-antithrombin complex formation with consequent hyperactivity of the thrombin[42]. A decrease in protein C has been reported in diabetes[76]. This decrease is directly caused by hyperglycemia, and affects the entire molecule, involving both the biological activity of the protein and its antigenic concentration [76]. Our results showed a significant decrease in ATIII (Figure 2) and protein C (Figure 3) in STZ-induced diabetic rats when compared with control rats. In addition, the current work revealed that L-carnitine treatment of diabetic rats elevated AT-III (Figure 2) and protein C (Figure 3) activities. Apparently, the anticoagulatory effects of this agent could be partially ascribed to enhancement of thrombolysis. These findings suggested that L-carnitine was able to reduce the risk of diabetes associated thrombosis.

A considerable body of evidence indicates that the generation of ROS may mediate the cytotoxic effect of streptozotocin on pancreatic β-cell. An increase of ROS production has also been pointed out

as a main instrument of destruction in streptozotocin-damaged pancreatic islets[47]. In agreement with these studies, the current work revealed that streptozotocin causes an increase of MDA level and decreased the anti-oxidants (GSH level, and GSH-Px, SOD and CAT activities) (Table 4). Diabetes-related increase in lipid peroxidation product (MDA) might be the reflection of the decrease in enzymatic and non- enzymatic antioxidant protection. L-carnitine administration caused a decrease in lipid peroxidation (MDA) (Table 4) in STZ-induced diabetic rats. This may be due to its active role in the transport of fatty acids for energy production, thereby lowering the availability of lipids for peroxidation[77]. In addition, the production of highly reactive oxygen species such as O2•–, H2O2 and OH• are also catalyzed by free iron through Haber-Weiss reaction [78]. Carnitine and its esters have been shown to partially inhibit iron-induced lipid peroxidation in liposomes[79] by forming complexes with free iron. Thus, the reduction in lipid peroxidation in the present study is due to the iron-chelating property of L-carnitine. In addition, the anti-oxidants (GSH level, and GSH-Px, SOD and CAT activities) (Table 4) were increased in STZ-induced diabetic rats with L-carnitine administration which could be the result of decreased ROS. These results indicate the important role of L-carnitine in the improvement of oxidative stress in STZ-induced diabetic rats. On the other hand, no change in lipid peroxidation and anti-oxidants (GSH level, and GSH-Px, SOD and CAT activities) (Table 4) was observed in diabetic rats with warfarin administration.

Conclusion

Hyperglycemia plays an important role in hypercoagulation state and oxidative stress in streptozocin-induced diabetes mellitus. L-carnitine improves oxidative stress and decreases the hypercoagulation state in diabetes mellitus. On the other hand, warfarin normalizes the hypercoagulation state in diabetes mellitus with no effect on oxidative stress.

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EFFECT OF ANTICOAGULANT (WARFARIN) AND L-CARNITINE ON HAEMOSTATIC FUNCTION AND OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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OUTCOME OF BAHA SURGERY USING THE INFERIORLY BASED PARTIAL THICKNESS SKIN FLAP

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Abstract

Objective: *To evaluate the outcome of bone anchored hearing aid (BAHA) surgery using an inferiorly based partial thickness skin flap.*

Introduction: *The wide application of BAHA surgery and the different surgical techniques necessitate the analysis of the outcome of this surgery and its safety and effectiveness as an auditory rehabilitation modality.*

Methods:

Study design: *Retrospective chart review.*

Patients and methods: *Patients subjected for BAHA surgery for the rehabilitation of their deafness. Clinical records were reviewed from August 2007 to April 2011. Skin healing was reviewed and post operative complications were analysed.*

Main outcome measures: *The incidence and grading of complications and their temporal development following BAHA surgery. A modified Holger's classification was used to measure post BAHA surgery complications.*

Results: *BAHA surgery was performed for 45 patients, with a male to female ratio of 3:5. No major complications were encountered in the first post operative month. Afterwards, complication rates decrease with time. Early complications were usually minor and medically treatable whereas late complications were major and required surgical intervention.*

Discussion: *Most of early complications of BAHA surgery are related to defective healing of skin flaps. These were mostly minor and reversible and did not disturb osseointegration. Long term complications are mostly related to defective osseointegration, were usually major and*

would mostly require surgical intervention to correct.

Conclusion: *BAHA surgery is safe and effective for auditory rehabilitation. The skin flap raising technique applied in this group of patients is safe and applicable compared to other skin preparation techniques.*

Introduction

The bone-anchored hearing aid BAHA) surgery is a well-accepted auditory rehabilitation modality for patients who can't tolerate the traditional air conduction hearing aids[1-3]. The BAHA is also better than bone conductor aids as it eliminates trans-cutaneous damping of sound transmission, associated with bone conductor aids, resulting in better aided thresholds[4,5]. The main principle of BAHA is the transmission of sound from the hearing aid (bone conduction vibrator) to skull bone and hence to the functioning cochlea. This is achieved via implanting a titanium fixture into temporal bone and allowing adequate time for osseointegration to take place.

The main component of surgical intervention is the preparation of implantation site by thinning the soft tissue down to the periosteum so that the trans-cutaneous abutment, implanted on top of the fixture, stands above skin level. This results in a percutaneous implant surrounded by thin, hairless and immobile skin adherent to the underlying periosteum[6]. This helps to reduce mechanical energy loss from the titanium implant to the surrounding myofascial tissues. It also creates hairless skin to enable easy use and cleaning of the BAHA.

There have been several surgical techniques for skin preparation for BAHA implantation. These include free retro-auricular full-thickness skin grafts[7,8], split-thickness skin grafts[9], single or multiple pedicled local flaps [8,10,11]. Simple vertical post aural skin incision and subcutaneous tissue reduction have also been proposed[12,13]. Graft and or flap raising was conducted either manually[8,12,14] or using dermatomes[8,9,11]. The choice of surgical technique depends mainly of the surgeon's experience and preference. Reported complications of BAHA surgery include soft tissue complications such as skin flap

necrosis, infection of the flap, skin growth over the abutment, and bone complications such as failure of osseointegration and implant extrusion[15,16]. The overall patients' satisfaction after BAHA surgery has been reported to be high[17].

The multitude of different techniques utilised for skin preparation for BAHA implantation indicate that none of these is ideal for all cases. There is continuous search of BAHA surgeons to establish a less complicated surgical approach for BAHA fitting. In this paper, an inferiorly based skin flap technique for BAHA surgery is described and the outcome of 45 adult BAHA implantations is analysed.

Methods

A retrospective review of clinical records of patients implanted with a BAHA from August 2007 to April 2011 was conducted. The indications for BAHA surgery were recorded. Patients were usually referred for audiological assessment for their suitability for BAHA before being considered for surgery.

Operative Technique

The surgical technique used in these cases included the creation of a partial thickness post auricular inferiorly based skin flap which was manually fashioned. Depending of the thickness of the retroauricular subcutaneous tissue, the flap dimensions were 4-5x3-4cm and the flap is centred on the point of optimal insertion 55mm behind and 30mm above the centre of external auditory canal (EAC) at the level of the supra meatal crest. A 15 blade was used to make an inverted U incision and the inferior based skin flap was reflected as thin as possible and kept moist with saline swabs to avoid tissue dehydration. Subcutaneous soft tissue was reduced down to periosteum and the excision edges are slopped to allow a smooth closure. A cruciate incision was then made to raise periosteum and expose the underlying bone for drilling and placement of the counter sink self tapping fixture followed by the abutment. This was performed following standard Branemark technique[6] and as recommended by the manufacturer. Then the periosteum was replaced and incision closed.

A linear incision was made in the skin flap to deliver the abutment. The abutment was a closed with a healing cap and mastoid dressing was applied to be removed the following day. The healing cap was removed after 5 days. Patients were reviewed in BAHA dressing clinic as and when required and routinely reviewed in BAHA clinic run by the surgeon in 3 weeks and the after 2 months. Further clinical visits were arranged as and when needed. In all cases, the operation was done in one stage. At least 3 moths were allowed for complete osseointegration before loading the implant.

Overall results and complications of BAHA surgery were studied. The main outcome measures were post BAHA implantation complications including soft tissue complications and defective osseointegration. Patients' satisfaction using the BAHA was also assessed.

Assessment of post operative complication

The author modified Holgers classification[18] to grade skin reactions around the BAHA implant as follows:

Grade 1: redness with slight swelling around the abutment

Grade 2: moist skin and moderate swelling – slight infections, minimal crustation, raw skin, slight skin gapping.

Grade 3: tissue granulation around the abutment – treatable infection.

Grade 4: overt signs of infection/pain resulting in removal of the implant – overt skin deficiency/overgrowth, excess scarring.

Grade 5: abutment or fixture extrusion.

Generally, grades 1 and 2 were considered minor, grades 3 intermediate and grades 4 and 5 major.

Temporal classification of post operative complications defined early complications as developing within the first 1 month, intermediate complications developing after one month up to one year and late complications developing after one year.

Results

The study included 45 patients, 16 men and 29 women with male:

female ratio ~5:9. BAHA was inserted for 26 right and 19 left ears. Demographic data are presented in table 1.

BAHA indications

The most common clinical indication for BAHA surgery was recurrent ear infections (24 patients, 53%) with which the patient was unable to wear an ordinary hearing aid. Nearly half of these had middle ear surgeries including 4 bilateral, 4 right and 3 left mastoidectomies. One patient had severely stenotic external auditory canal and one could not tolerate the ordinary behind-the-ear hearing aid. Nine patients had lost hearing due to acquired aetiologies (post infective or post traumatic, accidental or surgical) and 7 had either congenital or degenerative aetiologies of deafness. Three patients had otosclerosis.

The most predominant type of hearing loss was mixed encountered in over one third of patients (38%), whereas conductive and sensorineural loss together presented over one fourth of cases (27%). Profound hearing loss and dead ears were found in over one third of patients (35%). Of the 11 patients with dead ears 6 had dead right and 5 had dead left ears.

All cases were done under GA. No dural exposure, venous haemorrhage, mastoid cells exposure or skin flap damage was seen in any case. All implants osseointegrated and all patients proceeded to BAHA fitting. Follow up duration ranged from 7 to 56 months (mean 17.6 ± 23 months). No patient required removal of the abutment or fixture during the observation period of this study.

One patient had excess skin around the abutment which did not respond to local steroid application and required excision of the overgrowing skin. Abutment extruded in 2 patients after an average 22.3 months and fixture extruded in 3 patients after an average 43.6 months. In all cases, the cause of extrusion minor trauma. Extruded fixtures were reinserted under local anaesthesia, with limited exploration of the operative site. Incidence of post BAHA surgery complications, based on complication onset and

overall grading, is summarised in Table 2 and Figure 1.

Early complications developed within 1 month after surgery, intermediate complications developed from 1-12 months and late complications after 1 year. Complications were classified minor, intermediate and major based on a modified Holger's classification. The % quoted is calculated in relation to the total number of complications not the number of surgical procedures. Minor complications were reversible and treated conservatively. Major complications required either long term medical treatment or surgical intervention.

Normal skin healing was reported in 25 patients (56%) whereas complications developed in 20 patients (44%). In 3 patients, various onset complications developed and these were counted as multiple complications to result in a total of 21(47%) complications rate.

Considering the general grading of complications, minor complications represented more than half of the total complications (57%). Moderate and major complications represented 24% and 19% of total complications respectively. Minor complications required 1 or 2 clinical visits to resolve whereas intermediate complications required 1-3 visits ($p>0.05$). Major complications required either a prolonged medical or surgical management to correct. Considering the onset of complications, more than one fourth of complications (29%) developed in the first month and two thirds of these were minor. In the following 11 months (intermediate), 57% of total complications developed and more than $^1/_2$ of these were minor complications. Late onset complications developed in 3 cases representing 14% of total complications and these developed after 13-52 months (mean 30.8 months). Two out of the three late complications were major and these represented 50% of major complications and the other 50% developed in the intermediate period.

Patient's satisfaction was assessed qualitatively not quantitatively based on patients' comment and impressions about the impact

of their BAHAs on their hearing and quality of life. Patients' satisfaction was reported at their final clinical visits. The majority of patients (93%) were generally satisfied with the BAHA and their satisfaction ranged from being reasonably to extremely happy. Three patients were not satisfied with their BAHA: one patient was not able to use the hearing aid due to local discomfort and 2 patients were not satisfied with the hearing gain. One of these 2 patients had fixture extrusion and declined fixture reinsertion.

Table (1): Demographic data of 45 patients fitted with BAHA.

	Number	Age range	mean
Males	16	43-77 y	61.9 y ± 10.2 (SD)
Females	29	17-74 y	49.2 y ± 13.3 (SD)
Total	45	17-77 y	52.7 y ± 13.7 (SD)

Table (2): Overall complications after BAHA surgery in 45 patients.

Onset	Extent			
	minor	Intermediate	Major	Total
Early	4 (33%)	2 (40%)	------	6 (29%)
Mid	7 (58%)	3 (60%)	2 (50%)	12 (57%)
Late	1 (9%)	------	2 (50%)	3 (14%)
Total	12 (100%)	5 (100%)	4 (100%)	21 (100%)

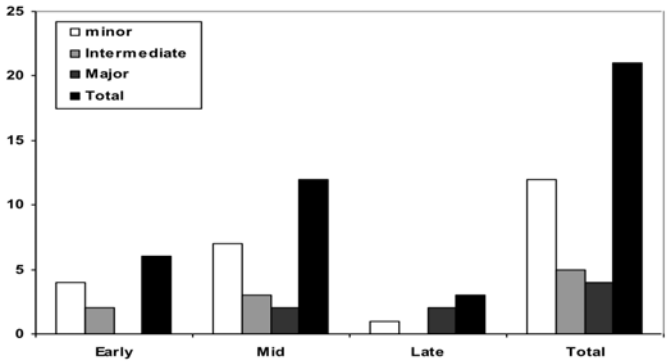

Fig. (1): Post BAHA complications in 45 patients.
Based on timing, post BAHA complications were classified as developing early (within 1 month), intermediate (after 1 month till the end of 1st year) and late (after 1 year). Complications were classified as minor, intermediate and major based on a Holger's classification modified by the author.

Discussion

The technique used in this series involves manual thinning of the skin flap. The author found that technique reasonably easy and safe. There was no occasion of flap damage or penetration due to careful flap thinning with frequent inspection of the skin side and assessment of flap thickness as the flap raising progresses. In the first few cases, flap raising was relatively slow paying more attention to avoid flap over thinning and/or penetration. As the technique was mastered, less time was required to raise the flap with the required thickness. The flap was centred on the supra-meatal crest. At this point, a suitable bone thickness can be found and a good sound conduction to the cochlea is achieved[12]. Dehydration of the flap was avoided by applying moist saline swabs on the under surface of the flap. This seems to help as there were no records of flap dryness or ischemia. Based on general surgical principles, a skin flap technique is expected to secure quicker and less complicated healing compared to a skin graft[7,16]. The linear incision technique has been reported to provide minimal disturbance to the skin and its blood supply with complication rates and long-term results comparable to other reported skin flap techniques [12,19]. However, soft tissue reduction and surgical exposure of periosteum and underlying skull bone can be relatively difficult. The creation of an inferiorly based flap allows wider exposure of the surgical field and easier and more controlled soft tissue reduction with no apparent compromise of flap vascularity.

All patients had their BAHA fitting as planned. Over half of patients enjoyed uneventful post operative course with complete wound healing and implant fitting as planned. In those who developed post operative complications, most of complications (81%) were either minor or intermediate and all these were reversible with careful medical treatment and did not interfere with the hearing aid use. This is comparable to previously reported BAHA outcome[7,11,15, 20,21].

The first postoperative month is the critical period for wound heal-

ing and infection at the site of implant could endanger osseointegration resulting in surgical failure[22]. No major complication was recorded in this period and only minor or intermediate complications were encountered and these represent over one fourth of total complications. After complete wound healing, the chance of developing minor complications decreased through the first year and became progressively less afterwards. Over the following 11 months there were increased absolute numbers minor and intermediate complications in addition to 2 major complications. However, considering the timescale and taking the average complication rate/month, the chance of developing complications after the first post operative month was less that one fifth that in the first postoperative month. After the first post operative year, the chance of developing a complication was nil as only one minor and 2 major complications were encountered with a mean follow up of 17.6 months.

Major complications in this series developed in 9% of patients which represented 19% of total complications. All major complications developed after complete osseointegration and sound processor fitting. The one patient who developed excess skin growth over the abutment received prolonged medical treatment with local steroids to try and reverse skin growth over the abutment as recommended before[23]. This patient required surgical intervention to excise the excess skin. The other 3 patients required reinsertion of the extruded fixture and the 2 patients who had this done proceeded to sound processor fitting and enjoyed satisfactory hearing rehabilitation.

Fixture extrusion occurred in 3 patients (7%) in this study and this is comparable to that reported in other studies[11,15,24]. It would be expected that fixture extrusion occurred much later than abutment extrusion as the latter is not implanted in skull bone and is more superficial and prone to external forces. The abutment is likely to fall out much easier and earlier than the bone anchored titanium fixture particularly after complete osseointegration. Howev-

er, fixture extrusion upon exposure to repeated external forces might indicate delayed failure or gradual loss of osseointegration. In these circumstances, the fixture would come out after a trivial trauma such as manipulation of a jammed sound processor or hitting the bed. Excessive external force could, on the other hand, force the fixture to extrude particularly in the presence of abutment with or without the sound processor as the latter 2 components would act then as a lever increasing the effect of applied external force. None of the patients had reported symptoms or signs of late infection. Whether fixture extrusion was due to gradual loss of osseointegration, subtle local inflammatory changes or a direct effect of external trauma can not be determined.

The majority of patients in this series were satisfied with their BAHA. As the vast majority of patients had previously worn air or bone conduction hearing aids, the BAHA relieved these patients, particularly those with recurrent ear infections, from the former troubles they had with the air or bone conduction aids. The hearing gain, improved communication and quality of life as well as the lack of inconvenience of fitting and removing the ordinary haring aids were among the satisfying factors for these patients. This is comparable to previous results reported before[17].

Although early BAHA fitting has been advocated in some studies[12,19,25-27], in this series, osseointegration was considered to be complete in 3 months and patients were fitted afterwards as traditionally followed[6]. Following this policy, none of the patients had failure of osseointegration and all patients were finally loaded with the sound processor as planned. The author thinks that early fitting should be taken with great caution given the fact that more than half of BAHA patients had already suffered from recurrent ear infections with or without hearing aids. After the first 3 months, the chance of developing post operative complications which could fail osseointegration decreases and it would be safer to allow complete wound healing and treat any post operative complica-

tions before fitting the patient with the sound processor. The author, however, has no reasons not to consider earlier fitting for selected patients when there is adequate supportive evidence to the safety and suitability of early fitting in BAHA surgery.

In this series, one fourth of patients had dead one ear and would not benefit from the ordinary hearing aids. They would only benefit from the contra lateral rerouting of sound (CROS) hearing aids or BAHA. Previous studies had reported greater improvement in speech intelligibility in noise from BAHA compared with CROS hearing aids[28]. Furthermore, CROS aids have the inherent inconvenience for patients in the presence of recurrent ear infections. Furthermore, as the sound is directly transmitted from the BAHA fixture to skull bone, there is no trans-cutaneous damping of sound transmission inherent in the bone conductor aids resulting in better aided thresholds using the BAHA[4,5]. It was not, therefore, the author's practice to try CROS hearing aids before considering the patients for BAHA surgery although all the options were always discussed with patients to chose from and to give an informed consent.

Weakness of the study: This is a retrospective study looking at a single operative technique practised by the author. The number of patients involved in this study is not large enough and the follow up period of this group of patients is not long enough to obtain an adequate insight to the potential triggering or precipitating factors involved in the development of post BAHA surgery complications. Further studies with large patients' number and long enough follow up are required to evaluate the long term clinical effectiveness of this surgical technique compared to other techniques employed in BAHA surgery.

Conclusion

The inferiorly based partial thickness skin flap technique utilised in this series of 45 patients is easy to master for skin preparation prior to BAHA implantation. It is a safe technique and with fairly satisfactory outcome (post operative complications and overall pa-

tients' satisfaction) comparable to other skin preparation techniques employed in BAHA surgery.

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EVALUATION OF THE ROLE OF LAPAROSCOPY IN THE MANAGEMENT OF BLUNT ABDOMINAL TRAUMA

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Abstract

Introduction: *In the setting of blunt abdominal trauma, laparoscopy is used mainly for diagnosis, and its role in definitive operative repair is still debated.*

Aim of the work: *The aim of our work was evaluation of the role of laparoscopy in the management of blunt abdominal trauma.*

Patients and Methods: *This is an interventional, prospective, non-randomized clinical study conducted on twenty patients with blunt abdominal trauma admitted at Mansoura emergency hospital over the period between April 2011 and April 2013.*

Results: *It was evident that the largest number of patients was between 20-30 years (40%) and the least number of patients between 50-55 years (10%). No patients were below 10 nor above 55 years. Ten cases had hollow viscus injuries four of them were ileal, two of them were jejunal, three of them were colonic injuries and one was duodenal. We had two cases of active internal bleeding, one was splenic and the other was hepatic injury. We had one case of diaphragmatic injury. There were 2 cases of missed injuries discovered during delayed laparotomy done 2 days or more after diagnostic laparoscopy. The sensitivity of laparoscope in detection of hollow viscus injury was 80% and detection of diaphragmatic injury was100% and in the detection of solid organ injuries was 80%.*

Conclusion: *The diagnostic and therapeutic role of laparoscopy is progressing with time but is mainly directed towards hollow vicera and diaphragmatic injuries.The role of laparoscopy in control of intra-abdominal bleeding is limited and should not be on the expense of safety.*

Introduction

Despite improved diagnostic tools such as computerized tomography (CT scan), conventional treatment of patients with abdominal trauma injures often requires exploratory laparotomy procedures to accurately diagnose and treat patients, injures. Studies showed that nontherapeutic (i.e., negative) laparotomy rates range from 5% to 40%, depending on the clinical situation. Many surgeons now perform diagnostic laparoscopic procedures in haemodynamically stable patients with abdominal trauma injuries. Although laparoscopy in patient with abdominal trauma injury does have limitations, it is an effective tool for preventing negative laparotomies.(1)

In the setting of blunt abdominal trauma, laparoscopy is used mainly for diagnosis, and its role in definitive operative repair is still debated.(2)

Aim of the Work

The aim of our work was evaluation of the role of laparoscopy in the management of blunt abdominal trauma.

Patients and Methods

This is an interventional, prospective, non-randomized clinical study conducted on twenty patients with blunt abdominal trauma admitted at Mansoura emergency hospital over the period between April 2011 and April 2013.

Patients' population:

Twenty patients (14 males and 6 females) were included in the study. Their ages ranged between 13 and 55 years with mean age 26 years.

Our inclusion criteria were blunt abdominal mono-trauma, haemodynamically stable and positive clinical or radiological signs for hollow viscus or diaphragmatic injuries.

Exclusion criteria were polytraumatized patient, haemodynamically unstable and general contraindications of laparoscopy.

Patients' evaluation:

All patients were subjected to primary survey, then secondary survey which localized the trauma to the abdomen.

All patients were investigated as follows:

A- Laboratory in the form of; CBC, serum creatinine, liver function studies, Coagulation profile, RBS, serum electrolytes and arterial blood gases.

B- Radiological studies included; Abdominal X-ray supine and erect showing the lower chest, FAST and aspiration from the free fluid or collection if accessible and CT abdomen with oral and IV contrast.

Follow up studies:

A- Clinical

• Vital signs every 3 hours, Serial abdominal examination every 6 hours.

B- Laboratory

• Hemoglobin, hematocrit value, TLC and ABG every 6 hours.

C- Radiological

• Serial FAST every 6 hours, which could be modulated according to the clinical situation.

• Follow up abdominal X-ray when the clinical picture was equivocal.

The study was conducted on one group including 20 patients who were subjected to a diagnostic laparoscopy after receiving the conservative measures done to stable patients with blunt abdominal trauma. Management was then tailored according to the laparoscopic findings.

We had 4 sub-groups:

a- Non therapeutic (only diagnostic) sub-group in which the laparoscopic findings did not necessitate therapeutic intervention.

b- Totally laparoscopic sub-group in which the laparoscopic findings were managed totally by laparoscopy.

c- Laparoscopically assisted sub-group in which the laparoscopic findings were managed partially by laparoscopy and the procedure was completed through a small laparotomy incision (3-4 cm).

d- Laparoscopic converted to laparotomy sub-group in which laparoscope failed to manage the injury so converted to laparotomy.

Diagnostic laparoscopy under general anesthesia with endotra-

cheal intubation was used in all cases.

Steps:

• 10 mm trocar was inserted in the left iliac fossa or peri-umblical region using an open technique (Hasson technique).

• A pneumo-peritoneum was produced using CO2 insufflation through the trocar at a rate of about 0.5ml per minute to a maximum intra-abdominal pressure of 9-12 mmHg.

• A 30 degree telescope was inserted in the abdominal cavity.

• An initial rapid tour was made inside the abdominal cavity searching for active bleeding which took priority in management.

• We determined the sites of port placement according to the operative findings and needs.

• For handling of the small intestine to explore, two additional 5 mm trocars were placed in the upper left and the lower right quadrants and 2 atraumatic graspers were inserted through the two trocars.

• One side of the bowel wall is observed by lifting up the bowel with the 2 bowel forceps the other side of the bowel is observed by turning over the bowel.

• The stomach, duodenum, colon and small intestine were carefully examined for perforation or ischaemic changes.

• If subserosal hematoma or dirty exudate was not seen in the retroperitoneal parts of the duodenum or the colon, the patient was judged not to have perforation.

• The transverse colon, splenic flexure and descending colon were inspected while the surgeon standing on the right side.

• The liver, stomach spleen and diaphragm were inspected while the patient in the reversed Trendlenburg position.

Therapeutic laparoscopic procedures:

Laparoscopic intestinal repair and resection anastomosis:

Cases with small intestinal or colonic injuries which were documented by laparoscopy were repaired either totally laparoscopically by intra-corporeal suturing technique or by laparoscopic assisted techniques in which complete laparoscopic mobilization of the small intestine was performed and the injured segments a were

pulled out through a small laparotomy incision (3-4cm) then the resection of the injured segment and anastomosis of the two ends of the small intestine was performed using conventional techniques (single layer extra-mucosal). Then we pushed the anastomotic segment back into the abdomen.

Laparoscopic repair of diaphragmatic injury;

We had one case of diaphragmatic repair done by the use of non absorbable sutures (Prolene 0) using intra corporeal suturing technique.

Follow up of the patients in the post-operative period with recording of the morbidity and mortality for 6 months after discharge. All data were collected in special preformed sheet for statistical analysis. The data included patient demographics, co-morbidities, mechanisms of injuries, indication of laparoscopy, findings in laboratory and radiological investigations, different types of laparoscopic intervention, hospital stay, number of units of blood transfusion, intensive care unit period, time of intervention and postoperative morbidity and mortality.

Statistical Analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 15. Qualitative data were presented as number and percent. Quantitative data were tested for normality by Kolmogrov-Smirnov test. Normally distributed data were presented as mean ± SD.

Results

Patients' profile:

This prospective study was conducted on twenty patients who had blunt abdominal trauma. These patients were managed by the usual conservative measures done to patients who are haemodynamically stable (admission in HDU after starting resuscitative measures then follow up using clinical, laboratory and radiological parameters) then diagnostic laparoscopy was done and management differed according to the laparoscopic finding.

Causes of conversion of laparoscopy to laparotomy (2 cases):

Failure of control of bleeding 1 case (50%).

Technical difficulties 1 case (50%).

Missed injury and delayed laparotomy:

There were 2 cases of missed injuries discovered during delayed laparotomy done 2 days or more after diagnostic laparoscopy so the incidence of missed injury is 10%. These 2 cases were belonging to the non-therapeutic group so non therapeutic laparotomy was avoided in 7 cases out of 20 (35%).

Demographic characters

Table (1): Demographic characters of patients of our study (N=20).

Age	**Range**	**13-55years**
	Mean +/- SD	**26.0 years ± 11.05**
Sex	Male	14 (70%)
	Female	6 (30%)

Indications of laparoscopy

Table (2): The different indications of the laparoscopic exploration and the corresponding laparoscopic finding (N=20).

Indication of lap`aroscopy	**Laparoscopic finding ± laparotomy**
Clinical indication of hollow viscus injury (4 cases)	**2 positive** (50%) **2 negative** (50%)
Radiological indication Plain x ray (2cases)	**2 positive** (100%)
FAST and aspiration (3 cases)	**1 positive** (33.3%) **2 negative** (66.6%)
CT (9 cases)	**5 positive (56%)** 3 by laparoscopy (33%) 2 by delayed laparotomy (23%) **4 negative (44%)** for hollow viscus injury
Radiological suspicion of diaphragmatic injury 2 cases	**1 positive** (50%) **1 negative** (50%) For diaphragmatic injury.

Table (3): Different laparoscopic procedures and their percent (N=20).

Type of laparoscopic procedure	**Number of patients**	**percent**
Non therapeutic (diagnostic only)	9	45%
Therapeutic (totally laparoscopic)	7	35%
Therapeutic (laparoscopic assisted)	2	10%
Laparoscopic converted to laparotomy	2	10%

Table (4): Different therapeutic procedures done to patients of our group their percent (N=20).

Operative procedure	Number of patients	Percent
Resection anastomosis of small intestine	2	10%
Repair of intestinal injury	4	25%
Ileostomy	2	10%
Colostomy	2	10%
Splenectomy	1	5%
Hepatorraphy	1	5%
Repair of diaphragmatic tear	1	5%

Table (5): Sensitivity, specificity, Positive predictive value (PPV), negative predictive value (NPV) and accuracy of different diagnostic modalities in the detection of hollow viscus injury.

	Sensitivity	Specificity	PPV	NPV	Accuracy
Clinical examination	20%	80%	50%	50%	50%
X ray	20%	100%	100%	55.6%	60%
FAST	10%	80%	33.3%	47.1%	45%
CT	50%	60%	55.6%	54.6%	55%
Laparoscopy	80%	100%	100%	83.3%	90%

Table (6): Sensitivity, specificity, Positive predictive value, negative predictive value and accuracy of different CT signs in the detection of hollow viscus injury.

	Sensitivity	Specificity	PPV	NPV	Accuracy
Extravasation of oral dye	10%	100%	100%	52.63%	55%
Extra-luminal gas	10%	100%	100%	52.63%	55%
Thickening of bowel loops and collection	20%	80%	50%	50%	50%
Intra-peritoneal fluid without solid organ injury	10%	80%	33.3%	47.1%	45%

Table (7): Sensitivity, specificity, Positive predictive value (PPV), negative predictive value (NPV) and accuracy of combined radiology and laparoscopy in the detection of diaphragmatic injury.

	Sensitivity	Specificity	PPV	NPV	Accuracy
X ray and CT	100%	94.74%	50%	100%	95%
Laparoscopy	100%	100%	100%	100%	100%

Table (8): Sensitivity, specificity, Positive predictive value (PPV), negative predictive value (NPV) and accuracy of CT and laparoscopy in the diagnosis of solid organ injury.

	Sensitivity	Specificity	PPV	NPV	Accuracy
CT	100%	100%	100%	100%	100%
Laparoscopy	80%	100%	100%	93.75%	95%

Discussion

Current trends in all areas of surgery are towards minimal invasive techniques. Laparoscopy is the best example of these minimally invasive techniques and its use as a diagnostic tool in trauma patients was introduced as early as 1976 by Gazzaniga.[3]

The definite goal of our study is to evaluate the role of laparoscopy in the diagnosis and treatment of cases of blunt abdominal trauma.

In our study, most of patients were of the middle age group where 70% of the patients of our study are between 20 and 50 years, 20% of patients are less than 20 years and 10% of patients are more than 50 years. While in a study by Cherkasov et al. (2007),[4] 62.6% of patients were between 20-50 years, while 10.6% and 14.5% were less than 19 and greater than 50years, respectively.

In our study the vast majority of cases were hollow viscus injuries (small intestine and colon) 10 cases 50% followed by the liver 4 cases (20%) followed by the retroperitoneum and mesentry 2 cases for each (10% for each) followed by spleen and diaphragm 1 case for each (5% for each) in contrast to this Alonso et al. (2003),[5] had demonstrated that the vast majority of cases of blunt abdominal trauma were to solid organs spleen and or liver followed by injury to the kidney, mesentry, small bowel, colon and pancreas.

The difference in the order of the different organs involved in blunt abdominal trauma between our study and the previous study of Cherkasov et al. (2007),[4] could be explained by the fact that we were dealing with a specific category of patients with blunt abdominal trauma who were haemodynamically stable and showed either clinical or radiological signs suggesting or suspecting either hollow viscus or diaphragmatic injuries and that patients who had solid organ injuries which mostly diagnosed by CT but do not show these signs were subjected to the traditional conservative measures done to patients of blunt abdominal trauma and were excluded from our study.

In another study by Day et al.

(2002),[6] they postulated that the sensitivity of initial abdominal examination was 16% and specificity was 52%in determining the need of laparotomy.

In our study we had 4 cases suspected to have hollow viscus injury by serial clinical abdominal examination depending on the previous signs. Laparoscopic evaluation revealed 2 positive cases for this while the other 2 cases were negative for this. Sensitivity was 20% and specificity was 62% which was higher than the previous study which could be explained by that in our study we evaluated serial examination not initial examination only. The problem in clinical examination is that it depends on the experience of the examiner also it needs serial abdominal examination also initial evaluation is not so helpful because contusion of the abdominal wall may produce signs similar to that of peritonitis also may be affected by drugs, analgesics, narcotics or diminished level of consciousness because of trauma.

Liu et al. (2003),[7] had stated that CT is a relatively non-invasive diagnostic modality. Its complication reported to date has been so infrequent. Both intra peritoneal and retroperitoneal structures are visualized and the vascular integrity of the solid organs (spleen- liver- kidney) can be evaluated but the sensitivity of CT remains questionable especially for hollow viscus injury.

Alan et al (2008),[8] had demonstrated that CT has a significant false negative rate in the diagnosis of small bowel injuries, findings suggestive of small bowel injury include fluid collection without solid organ injury, bowel wall thickening, mesenteric infiltration, free intra-peritoneal air and oral dye extravasation.

In our study we found that the sensitivity and specificity of CT in the diagnosis of solid organs was 100% in agreement with most of the literature but its sensitivity for hollow viscus injury was 50% but its accuracy in detection of the exact site is questionable. In our study it was 20% where only one case out of five show CT findings similar to laparotomy regarding the site of leak and was the duodenum.

In a study by Feliz et al. (2006), [9] there were 7127 trauma admissions, of which 113 had abdominal explorations for blunt (88%) and penetrating (12%) trauma. Thirty-two (28%) patients had laparoscopy performed. Laparotomy was avoided in 56% of these patients.

In our study, laparotomy was avoided in 70% of patients 35% was because of laparoscopic findings that do not necessitate therapeutic intervention and 35% was because laparoscopic findings that were managed totally by laparoscopy but the ratio is higher in our study because all our patients are blunt abdominal trauma unlike the previous study which included patients with blunt and penetrating abdominal trauma and it is well established that the incidence of intra-abdominal injury is less in blunt abdominal trauma than that of penetrating abdominal trauma and so conservative management is more applicable in blunt than in penetrating.

In the previous study by Feliz et al. (2006),[9] Laparoscopy was negative in 9 (28%) patients, 5 of whom had blunt trauma and 4 had penetrating trauma. Laparoscopy was diagnostic and nontherapeutic in 3 (10%) patients with nonexpanding hematomas. Laparoscopic therapeutic interventions were performed in 6 (19%) patients. Laparotomy was avoided in 17 (56%) patients. Laparoscopy assisted in the diagnosis and subsequent conventional repair of 10 cases of perforated viscera, diaphragmatic ruptures, and 1 distal pancreatic injury. Thirteen (68%) of the injuries diagnosed by laparoscopy were an injury to a hollow viscus. No injuries were missed, or technical complications occurred, as a result of laparoscopic explorations. No patients who underwent laparoscopy died.

In our study, we had no negative cases by laparoscopy. This could be explained by the fact that we dealt with patients already having either clinical or radiological signs suggesting hollow viscus or diaphragmatic injuries we did not do laparoscopy for all patients with blunt abdominal trauma. Initially we had 9 (45%) cases with nontherapeutic (only diagnostic laparoscopy) with liver, retroperi-

toneal and mesenteric injuries with no active bleeding. laparoscopy assisted in the diagnosis of 2 cases (10%) of intestinal injuries which enabled us to make a small laparotomy incision and completing the procedure (laparoscopic assisted). We made diagnostic laparoscopy then completing the therapeutic intervention totally by laparoscopy in 7cases (35%). We had 10 cases (50%) of the whole group with intestinal injury 8 cases (80%) of them were diagnosed by laparoscopy 2 cases (20%) were missed by laparoscope and detected during delayed laparotomy, 6 cases (60%) of them were managed totally laparoscopic, 2 cases (20%) were managed by laparoscopic assisted technique.

Laparoscopy determined the need for laparotomy and conventional repair was done after laparotomy in 2 cases (10%) and missed injuries occurred in 2 cases out of 20 which is the total number of the patients in our study (10%).

In a study by Elliot et al. (1998),[10] they assessed 47 patients selected for laparotomy after trauma all patients underwent diagnostic laparoscopy followed by laparotomy. Sensitivity was poor less than 50% for injuries of hollow viscera however laparoscopy had excellent sensitivity 96.2% and specificity 100% for determining the need for therapeutic laparotomy.

In our study, the sensitivity of laparoscope for detection of hollow viscus injury was 80% and specificity for this was 100%.

In a study by Chersakov et al. (2007),[4] conversion to laparotomy was performed in 26.7% of patients, massive solid organ involvement multiple abdominal organs injuries hemorrhage greater than 1500ml, haemoperitoneum greater than 500 ml and continuing heavy internal bleeding were the absolute indications for conversion to laparotomy in haemodynamically unstable subjects. Comorbid conditions such as respiratory cardiovascular and renal insufficiency were relative indications for conversion to laparotomy.

In our study, conversion to laparotomy immediately after lapa-

roscopy was done in 2 cases only (10%) there were two reasons for this conversion, the first was inability to control bleeding for more than 10 minutes.

The second reason was technical difficulties where duodenal injury was suspected and mobilization of the duodenum was required and could not be done by laparoscopy.

In the previous study by Chersakov et al. (2007),[4] the average time for diagnostic laparoscopy was 22±8 minutes and that of laparoscopic surgery was 60±12 minutes the average postoperative hospital stay following video assisted laparoscopy 12.2±3.8 days and 18.44±3.8 days after laparotomy.

In our study, the average time for diagnostic laparoscopy was 40±8minutes which is more than that of the previous study which may be due to our starting limited experience in the use of laparoscope in blunt abdominal trauma and this was reflected on our results as the starting limited experience made us more meticulous during exploration so the sensitivity and specificity of laparoscope was better than other studies. The post-operative hospital stay was variable between the different subgroups of our study it was 7.2±1.9 days for the non-therapeutic subgroup it was 10±2.8 days for totally laparoscopic sub-group and it was 8.1±1.7 it was 12±2.5days for the subgroup in which laparoscopy is converted to laparotomy and all these values were less than that of the previous study. We do not have clear explanation for this but could be due to the use of principles of fast track surgery in most cases of our study.

In the study by Cherkasov et al (2007),[4] the complications of laparoscopy group were as follows chest infection was 1.2% of patients, wound infection 2.8% of patients, evisceration 0% intestinal bands and obstruction 0% and the complications of the other group who underwent laparotomy were as follows chest infection 5.3% wound infection3.8% bowel evisceration 0.4% intestinal obstruction 0.6%.

In our study, wound infection

occurred only in the 2 subgroups in which a laparotomy incision was done. This can be explained by the fact that incisions of totally laparoscopic subgroups mostly are very small 1cm or less, but intra-peritoneal collection occurred only in other 2 subgroups which were done totally laparoscopically this could be attributed to that laparoscopy is less efficient in drainage of intra-peritoneal collection than open drainage and breaking of septa by hand dissection. We had only one case of chest infection in the subgroup in which laparoscopy converted to laparotomy. This can be explained by that laparotomy incision made patient feel postoperative pain that limits respiratory movement predisposing to chest infection. This could be considered one of the major advantages of laparoscopy. No other chest complications related to CO2 insufflation in the studied group were detected.

No cases of evisceration nor adhesive intestinal obstruction were detected in our studied group unlike the previous study because of the small number of patients in subgroups subjected to laparotomy and relatively short period of follow up and can be explained also as an advantage of laparoscopy. We had 2 cases of intestinal injury which were missed by diagnostic laparoscopy. There was 1 more case of intestinal leak in the subgroup which was done totally laparoscopic which may be explained by error in the technique of repair because of our limited experience in intra-corporeal suturing technique.

Laurance and Gerard (2003). [11] stated that blunt abdominal injuries is usually associated with serious complications and high mortality of about 25- 65% as a result of difficulty in prompt diagnosis and management of other injuries.

In our study, the mortality rate was 0% in our patients' population, this can be explained by the fact that we dealt with a specific category of patients in which the abdomen was only involved in trauma and all patients were haemodynamically stable. Extra-abdominal injuries were absent in our study. It is well known that extra-abdominal injuries account

for a significant share of mortality rate among trauma patient.

Conclusion and Recommendations:

The diagnostic role of laparoscopy is progressing with time but mainly directed towards diagnosis of hollow vicera and diaphragmatic injuries.

The role of laparoscopy in control of intra-abdominal bleeding is limited and should not be on the expense of safety

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EVALUATION OF THE ROLE OF LAPAROSCOPY IN THE MANAGEMENT OF BLUNT ABDOMINAL TRAUMA

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IMMUNOHISTOCHEMICAL PROFILING OF B-CELL LYMPHOMA INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND CLASSICAL HODGKIN LYMPHOMA

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Abstract

Aim: *Currently, WHO classification 2008 approved the term B-cell lymphoma intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (cHL).This study was urged by scarcity of data about such cases to explore their immunohistochemical features.*

Methods and Results: *Thirteen cases of B-cell lymphoma cases with intermediate features were identified over a 9-year period. HRS-like cells were CD30+ with a high rate of CD20+ (100%) and lower rates of CD15+ (25%) and Fascin+ (50%).*

Conclusion: *B-cell lymphoma intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (cHL) is a distinct entity of lymphoma characterized by intermediate histological and immunophenotypical features between cHL and DLBCL.*

Key words: *Lymphoma, Grey zone, Hodgkin.*

Introduction

Recently more light has been shed on the overlap in biologic and morphologic features between classic Hodgkin lymphoma (cHL) and mature B-cell lymphoma[1]. This overlap was further substantiated by analyzing the neoplastic

nature of the malignant cells of cHL, which were proven to be of B-cell origin in nearly all cases[2]. This advocated the introduction of the new term "grey zone lymphoma", which was used for the first time in 1998 in the "Workshop on Hodgkin's disease and related diseases"[3].

According to WHO classification 2008, the term "grey zone lymphoma" designates anterior mediastinal involvement with lymphoma intermediate between DLBCL and cHL[4]. On morphologic basis this lesion is characterized by sheet like growth of pleomorphic tumor cells with Hodgkin-Reed-Sternberg (HRS)-like morphology admixed with a varying degree of inflammatory background formed of mature lymphocytes, eosinophils and plasma cells[5]. The tumor characteristically shows fibrous stroma reminiscent of nodular sclerosis variant of Hodgkin lymphoma[1]. Geographic necrosis is usually present which, unlike necrosis of cHL, do not show neutrophilic infiltrate[4]. Immunophenotypically, the tumor cells show combined expression of B-cell markers (CD20, CD79a, PAX5) and Hodgkin markers (CD30, CD15)[6]. ALK-1 is consistently negative. Surface or cytoplasmic immunoglobulins are also absent. EBV has been detected in less than 20% of cases[4].

Materials and Methods

Patient samples:

Thirteen consultation cases of B-cell lymphoma intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (cHL) lymphoma diagnosed between 2003 and 2011 were retrieved from the files of the Department of Pathology and Laboratory Medicine, Nagoya University hospital, Japan. This study was approved by the institutional review board of Nagoya University Hospital.

All the examined slides were from excisional biopsies. The histological features were evaluated in 4μm thick hematoxylin and eosin-stained sections of formalin-fixed paraffin-embedded tissue.

Immunohistochemistry:

Tissue sections were stained with antibodies directed against

CD20, CD79a, CD15, CD30, PAX5, MUM1, Fascin, ALK1, CD3, CD10, BCL-2, BCL-6, P53, Ki-67, CD45RO, EMA, κ and λ. Appropriate positive and negative controls for all the selected immunostains were used. Histological and immunohistochemical data were assessed by three pathologists of the authors (Megahed, Asano and Kato).

In situ hybridization study:

The presence of EBV small ribonucleic acids was examined by in situ hybridization using EBV-encoded small nuclear early region (EBER) oligonucleotides on formalin- fixed, paraffin-embedded sections. Briefly, a DAKO hybridization kit was used with a cocktail of fluorescein isothiocyanate-labeled EBER oligonucleotides (one oligonucleotide corresponding to EBER1 and the other to EBER2, both 30 bases long; DAKO A/S code Y 017). Hybridization products were detected with a mouse monoclonal anti- fluorescein isothiocyanate (DAKO M878), a Vectastain ABC Kit (Vector, Burlingame, CA) using immunoperoxidase techniques and 3, 3'-diamino- benzidine (DAB) as the chromogen. RNase A or DNase I pre- treatment was used for the negative controls, and EBER-positive Hodgkin disease specimens were used as positive controls.

Statistical Analysis

Differences in characteristics between the two groups were examined by the chi-squared test, Fisher exact test, Student t test, or Mann-Whitney U test as appropriate. Patient survival data were analyzed by the Kaplan-Meier method. Differences in survival were tested by the log-rank test. Survival for this study was evaluated in terms of disease-specific survival (DSS), measured from the date of diagnosis to the date of death due to a lymphoma-related cause. In DSS analysis, patients were examined at the time of death if this was from a lymphoma-unrelated cause, while deaths from treatment- related causes were classified as death from lymphoma. All data were analyzed with the aid of STATA software (version 10.0; Stata Corp., College Station, Texas).

Results

Histopathological examination of the cases showed 4 cases re-

sembling DLBCL while 9 cases were closer in their histopathology to cHL. All the cases were characterized by sheets of highly pleomorphic cells; some of them were Hodgkin-Reed- Sternberg like cells (HRS-like cells). The background showed mixed inflammatory infiltrate formed mainly of lymphocytes, plasma cells and eosinophils (figure 1; A, B). The density of the inflammatory infiltrate was mild in 4 cases, moderate in 2 cases and severe in 7 cases. All the cases showed sclerosis in the form of admixture of thick compartmentalizing (Figure 1; C) and thin mesh-like fibrous bands (Figure 1; D).

Necrosis was recognized in all the cases and constituted less than 20% of the tumor area except one case in which necrosis reached up to 40% of the tumor area (Figure 1; E). In contrast to cHL, the necrosis in all the cases was devoid of any neutrophilic infiltrate.

The pattern of the tumor growth was diffuse in 7 cases, sinusoidal in 1 case, nodular in 4 cases and vaguely nodular in 1 case. Two cases showed associated granulomatous response in the form of multiple epithelioid granulomas.

Immunohistochemical staining summarized in Table (1) revealed that CD20 was positive in more than 30% of the malignant cells in all the examined cases (figure 1;F). Other B-cell markers also, namely CD79a and PAX5, were positive in all cases. CD30 was positive in all but 2 cases (figure1; G). CD15 was positive in 4 cases including the two CD30-negative cases (figure1; H). Fascin was positive in 7 cases including one of the CD30-negative cases. Mum1 was positive in all but one case (case no.8). None of the examined cases showed EBV, ALK-1, CD10, κ or λ positivity. P53 was positive in 8 cases. EMA was detected in less than 10% of the tumor cells in 3 cases. Ki-67 index ranged from 40% to 90% (mean=64%). Three cases showed Bcl-6 positivity.

Table (1): Immunophenotypic features of B-cell lymphoma intermediate between DLBCL and cHL.

Case no.	CD20	CD79a	PAX5	CD30	CD15	Fascin	MUM1	EMA	Bcl2	P53	Ki67	CD45RO	Bcl6
1	+	+	+	-	+	+	+	-	+	-	90%	-	-
2	+	+	+	+	-	-	+	-	-	+	80%	-	+
3	+	+	+	+	-	+	+	-	-	+	50%	-	-
4	+	+	+	+	-	+	+	10%	+	+	50%	-	+
5	+	+	+	-	+	-	+	10%	+	+	90%	-	-
6	+	+	+	+	-	-	+	-	-	-	50%	-	-
7	+	+	+	+	-	-	+	-	+	-	70%	-	-
8	+	+	+	+	-	+	-	-	-	+	60%	-	+
9	+	+	+	+	-	+	+	-	+	-	40%	-	-
10	+	+	+	+	-	-	+	-	+	+	40%	-	-
11	+	+	+	+	-	-	+	-	-	+	80%	-	-
12	+	+	+	+	+	+	+	-	+	-	70%	-	-
13	+	+	+	+	+	+	+	5%	-	+	60%	-	-

All cases were negative for CD3, CD5, CD10, kappa, lambda, EBER in situ hybridization, and ALK1.

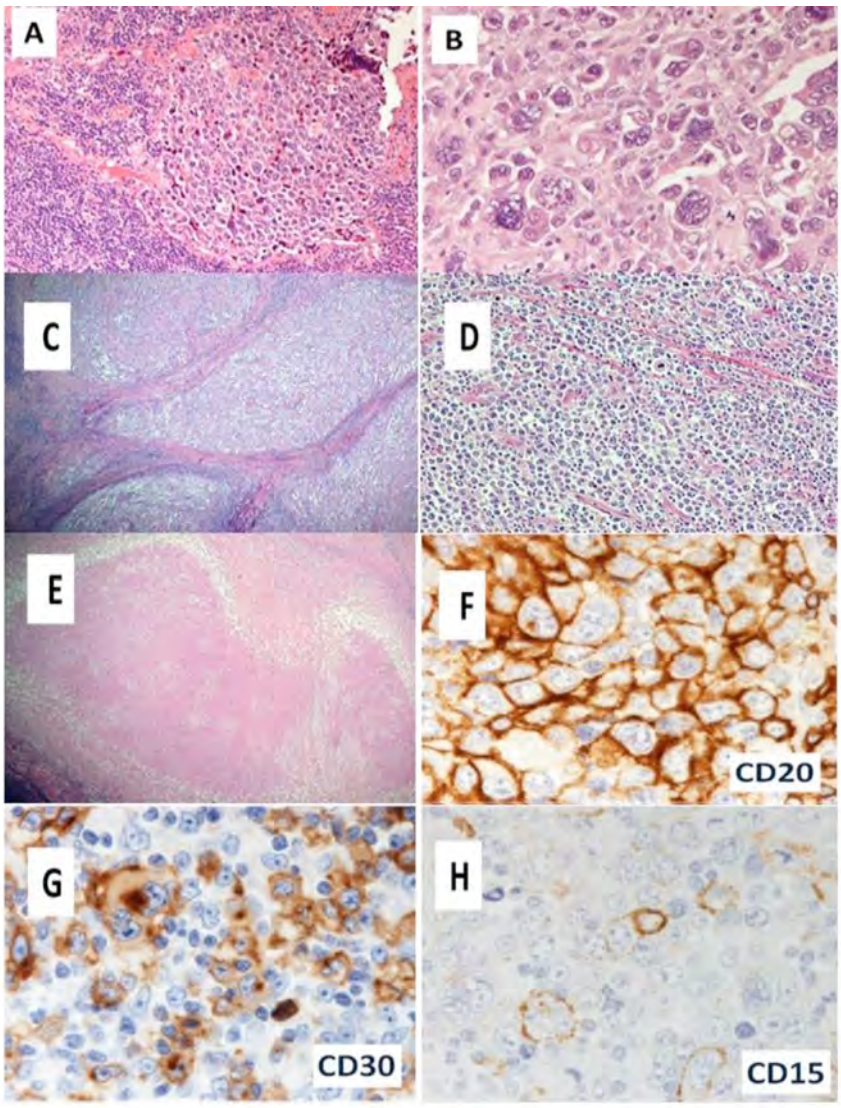

Fig. 1: Hisologic and immunophenotypic features of the cases of avDLBCL. Sheets of pleomorphic cells arranged in sinusoidal pattern (A) The cells show high degree of anaplasia with HRS-like cells and giant cell formation admixed with inflammatory background (B) Thick compartementalizing fibrous bands giving the tumor nodular pattern(C) Thin mesh like fibrous bands (D) Wide areas of geographic necrosis (E) Strong membranous staining with anti-CD20 (F) Membranous and Golgi staining with anti-CD30 (G). Some cases showed membranous staining with anti-CD15 (H).

Discussion

Distinguishing between Hodgkin and non-Hodgkin lymphoma has always been the main task of hematopathologists for ages. Their decision is the main guide for clinicians to implement the strategy plan of chemotherapy. Although clearer cut criteria are currently used in the WHO lymphoma classification 2008; which is attributed to the advances in immunohistochemistry, cytogenetic and molecular pathology; segregation between Hodgkin and Non-Hodgkin lymphoma cases has not always been an easy job. Cases with intermediate histologic and immunophenotypic features are now on the record. Many of these cases have been noticed in the mediastinum and the term "mediastinal grey zone lymphoma" (MGZL) is now officially applied in the WHO classification 2008 to designate such intermediate cases. In the study performed by Traverse-Glehen et al about MGZL they reported 6 cases of mediastinal composite lymphoma of cHL and mediastinal large B-cell lymphoma (MLBCL), and 9 cases of sequential cHL and MLBCL from which they concluded that such cases might represent a continuum[8]. Gracia et al postulated that the recognition of such cases in the mediastinum might signify existence of favorable mediastinal microenvironment for their development[5].

However, cases of such morphologic and immunophenotypic features have been recognized recently at extramediastinal sites[5]. Gracia et al reported 9 cases of DLBCL with Hodgkin features of which 3 cases occurred extramediastinally. Portlock et al also reported a series of 25/248 cases of cHL showing more than 50% positivity of the tumor cells for CD20[9].

The main pathological characteristic feature of our cases was the discrepancy between morphologic and immunophenotypic features; cases of cHL-like morphology showed pan B-cell marker positivity (CD20, CD79a, PAX5) while cases morphologically closer to DLBCL showed CD30 and CD15 positivity[10,11]. In contrast to cHL [12,13,14], our series showed P53 and Bcl-6 positivity in a number of cases which are usually detected among high-grade B-cell tu-

mors or among cases of aggressive transformation of cHL[15].

The underlying pathogenesis of evolution of grey zone lymphoma is still mysterious. However, recent reports analyzing molecular biology of cHL and MLCL revealed similarities in genetic alterations. This was explained by Traverse-Glehen et al that B-cells are capable of transformation to either HRS cells or neoplastic large B-cell[1]. The precise molecular events deciding such transformation are yet undetermined. During such complex steps of transformation the progression of B-cell might stop in an intermediate-stage giving rise to grey zone lymphoma. On the other hand, Gracia et al suggested that such intermediate lymphomas might arise by aggressive progression from cHL [5]. Further study of such cases in the future will contribute to lift the curtain on the biologic links between Hodgkin and non-Hodgkin lymphoma.

In conclusion, B-cell lymphoma intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (cHL) represents a distinctive subtype of lymphoma showing histological and immunophenotypic features intermediate between DLBCL and cHL. Further studies of these cases will reveal more links between Hodgkin and non-Hodgkin lymphoma and offer better treatment strategies.

Conflict of interest:

The authors declare to have no conflict of interest.

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IMMUNOHISTOCHEMICAL PROFILING OF B-CELL LYMPHOMA INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND CLASSICAL HODGKIN LYMPHOMA

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EFFECT OF ERYTHROPOIETIN AND STEM CELLS ON ANIMAL MODEL OF CHRONIC NEPHROPATHY

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Abstract

Aim: *the purpose of this study was to assess the effects of bone marrow derived mesenchymal stem cells and Darbopoietin alpha on adriamycin- induced chronic renal disease in rats.* ***Methodology:*** *80 male Sprague Dwaley rats divided into 4 groups (20 rats each); (Group I): normal (negative) control group received saline as a vehicle, (Group II): positive control (twice intravenous injection of ADR via penile vein at fourteen days interval with no treatment given. (Group III): twice injection of ADR plus darbepoetin alpha (Aranesp) subcutaneously once weekly for twelve weeks. (Group IV): twice injection of ADR plus twice intravenous injection of MSCs each one was administrated 5 days after each adriamycin injections.* ***Results:*** *Administration of subcutaneous darbepoietin alpha favored survival of adriamycin nephrosis in rats and significantly improved animal body weight when compared with positive control group and also had beneficial effect on histolopathological and biochemical parameters such as blood hemoglobin level, renal functions manifested by decrease in BUN, prevented glomerular filtration barrier damage by adriamycin as shown by amelioration of proteinuria, while, twice intravenous injection of MSCs produces non significant improvement of animal survival rate, body weight, blood hemoglobin level, proteinuria, hypoalbuminemia of adriamycin nephropathy model in rats while, it produced significant improvement in BUN and hyperlipidemia.* ***Conclusion:*** *Concomitant administration of darbepoietin alpha with adriamycin treatment from the first day had a protective effect manifested by improvement in both biochemical and histopathological parameters. On the other hand, MSCs injection significantly produces histological protection on both glomeruli and tubu-*

lointerstitial region of the kidney but couldn't modify clinical parameters as (body weight, animal mortality, anemia, proteinuria) indicating that MSCs only provide partial protection that did not modify outcome of adriamycin nephrosis.

Introduction

The prevalence of chronic kidney disease has been growing consistently for the past decades. This alarming increase in chronic and end-stage renal disease is accompanied and promoted by a growing prevalence of cardiovascular risk factors such as obesity, diabetes, and hypertension that increase the overall morbidity and mortality in these patients. Therefore, there is an urgent need to identify the mechanisms that perpetuate and aggravate renal dysfunction and scarring, and to develop strategies to prevent and attenuate them[1]. ADR-induced renal failure is a well-accepted chronic disease model of progressive glomerulosclerosis in rats[2] which mirrors that seen in human CKD due to primary focal segmental glomerulosclerosis[3]. It is characterized by rapid development of proteinuria and glomerulosclerosis and evolves to renal fibrosis and renal failure. The use of cell therapy has been suggested as a potential modality to improve the course and outcome of kidney injury[4]. Bone marrow derived mesenchymal stem cells(MSCs) are a source of multipotent cells having the potential of tissue regeneration in experimental models of myocardial infarction[5] neurological disease[6], and acute kidney injury[7]; infusion of murine[8] and human MSCs[9] in mice with acute kidney injury decreased renal tubular injury and ameliorated renal function impairment, which translated into reduced animal mortality. But, the therapeutic potential of MSCs in animal models of chronic nephropathies has not been completely established so far[10]. EPO is a glycoprotein hormone, primarily produced by renal cortical and outer medullary fibroblasts in response to hypoxia[11]. The expression of EPO R in non-erythroid tissues such as the brain[12], retina[13], heart[14], kidney[15], smooth muscle cells[16], myoblasts[17] and vascular endothelium[18] has been associated with the discovery of novel biological functions of endogenous Epo

signalling in non-haematopoietic tissues. Studies on acute renal failure have demonstrated the beneficial effect of erythropoietin on renal function[19] However, the effects of EPO on chronic renal failure have been poorly investigated[20].

So, the present work was conducted to investigate the possible protective effects of darbepoietin alpha and mesenchymal stem cells on the outcome of chronic adriamycin nephropathy model in rats.

Materials and Methods

1. Experimental Animals:

The materials of this work comprised of eighty male Sprague-Dwaley rats weighing 200-250 gm, aging 2-3 months, bred and housed in the animal house of Mansoura Experimental Research Center (MERC). These animals were housed at temperature 20°C-25°C and fed standard laboratory chow, and had free access to tap water. The experimental protocol was approved by the Local Ethical Committee, Faculty of Medicine, Mansoura University.

Study Design:

Eighty rats were assigned to the following four experimental groups (n = 20/group):-

1. Group A: vehicle (negative control):

Rats received tail-vein injection of comparable volume of 0.9% saline.twice at a 14-day interval.

2. Group B: Adriamycin (positive control):

Rats received tail-vein injection of ADR (pharmacia Italia,SPA) (4 mg/kg body weight saline) twice at a 14-day interval.

3. Group C:(ADR+darbepoetin alpha):

Rats received tail-vein injection of ADR (4 mg/kg body weight) twice at a 14-day interval plus 0.3 μg/kg body weight darbepoetin alpha (Aranesp) (Amgen Europe B.V Breda, Netherlands) subcutaneously once weekly for four weeks.

4. Group D: (ADR + MSC):

Rats received tail-vein injection of ADR (4 mg/kg body weight) twice at a 14-day interval) plus twice injection of MSCs ($2x10^6$) cells at a 14-day interval, each one administrated 5 days after adriamycin injections.

Collection of blood and urine samples:

By the end of the experiment, rats were weighed and sacrificed using an overdose of thiopental (75 mg/kg body weight, i.p.). Blood samples were taken by heart puncture then placed into 2 sets of tubes; one set is EDTA-containing tubes for assessment of haemoglobin and the other set are tubes without anticoagulant that were centrifuged at 3000x g for 15 minutes, and stored at -20°C till the time of biochemical analysis.

Hemoglobin content was assessed at the time of sacrifice for each group, but the other investigated biochemical parameters such as serum albumin, creatinine, BUN, serum triglyceride and serum cholesterol were assessed for all groups by the end of the study.

The rats were placed in a metabolism cage for 24 hours in order to collect 24-hour urine from 8 a.m. to 8 a.m. of the next day. The urine volume was measured, and then a sample was taken for estimation of urine protein.

Harvesting kidney specimens:

By the end of the experiment both kidneys were removed and bisected into 2 equal halves. Then they rapidly fixed into 10% neutral formalin.

Histopathological examination:

The kidney specimens were processed for paraffin blocks and sections of 3-µm thickness were made and stained with haemtoxylin and eosin and prussian blue.

Results

Effect of DPO and MSC on animal survival:

Nineteen of twenty rats (95%) in the negative control group (group I) survived 12 weeks, while survived rats were eight of twenty (37%) in positive control group (group II), fifteen of twenty (75%) in DPO-treated group (group III) and eleven of twenty (55%) in MSC treated group (group III) (Fig.1). Compared with negative control group, positive control group showed significant increase in animal mortality ($p=0.001$). Treatment with DPO caused significant attenuation in animal mortality rate ($p=0.02$), while MSCs therapy caused non-significant attenuation in animal mortality rate ($p=0.3$).

Effect of DPO and MSC on animal weight:

Compared to negative control group (group I), positive control group (group II) and MSCs treated group (group IV) showed significant decrease in body weight (P<0.001) respectively, while DPO treated group (group III) showed no significant decrease in body weight.

As regard to the effect of treatment on body weight, DPO treated group showed statistically significant increase in body weight (P<0.001) when compared with positive control group while MSCs treated group showed non-significant increase in body weight. In addition, there was statistically significant difference in body weight between DPO treated group and MSCs treated group (P=0.01) being higher in DPO treated group (table 1).

Effect of DPO and MSC on blood hemoglobin level:

There was statistically significant decrease in haemoglobin level in positive control group (group II) (P<0.001), DPO treated group (group III) (P=0.004) and MSCs treated group (group IV) (P<0.001) when compared to negative control group (group I).

Compared with positive control group (Group II), it was shown that, there was statistically significant increase in haemoglobin level in DPO treated group (group III) (0.002), while MSCs treated group (group IV) showed no significant increase in hemoglobin level, in addition, there was no statistically significant difference between MSCs treated group (group IV) and DPO treated group (Group III) (Table 2).

Effect of DPO and MSC on serum BUN & creatinine:

Positive control group (group II) had significant higher BUN level (P<0.001) when compared to negative control group (group I). As regard to effect of DPO injection (group III), there was statistically significant decrease in BUN level when compared to positive control group (group II) and to MSCs treated group (group IV) (P<0.001 and P=0.016) respectively with no statistically significant difference DPO treated group (group III) and negative control group(group I). As regard to effect of MSCs injection,

there was statistically significant decrease in BUN level when compared to positive control group (group II) (P=0.014) but still significantly higher than DPO treated group (group III) (P=0.016), while, there was no statistical significant difference among all groups as regard to serum creatinine(table 3) .

Effect of DPO and MSC on Serum cholesterol and serum triglycerides:

Positive control group (group II) showed significant increase in serum triglycerides (P<0.001) and cholesterol (P<0.001) when compared to negative control group (group I).

As regard to effect of subcutaneous DPO treatment, DPO treated group (Group III) showed significant reduction in serum triglycerides (P<0.001) and serum cholesterol (P=0.002) when compared with positive control group (group II) but when compared with negative control group (group I), serum triglycerides still had significant higher value (P=0.005) while there was no statistically significant difference as regard to serum cholesterol.

In MSCs treated group (group IV), there was significant reduction in serum triglycerides (P=0.017) and serum cholesterol (P=0.011) when compared with positive control group (group II) but when compared with negative control group (group I), serum cholesterol still had significant higher value (P=0.005) while there was no statistically significant difference as regard to serum triglycerides. In addition, there was statistically significant difference between serum triglycerides or cholesterol between DPO treated group (Group III) and MSCs treated group (group IV).

Effect of DPO and MSC on urinary protein excretion (mg/ 24 hr) and serum albumin (g/ dl):

Positive control group (group II) had marked and significant increase in urinary protein excretion (P<0.001) when compared to negative control group (group I).

As regard to effect of subcutaneous DPO treatment, DPO treated group (group III) showed significant reduction in urinary protein excretion (P<0.001) when com-

pared with positive control group (group II) but when compared with negative control group (group I) there was no statistically significant difference.

In MSCs treated group (group IV), there was significant reduction in urinary protein excretion (P<0.001) when compared with positive control group (Group II) but when compared with negative control group (group I) and DPO treated group (Group III), urinary protein excretion still had significant higher value (P<0.001 and 0.004) respectively (table 5).

As regard to serum albumin, positive control group (group II) had statistically lower serum albumin level (P<0.001) when compared with negative control group (group I).

As regard to effect of subcutaneous DPO treatment, DPO treated group (group III) showed statistically higher serum albumin level (P=0.012) when compared with positive control group (group II) but when compared with negative control group (group I) there was no statistically significant difference.

In MSCs treated group (group IV), there was no significant reduction in serum albumin level (P<0.001) when compared with negative control group (group I). Also, there was no statistically significant difference in serum albumin level when compared with either positive control group (Group II) or DPO treated group (Group III).

Effect of DPO and MSC on renal morphology:

Light microscopic examination revealed that kidney lesions are present mainly in the cortex, outer strip of the outer medulla and to less extent in inner strip of outer medulla and inner medulla.

In the cortex, the lesion was mainly glomerular where the positive control group (group II) showed significant higher number of podocyte detachment (P<0.001), podocyte vacuolization (P<0.001), capillary dilatation and also, parietal epithelial cell vacuolization of bowman's capsule (P<0.001) when compared to negative control group (group I) (Fig.2).

As regard to changes in tubulo-

intestitial region of positive control group (group II) in the cortex and outer strip of outer medulla, there was significantly higher number of necrotic dilated tubules filled with hyaline protein casts (P<0.001), when compared to negative control group (group I). Also, there was mild degree (grade I) of inflammatory cell infiltrate and fibrosis in periglomerular region, deep cortex and medullary rays in positive control group (group II) but theses lesions were absent in normal control group (group I). Also, in inner strip of outer medulla and inner medulla region of positive control group (group II) there was only necrotic dilated necrotic dilated tubules filled with hyaline protein casts with no fibrosis or inflammatory cell infiltrate (fig.3).

As regard to effect of treatment on chronic adriamycin nephropathy, both DPO treated group (group III) and MSCs treated group (group IV) significantly decrease all signs of glomerular damage and number of necrotic dilated tubules, also, decrease degree fibrosis and inflammatory cell infiltrate but effect on fibrosis not reach statistically significant when compared with positive control group (group II) (fig.4).

When comparing DPO treated group (group III) with MSCs treated group (group IV), there was no significant difference between them except for necrotic tubules in OSOM which was higher in MSCs treated group (group IV). Another characteristic finding of MSCs treated group (group IV), that there was significant increase in all signs of regeneration in OSOM as, solid sheets of cells, tubules with large vesicular nuclei, solid tubules and dilated tubules with festooned nuclei when compared with other groups (fig.5).

Table (1): Animal weight (g) among the studied groups.

		Negative Control group (no=19)	Positive control group (no=8)	DPO group (no=15)	MSCs group (no=11)
Body weight (g)		**294.44±16.09**	**220.33±21.25**	**273.64±24.60**	**232±25.40**
P value	**vs. negative control group**	----------	<0.001	NS	<0.001
	vs. positive control group	<0.001	----------	<0.001	NS
	vs. DPO group	NS	<0.001	----------	0.01
	vs. MSCs group	<0.001	NS	0.01	----------

Table (2): Hemoglobin (gm/dl) among the studied groups.

		Negative Control group (no=19)	Positive control group (no=8)	DPO group (no=15)	MSCs group (no=11)
Body weight (g)		**294.44±16.09**	**220.33±21.25**	**273.64±24.60**	**232±25.40**
P value	**vs. negative control group**	----------	<0.001	NS	<0.001
	vs. positive control group	<0.001	----------	<0.001	NS
	vs. DPO group	NS	<0.001	----------	0.01
	vs. MSCs group	<0.001	NS	0.01	----------

Table (3): Comparison between blood urea nitrogen and serum creatinine among the studied groups.

		Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
Negative control group		**0.56±0.08**	**39.79± 5.32**
Positive control group		**0.95±0.55**	**261.18±116.52**
Darbepoietin group		**0.79±0.42**	**64.36± 17.40**
MSCs group		**0.82±0.33**	**160.90±84.08**
P value	**gp I vs. gp II**	NS	<0.001
	gp I vs. gp III	NS	NS
	gp I vs. gp IV	NS	<0.001
	gp II vs. gp III	NS	<0.001
	gp II vs. gp IV	NS	0.014
	gp III vs. gp IV	NS	0.016

Table (4): Comparison between serum cholesterol and serum triglycerides among the studied groups.

		Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)
Negative control group		77.76± 28.86	63.62± 7
Positive control group		408.40±126.54	536.87 ±313.52
Darbepoietin group		225.22±116.59	205.78 ±86.23
MSCs group		261.63±87.45	255.60±78.02
P value	**gp I vs. gp II**	<0.001	<0.001
	gp I vs. gp III	0.012	NS
	gp I vs. gp IV	0.005	NS
	gp II vs. gp III	<0.001	0.002
	gp II vs. gp IV	0.011	0.017
	gp III vs. gp IV	NS	NS

Table (5): Comparison between urinary protein excretion mg /24 h and serum albumin g/dl among the studied groups.

		Urinary protein excretion (mg /24 h)	Serum albumin (gm/dl)
Negative control group		**2.70±1.81**	**3.76±0.61**
Positive control group		**90.82± 46.21**	**2.22±0.75**
Darbepoietin group		**21.73± 14.46**	**3.14±1.14**
MSCs group		**69.53± 27.78**	**2.33±0.42**
P value	**gp I vs. gp II**	<0.001	<0.001
	gp I vs. gp III	NS	NS
	gp I vs. gp IV	<0.001	0.003
	gp II vs. gp III	<0.001	0.012
	gp II vs. gp IV	NS	NS
	gp III vs. gp IV	0.004	NS

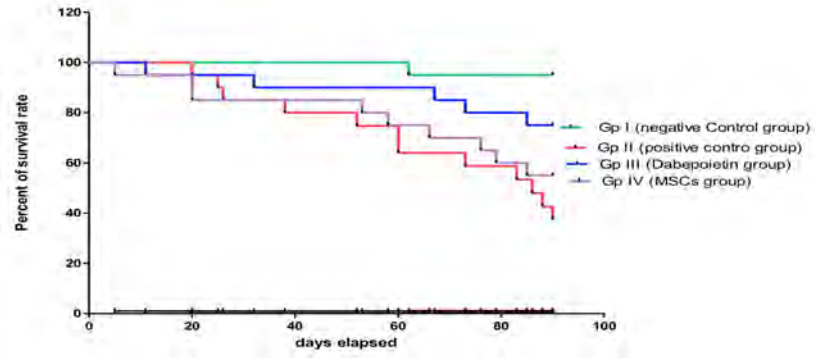

Fig. 1: Kaplan-Meier survival curves of the studied groups.

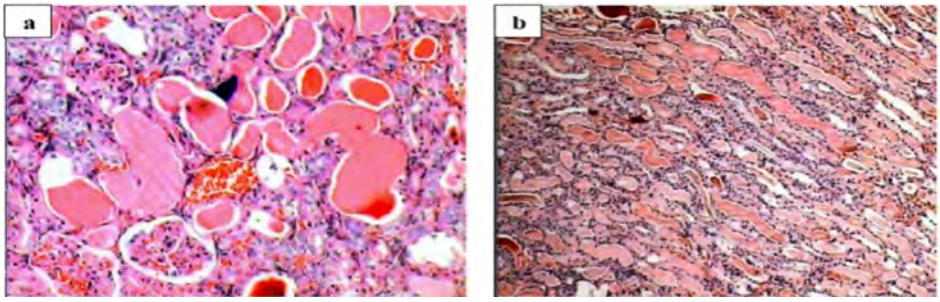

Fig. 2: kidney sections of positive control group showing dilated atrophied tubules filled with protein casts in deep cortex (a) and in medulla(b)
(a, magnification x400), (b, magnification x100).

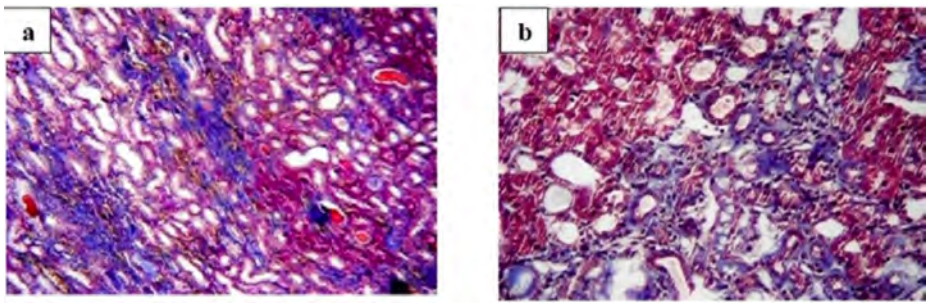

Fig. 3: kidney sections of positive control group showing fibrosis in deep cortex and outer strip outer medulla (MT) (a,b magnification x400).

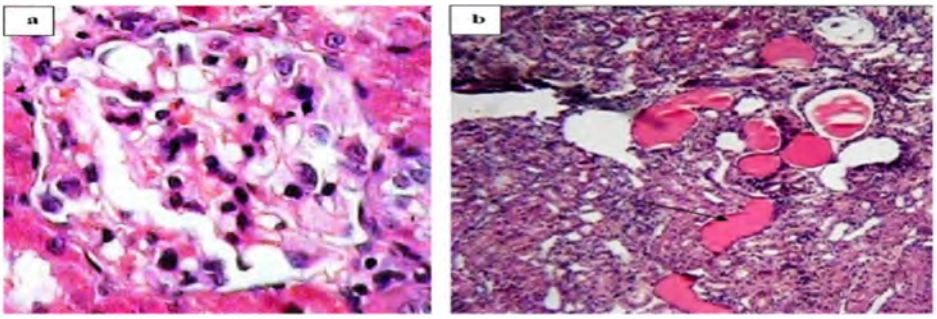

Fig. 4: kidney sections of DPO treated group showing partially preserved glomerular architecture(a) and lesser dilated tubules that filled with casts (H&E) (a, magnification x400), (b, magnification x100).

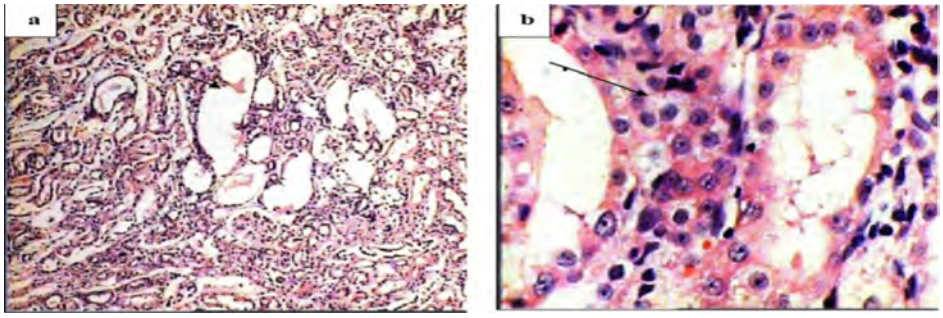

Fig. 5: kidney sections of MSCs treated group showing lesser degree of inflammatory cell infiltrate (H&E) (a) solid sheets of cells indicating regeneration (H&E).

Discussion

In the present study, administration of adriamycin to rats caused significant increase in mortality rate, reduction in body weight and decrease in blood hemoglobin level when compared with those of the negative control group at the end of experimental period. In agreement with these findings Ammar et al.,[21] found that adriamycin exposure resulted in a significant decrease in hemtocrite levels, thus reducing the oxygen-carrying capacity of the blood.

Moreover, adriamycin caused marked elevation in blood urea nitrogen without significant change in serum creatinine level. These results are in agreement with many investigators. Liu et al.,[22] mentioned that adriamycin injection caused renal injury as evi-

denced by marked elevation of BUN while serum creatinine level was normal. Moreover, Magnasco et al.,[23] found that after adriamycin injection renal failure developed after 3 months of follow-up in all rats although without reaching statistical significance in serum creatinine comparison with con¬trol rats, because of a wide dispersion of creatinine values. Furthermore, Marshall et al.,[24] who studied the effect of warm ischemia reperfusion on kidney functions, found that after 14 days serum creatinine return to normal, while blood urea nitrogen remained elevated and he speculated that Serum urea appeared to be a more sensitive index of renal damage than creatinine.

Also, in the present work, adiamycin injection caused hyperlipidemia in the form of hypercholesterolemia and increased triglyceride. This is consistent with Hong et al.,[25] who speculated that this may be due inhibition of carnitine palmitoyl transferase system (CPT I) and lowered the level of cytochrome P450 which may in turn depress cholesterol 7α hydroxylase activities, the key enzyme in conversion of cholesterol to bile acids. In addition, The lipoprotein lipase activity, which varies inversely with free fatty acid levels, decreased dramatically in adriamycin-treated rats. Moreover, cholesterol ester synthase was increased[26]. Also, O'Donnell and Michael,[27] reported that proteinuria, through some unknown mechanism, appears to cause HDL abnormalities that facilitate accumulation of triglyceride-rich VLDL.

In the present study, urinary protein excretion was used to assess the function of the glomerular filtration barrier, it was found that all rats treated with ADR had marked proteinuria, this proteinuria is consistent with the presence of hyaline protein casts presented in the tubular lumen. The impairment of glomerular filtration barrier in adriamycin nepropathy model is consistent with many investigators and could be attributed to is reduction of glomerular charge selectivity and the restriction of larger solutes impaired, causing proteinuria[28].

In the current work, all rats of

adriamycin nephropathy rats had lower plasma albumin as a consequence of losing protein in urine. This glomerular dysfunction was also confirmed by histolopathological examination under light microscope showing significant increase in number of detached podocytes due to either apoptosis or loss of negative charge. Also, there was an increase in number of vacuolized podocytes and epithelial cells in the adriamycin nephropathy when compared with normal control group. Vacuolization of podocytes and parietal epithelial is consistent with general proteinuric condition in this model in which, studies by Weening et al.,[29] on experimental models of protein overload proteinuria showed that increased transcapillary movement of proteins causes degenerative changes of glomerular epithelial cells characterized by swelling, vacuolization, increased reabsorption droplets, loss of foot processes, and lifting from the underlying glomerular basement membrane.

In addition to glomerular lesion in this model, there is also involvement of tubules and tubulointerstitial region, this is in agreement with Noiri et al.,[30] who reported in a study done on adriamycin nephropathy in rats that tubules and interstitium play a pivotal role in progressive kidney disease and are more predictive of the renal outcome.

The present study provides strong evidence that darbepoietin alpha protects the rat kidney in a model of adriamycin nephropathy. The results of this work demonstrated that the administration of darbepoietin alpha produces a significant increase in the body weight, hemoglobin level and serum albumin and significant decrease in the blood urea nitrogen, serum triglycerides, serum cholesterol, level of urinary protein excretion.

In consistence with the laboratory findings, the morphological changes showed that darbepoietin alpha reduced the renal glomerular damage at the end of experimental period. By significant decrease of number of detached podocytes, number of apoptotic bodies and number of dilated capillaries and vacuolized epithelial

cells. Also, the morphological changes showed that darbepoietin alpha reduced the renal tubular-interstistial damage in the form of reducing urinary protein cast in tubules, inflammatory cell infiltrate and fibrosis though not reaching statistitically significant difference when compared with adriamycin non treated group (group II). These findings are in agreement with those reported by others who demonstrated that EPO treatment improved the functional and morphologic glomerular and tubular injury in rats subjected to adriamycin nephropathy[31,32].

The improvement of body weight, hemoglobin level and BUN in adriamycin model by DPO is consistent with Noiri et al.,[33] who found similar results against adriamycin induced cardio renal injury in rats. Furthermore speculated that correction of anemia to the normal level by DA is an important mechanism of action because it retards the progression of CKD and cardiovascular diseases.

In the current work, in order to elucidate the effect of darbepoetin on the glomerular barrier function, urine protein level was assessed, it was found that darbepoetin ameliorates proteinuria when comparing DPO treated group with positive control group. The effect of DPO on proteinuria may be indirect effect due to an increase in oxygen-carrying capacity might improve structures and functions of podocytes,or direct effect as suggested by Eto et al.,[34] who reported that darbepoietin may provide direct protection on podocytes, the major culprit accounting for proteinuria through acting on EPO-R in podocytes and that DPO treatment ameliorated podocyte injury and reduced proteinuria by preventing the disruption of actin cytoskeleton and the reduction of nephrin through the binding to the EPOR to activate Janus-tyrosine kinase 2, phosphoinositide 3 kinase, and protein kinase B (Akt). It is noteworthy that several reports showed the direct interaction of Akt and actin.

Twice intravenous injection of MSCs produced improvement in, not reach statistically significant value, animal survival rate, body weight. blood hemoglobin level, proteinuria, hypoalbuminemia of

adriamycin nephropathy model in rats while, it produced significant improvement in BUN and hyperlipidemia. Also, histipathlogical finding revealed that MSCs provided histological protection of glomeruli manifested by decrease in detached podocytes per glomerulus, apoptotic bodies, aneurysmal dilated capillaries and vacuolized podocyte or parietal epithelial cells, while in tubulointerstitial region. Also, there was a significant decrease in number of necrotic tubules. Inflammatory cell infiltrate showed non-significant decrease in MSC-treated rats. As regard to fibrosis in this group it was minimal or absent, with appearance of histological findings of regeneration as Solid sheets, tubules with large vesicular nuclei and solid tubules.

This might be attributed to either failure of anemia correction by MSCs, time of stem cells administration or due to decrease number of engrafted cells in kidney after injection. Noiri et al.,[36] mentioned that anemia can share in progression of CKD, in the present study it was chosen to administrate cells five days post adriamycin injection to provide sufficient time for injury process to occur, it is known that injury is essential for homing to occur and also MSC and also in a more clinically relevant setting of intravenous infusion via intravenous route at the time of evident proteinuria.

Adequate homing of MSC to the injured tissue is important for effective therapy. As regard to efficient homing iron labeled cells technique was used to track cells in the kidney, it was found that homing occurred but few MSCs was detected in the glomeruli and tubulointerstitial region.

As regard route of administration, in the current, MSC is administered through a standard intravenous route. A disadvantage of the systemic intravenous delivery of MSC can be low uptake at the site of injury. Indeed, significant engraftment of injured tissue was observed in some studies [37,38], but Schrepfer et al.,[39] demonstrated that the systemic intravenous route of administration was not appropriate for MSC to reach their site of activation.

Zonta et al.,[40] showed that the intra-arterial administration of MSC were the most effective route to achieve immunomodulating effects in experimental kidney transplantation, which primarily occurs because large MSC (15 mm to 19 mm) remain trapped in the capillaries of the small lung filter, which in turn causes the inadequate homing of MSC to the injured tissue. However, using the renal artery as the injection route to administer MSC to treat DN may be associated with the following 2 major complications: (1) renal infarcts and loss of function, and (2) ectopic differentiation into adipocytes within glomeruli[41].

Conclusion

Concomitant administration of darbepoietin alpha with adriamycin treatment from the first day has a protective effect manifested by improvement in both biochemical and histopathological parameters,while MSCs injection significantly produces histological protection on both glomeruli and tubulointerstitial region of the kidney but couldn't modify clinical parameters as animal mortality, anemia, proteinuria and hyperlipidemia indicating that MSCs only provide partial protection that did not modify outcome of adriamycin nephrosis.

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EFFECT OF ERYTHROPOIETIN AND STEM CELLS ON ANIMAL MODEL OF CHRONIC NEPHROPATHY

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EFFECT OF ISS-ODN-INDUCED TLR9 STIMULATION ON EXPERIMENTALLY INDUCED AIRWAY INFLAMMATION IN MICE

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Abstract

We have used a mouse model of allergen-induced airway inflammation to demonstrate that ISS-ODN inhibit airway inflammation and remodeling in acute and chronic asthma. Inflammatory cells in mice treated with ISS-ODN decreases in acute and chronic asthma compared to positive control mice. Also, more than one dose of ISS-ODN decrease IL-4. While one dose increases Th1 cytokines (IFN-γ). ISS-ODN decreases airway remodeling such as thickness of epithelium and peribronchial fibrosis which is related to the increase of IFN-γ but not the decrease of IL-4 as other studies. So ISS-ODN before allergen exposure provide a new immunotherapy in asthma.

Introduction

Asthma is associated with a wide range of symptoms and signs, including wheezing, cough, chest tightness, shortness of breath and sputum production. The symptoms and signs evolve from three basic characteristics that underlie the disease: airway obstruction, AHR, airway inflammation[1]. Airway inflammation in asthma may represent a loss of normal balance between two "opposing" populations of Th lymphocytes. A cytokine imbalance towardTh2 will set the stage to promote the production of IgE antibody and subsequent allergic inflammation[2].

Toll-like receptors (TLRs) are a type of pattern recognition receptor (PRR) that recognize foreign substances, activate immune cell responses and play a key role in the innate immune system, and receive its name from their similarity to the Toll gene identified in

Drosophila[3]. TLRs Form a receptor superfamily with interleukin-1receptors (Interleukin-1 receptor/Toll-like receptor superfamily) so called TIR (Toll-IL-1 receptor). TIR domain recruits four adapters' molecules which are MyD88, Tirap, Trif and Tram in order to propagate a signal. These adapters activate other molecules including certain protein kinases (IRAK1, IRAK, TBK1, and IKKi) leading to the activation of two pathways both lead to nuclear translocation of transcription factor, NF-?B which lead to induction of inflammatory cytokines such as TNF-α and IL-12[4,5].

Synthetic (ODN) bearing unmethylated CpG motifs can mimic the immune-stimulatory effects after recognized by TLR9. Strong immunostimulatory effects are driven by sequences containing unmethylated CpG motifs these motifs appear to function as Th1-promoting adjuvants capable of switching the usual Th2 response toward a Th1 response[6]. CpG DNA directly activates denteritic cells and B-cells that express TLR9, binds CpG DNA and transduces its immune-stimulatory effects and indirectly activates other cells of the immune system such as T- lymphocytes cell, Natural killer cells and neutrophils, by altering the expression of specific cytokines, receptors, and adhesion molecules[7]. ISS-ODN activates the TLR- 9 pathway and promotes the development of a Th1 response and generates cytokines, such as TNF-α, IL-12, IFN-α and indirectly, IFN-γ. Long term treatment with ISS through induction of IL-12 and IFNs would lead to a rebalancing of Th1/Th2 response [8], which leads to decrease in airway inflammation Th2cytokine expression and airway hyperresponsiveness, and increases the expression of Th1 cytokines (IFN-γ, IL-12). It has also been shown that ISS-ODN attenuate allergen-induced airway remodeling in mice as manifested by decreased subepithelial collagen deposition[9].

Materials and Methods

1. Materials

A. Drug: Oligonucleotides

Endotoxin-free (<1ng/mg DNA) phosphorothioate ISS-ODN (5'-TGACTGTGAACGTTCGAGATGA-3') (Trilink, San Diego, CA) were used in the in vivo experiments.

B. Animals

One hundred and twenty Female BALB/c mice were obtained from Theodor Bilharz Research Institute, Cairo and were used when reached 8-10 weeks of age (25-30 gm weight) and fed normal diet.

C. Reagent: Imject alum

Aqueous solution of aluminum hydroxide (40 mg/ml) and magnesium hydroxide (40 mg/ml), (50 ml) (Pierce Biotechnology, Inc., USA).

2. Experimental Design

A. Induction of acute asthma

A total number of sixty BALB/c mice were randomly divided into the following three groups (20 mice per group).

Group A: Control Group (n=20)

Mice were sensitized subcutaneously on Days 0, 7, 14, and 21 with 100µl PBS to which 100µl imject alum is added. The inhalation challenge (days 27,29 and 31) consisted of three 30 minutes inhalations separated by 30 minutes rest intervals of PBS in an inhalation chamber, the nebulizer was set up to aerosolize 100 ml in the 30 minutes inhalation time period[10].

Group B: Positive Control Group (n =20)

Mice were sensitized subcutaneously on Days 0, 7, 14, and 21 with 100 µg of OVA(OVA, grade V; Sigma, St. Louis, MO) per mouse in 100µl PBS to which 100µl imject alum is added drop wise and mixed for 30 minutes to allow adsorption of OVA. The OVA inhalation challenge (days 27,29 and 31) consisted of three 30 minutes inhalations separated by 30 minutes rest intervals of OVA at a concentration of 10 mg/ml in an inhalation chamber the nebulizer was set up to aerosolize 100 ml in the 30 minutes inhalation time period [10].

Group C: Treated Mice group (n=20)

Mice were administered intraperitoneal ISS-ODN (100 µg in 100 µl of PBS) starting one day before the first intranasal OVA challenge on day 26[10].

B. Induction of chronic Asthma

A total number of sixty BALB/c mice were randomly divided into the following three groups (20 mice per group).

Group A: Control Group (n=20)

Mice were sensitized subcutaneously on Days 0, 7, 14, and 21 with 100µl PBS to which 100µl imject alum is added. Intranasal challenges with PBS were administered on Days 27, 29, and 31 under ether anesthesia. Intranasal OVA challenges were then repeated twice a week for six months[11].

Group B: Positive Control Group (n=20)

Mice were sensitized subcutaneously on Days 0, 7, 14, and 21 with 100 µg of OVA per mouse in 100µl PBS to which 100µlimjectalum is added drop wise and mixed for 30 minutes to allow adsorption of OVA. Intranasal OVA challenges (20 µg/50 µl in PBS) were administered on Days 27, 29, and 31 under ether anesthesia. Intranasal OVA challenges were then repeated twice a week for six months[11].

Group C: Treated Mice group (n=20)

Mice were administered intraperitoneal ISS-ODN (100 µg in 100 µl of PBS) starting one day before the first intranasal OVA challenge on Day 27, and then continuing every other week one day before intranasal challenges for six months[11].

3- Methods

Histopathological Study

For histopathological examination lungs were collected 24 hours after the last ovalbumin exposure. Animals were anesthetized with ether and dissected then lungs were collected. Sections were stained with haematoxylin and eosin (H&E)[12]. Quantitive analysis of inflammatory response was examined using Olympus camedia (Olympus imaging corp, japan), this include number of inflammatory cells, thickness of epithelium and thickness of collagen layer.

Blood collection

Blood was collected from the heart into open dry tube kept for thirty minutes at room temperature to clot and centrifuged at 4000 rpm for fifteen minutes then the serum was collected and stored at -70°C to be used for measurement of IL-4 and IFN-γ.

Tissue Homogenization

Lung tissue homogenized ac-

cording to ELISA kits manufacturer method, Tissue samples are collected, weighed (400-500mg) and added to lysis buffer (100 mM sodium phosphate, 150 mMNaCl, pH 7.4 at 1 ml PBS/100 mg tissue) then tissues are homogenized using glass grinder, Following homogenization, the tissue preparation is clarified by centrifugation for 15 minutes at 1,500 x . The supernatant should be removed from the pellet, aliquot and stored at –70°C or below until it is analyzed for cytokine content.

Measurement of Serum and-Lung Cytokines (IFN-γ, IL-4)

The concentrations of IFN-γ and IL-4 were assayed in serum and lung tissue by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Pierce biotechnology, Rockford, USA).

Statistical Analysis

Statistical calculations were carried out using GraphPad Instat3 computer program (GraphPad software Inc., version 3.05, 2000 California, USA).

Statistical analysis was carried out using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.

Results

1- Effect of ISS-ODN (100µg/100µl) on IFN-γ and IL-4 levels in both serum and lung homogenate in acute cases.

- Table (1) Figure (2) and (3) show that in control mice IFN-γ serum level was (213.4±0.7 pg/ml) and in lung homogenate was (132.3±1 pg/ml).

- The concentration of IFN-γ in mice treated with ISS-ODN increased significantly in serum compared to control mice (610±39.8 pg/ml vs 213.4±20.8 pg/ml) and increased significantly in lung homogenate compared to control mice (552±53 pg/ml vs 132.3± 23.2 pg/ml).

- The concentration of IFN-γ in mice treated with ISS-ODN increased significantly in serum compared to positive control mice (administered ovalbumin) (610±39.8 pg/ml vs 269.4±32.3 pg/ml) and increased significantly in lung homogenate compared to positive control mice (adminis-

tered ovalbumin) (552 ±53 pg/ml vs 207±15.8 pg/ml).

- And in figure (4) and (5) show that in control mice IL-4 serum level was (5.7±1.8 pg/ml) and in lung homogenate was (15.5±3.1 pg/ml).

- Concentration of IL-4 in positive group (administered ovalbumin) increased insignificantly compared to control group in serum (6.3±1.1 vs 5.7±1.8 pg/ml) but increased significantly compared to control group in homogenate (113±28.5 vs 15.5±3.1 pg/ml).

- Concentration of IL-4 in mice treated with ISS-ODN decreased insignificantly compared to positive group in serum (6.2±0.9 vs 6.3±1.1 pg/ml) and decreased insignificantly compared to positive group in lung homogenate (102±15 vs 113.7±28.5 pg/ml).

- Concentration of IL-4 in mice treated with ISS-ODN was insignificantly higher than control group in serum (6.2±0.9 vs 5.7± 1.8 pg/ml), and significantly higher than control group in lung homogenate (102±15 vs 15.5±3.1 pg/ml).

2- Effect of ISS-ODN (100µg/100µl) on IFN-γ and IL-4 levels in both serum and lung homogenate in chronic cases.

- Table (2), Figure (6) and (7) show that serum level of IFN-γ in control mice was (213.4 ± 20.8 pg/ml) and in lung homogenate was (132.3±23.2 pg/ml).

- The concentration of IFN-γ in mice treated with ISS-ODN increased significantly compared to positive group (administered ovalbumin) in serum (1313.4±229.5 pg/ml vs 213±27 pg/ml) and increased significantly compared to positive group in lung homogenate (386.5±55.3 pg/ml vs 148.7±2.8 pg/ml).

- The concentration of IFN-γ in mice treated with ISS-ODN increased significantly compared to control group in serum (1313.4 ±229.5 pg/ml vs 213±27 pg/ml) and increased significantly compared to control group in lung homogenate (386.5 ±55.3 pg/ml vs 132.3±23.2 pg/ml).

- While in figure (8) and (9) show that level of IL-4 in control mice was in serum (5.7±1.8 pg/

ml) and in lung homogenate (15.5±3.1 pg/ml).

- Concentration of IL-4 in positive group (administered ovalbumin) increased significantly compared to control mice in serum (57.1±27.vs 5.7±1.8 pg/ml) and increased significantly compared to control mice in lung homogenate (35.2±3.4 vs 15.5±3.1 pg/ml).

- Treatment with ISS-ODN decreased significantly the concentration of IL-4 compared to positive group in serum (6.8±1 vs 57.1±27.4 pg/ml) and decreased significantly the concentration of IL-4 compared to positive group in lung homogenate (19.5±4 vs 35.2±3.4 pg/ml).

3- Effect of ISS-ODN (100µm/ 100µl) on airways inflammatory and epithelial changes in mice with acute asthma.

- During one month of sensitization and challenge with ovalbumin and treatment with ISS-ODN the mortality rate was 30%.

As shown in table (3) and in figure (10) sensitization and inhalation challenge with ovalbumin for one month increase the number of inflammatory cells significantly in this positive group (171.6±3.3) compared to control group (23.2±4.1); these inflammatory cells are mainly neutrophils , few eosinophils are found. A single dose of ISS-ODN (100µm/100 µl) decreases the number of inflammatory cells significantly (94.8±4) compared to positive group (administered ovalbumin) (171.6±3.3).

As shown in table (3) and in figure (11) sensitization and challenge with ovalbumin increase the thickness of epithelial layer significantly (39±3.3 µm) compared to control group (19±1.9µm). Treatment with ISS-ODN decreases the thickness of epithelial layer significantly (25.1±6.2µm) compared to positive group (39±3.3µm).

As shown in table (3) and figure (12) peri-bronchial fibrosis increases significantly in positive group mice which sensitized and challenged with ovalbumin (10.4±3µm) compared to control group mice (3±0.25µm). Single dose of ISS-ODN (100?g/100µl) decreases fibrosis to normal thickness.

4- Effect of ISS-ODN (100µm/ 100µl) on airways inflammatory and epithelial changes in mice with chronic asthma

During six months of sensitization and inhalation challenge with ovalbumin and treatment with ISS-ODN the mortality rate was 25%.

As shown in table (4) and figure (13) number of inflammatory cells increases significantly in mice sensitized and challenged with ovalbumin for six months (175.8±11.2) compared to control mice (23.2±3.6) while in mice treated with ISS-ODN, the number of inflammatory cells decreases significantly (59±2.3) compared to mice sensitized and challenged with ovalbumin (175.8±11.2), These inflammatory cells are mainly lymphocyte and macrophages (foam cells). Most mice administered ovalbumin for six weeks form lymphoid follicles which are aggregation of inflammatory cells.

As shown in table (4) and figure (14) the mean thickness of epithelium in positive group mice (administered ovalbumin) (57.3 ±7.4µm) increases significantly compared to control mice (19±1.9µm). on the other hand mice treated with ISS-ODN shows significant decrease in the thickness of epithelium (31.3±1 µm) than in positive group mice (57.3±7.4µm).

As shown in table (4) and figure (15)sensitization followed by inhalation challenge with ovalbumin for six months results in collagen layer formation either peribronchial or parenchymal fibrosis. The thickness of sub-epithelial layer of collagen in mice treated with ISS-ODN (12.6±1.9 µm) decreases significantly compared to positive group mice (27.7±4 µm).

Table (1) : Effect of ISS-ODN (100 µg/100 µl) on IFN- and IL-4 level in both serum and lung homogenate in acute cases.

parameter	Treatment / Biological Fluid	Control Group (PBS) Pg/ml	Positive Group (OVA) Pg/ml	Treated Group (OVA+ISS-ODN) Pg/ml
IFN- γ	Serum	213.4±0.7	269.4 ±32.3	610 ±39.8*#
	Lung Homogenate	132.3 ±1	207 ±15.8	552 ±53*#
IL-4	Serum	5.7 ±1.8	6.3 ±1.1	6.2 ±0.9
	Lung Homogenate	15.5 ±3.1	113.7 ±28.5*	102 ±15*

- * significant compared to Control group at p<0.001
- # significant compared to positive group at p<0.001

Table (2): Effect of ISS-ODN (100 µg/100 µl) on IFN- and IL-4 levels in both serum and lung homogenate in chronic cases.

Parameter	Treatment / Biological fluid	Control Group (PBS) Pg/ml	Positive Group (OVA) Pg/ml	Treated Group (OVA+ISS-ODN) Pg/ml
IFN- γ	Serum	213.4 ±20.8	213 ±27	1313.4 ±229.5*#
	Lung Homogenate	132.3 ±23.2	148.7 ±2.8	386.5 ±55.3*#
IL-4	Serum	5.7 ±1.8	57.1 ±27.4*	6.8 ±1#
	Lung Homogenate	15.5 ±3.1	35.2 ±3.4*	19.5 ±4#

- * significant compared to Control group at p<0.001
- # significant compared to positive group at p<0.001

Table (3): Effect of ISS-ODN (100 µm/100 µl) on airways inflammatory and epithelial changes in mice with acute asthma.

Treatment / Body Fluids	Control group (PBS)	Positive group (OVA)	Treated group (OVA+ISS-ODN)
No.of inflammatory Cells /field at 400x	23.2 ±4.1	171.6 ±3.3*	94.8 ±4#
Thickness of epithelium (µm)	19 ±1.9	39 ±3.3*	25.1 ±6.2#
Thickness of Sub-epithelium Layer of Collagen (µm)	3 ±0.25	10.4 ±3*	3 ±0.25#

- * significant compared to Control group at p<0.001
- # significant compared to positive group at p<0.001

Table (4): Effect of ISS-ODN (100 μm/100 μl) on airways inflammatory and epithelial changes in mice with chronic asthma.

Treatment / Body Fluids	Control group (PBS)	Positive group (OVA)	Treated group (OVA+ISS-ODN)
No.of inflammatory Cells /field at 400x	23.2 ±3.6	175.8 ±11.2*	59 ±2.3*#
Thickness of epithelium (μm)	19 ±1.9	57.3 ±7.4*	31.3 ±1*#
Thickness of Sub-epithelium Layer of Collagen (μm)	3 ±0.25	27.7 ±4*	12.6 ±1.9*#

- * significant compared to Control group at p<0.001
- # significant compared to positive group at p<0.001

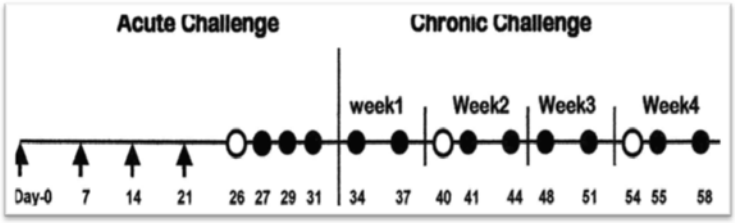

Fig. (1): Illustrated design for acute and chronic induction of asthma.

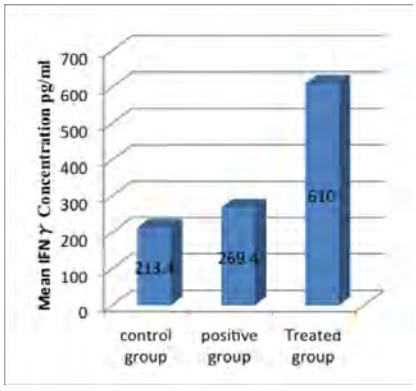

Fig. (2): Effect of ISS-ODN (100μg/100μl) on IFN-γ level in serum in acute cases.

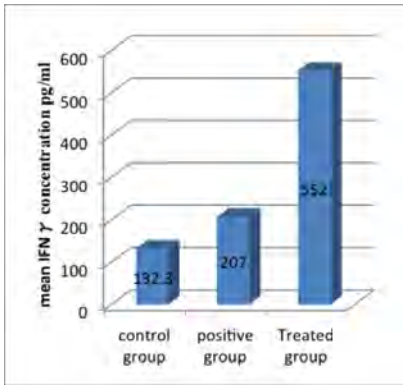

Fig. (3): Effect of ISS-ODN (100μg/100μl) on IFN-γ level in lung homogenate in acute cases.

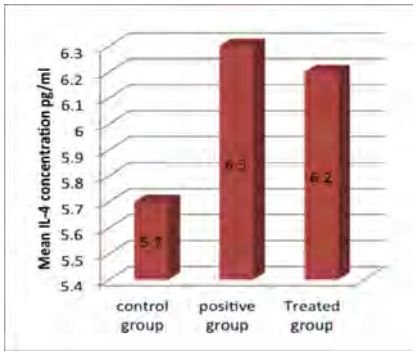

Fig. (4): Effect of ISS-ODN (100µg/100µl) on IL-4 level in serum in acute cases.

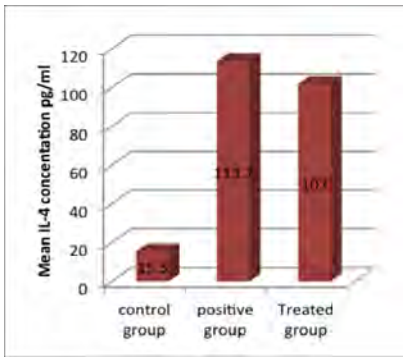

Fig. (5): Effect of ISS-ODN (100µg/100µl) on IL-4 level in lung homogenate in acute cases.

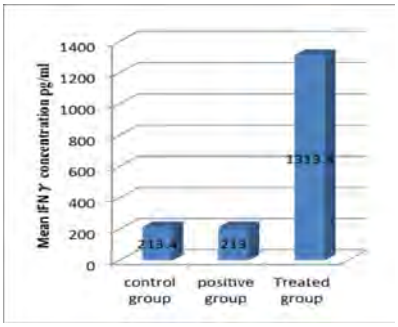

Fig. (6): Effect of ISS-ODN (100µg/00µl) on IFN-γ level in serum in chronic cases.

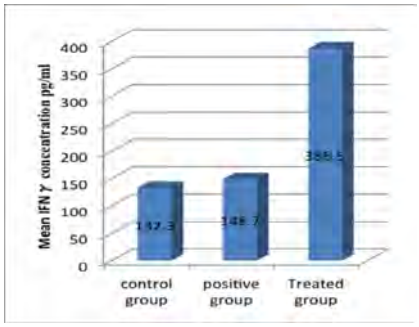

Fig. (7): Effect of ISS-ODN (100µg/100µl) on IFN-γ level in lung homogenate in chronic cases.

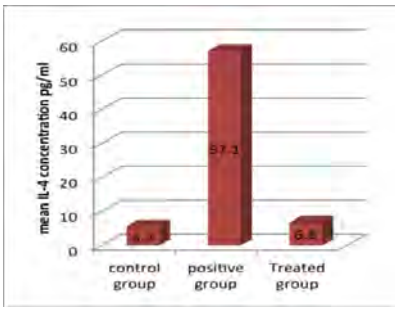

Fig. (8): Effect of ISS-ODN (100µg/100µl) on IL-4 level in serum in chronic cases.

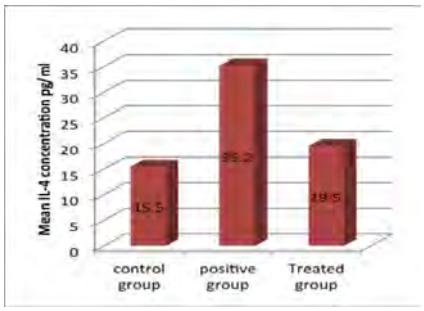

Fig. (9): Effect of ISS-ODN (100µg/100µl) on IL-4 level in lung homogenate in chronic cases.

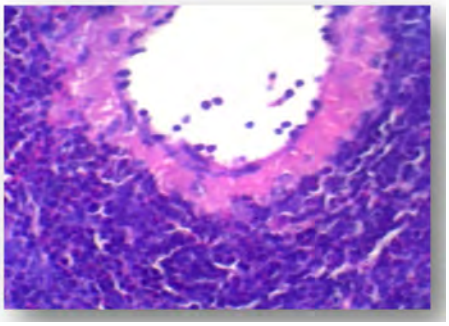

Fig. (10): Significant increase in number f inflammatory cells (neutrophils) in challenged mice with OVA x10.

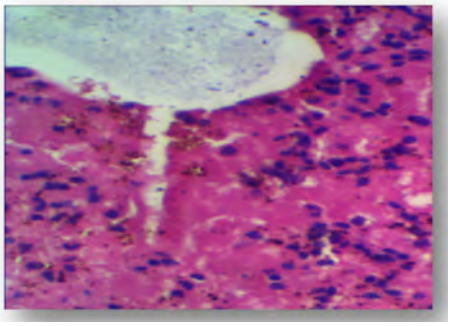

Fig. (10): Marked decrease in number of inflammatory cells (neutrophils) in mice treated with ISS-ODN. x10.

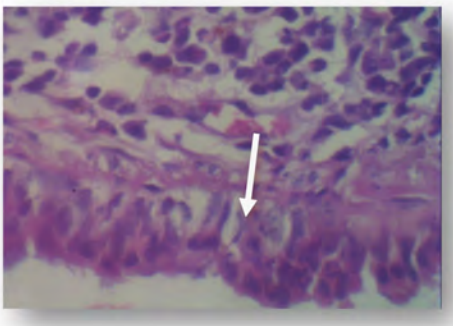

Fig. (11): Significant increase in thickness of epithelium in mice challenged ovalbumin. x40.

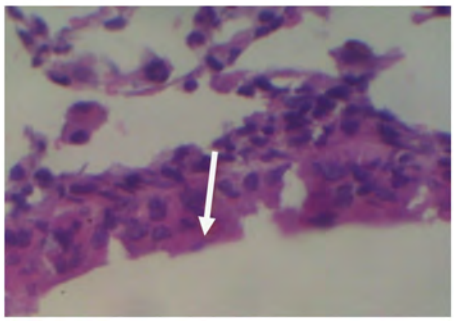

Fig. (11): Significant decrease in thickness of epithelium in mice treated with ISS-ODN. x40.

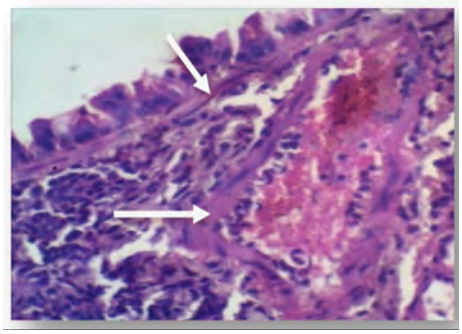

Fig. (12): Peri-bronchial fibrosis in mice challenged with ovalbumin x10.

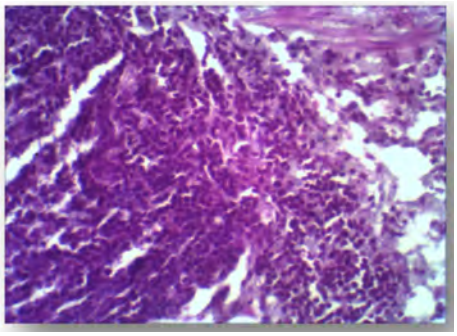

Fig. (13): Marked increase in number of lymphocytes in mice induced with ovalbumin x10.

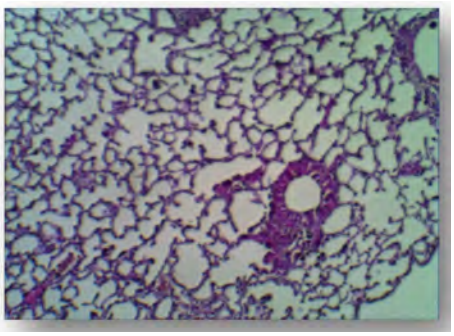

Fig. (13): Significant decrease in number of lymphocytes in mice treated with ISS-ODN x10.

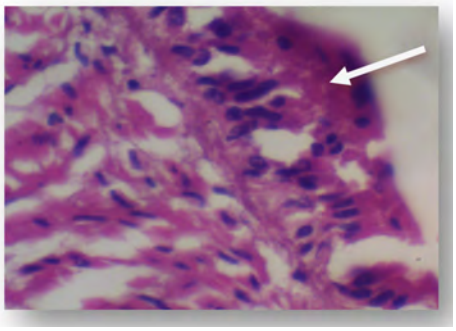

Fig. (14): Marked increase in thickness of epithelium in mice challenged with OVA. x40.

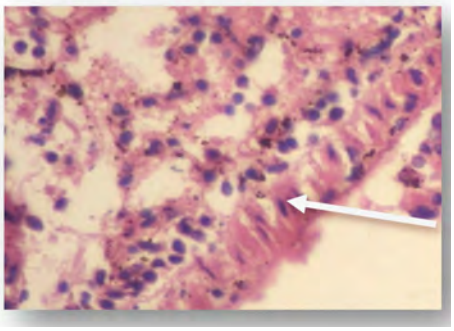

Fig. (14): significant decrease in thickness of epithelium in mice treated with ISS-ODN x40 .

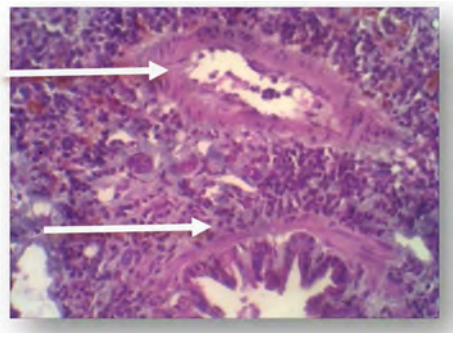

Fig. (15): Peri-bronchial fibrosis in mice challenged with ovalbumin. x10.

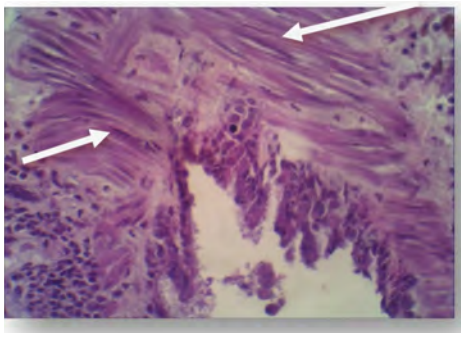

Fig. (15): Peri-bronchial fibrosis in mice challenged with ovalbumin. x10.

Discussion

Asthma is not only an airway obstruction but it is a more complicated inflammatory disorder in which many inflammatory cells and cellular elements play a role, [13]. Airway inflammation in asthma is a multicellular process involving mainly neutrophils, eosinophils, T lymphocytes and mast cells[14] and in this study we agree with many recent studies that show neutrophil infiltration after induction of asthma by ovalbumin in BALB/c mice and that neutrophils not eosinophils involved in induction of late asthma response after the fourth challenge[15]. The increased level of neutrophil is related to acute; sever asthma and a lower level of eosinophils[16,17]. Also, a study shows that after OVA challenge of sensitized BALB/c mice neutrophils are the first inflammatory cells detected in the mice airways[18]. Also, we agree with the results that stated that aggregation of inflammatory cells (mostly lymphocytes) was observed in the lung tissue of mice after chronic challenge with low dose of ovalbumin[19,11].

We agree with the results that showed that treatment with ISS-ODN restrict inflammatory cell infiltration in murine airways in both acute asthma[10] and chronic asthma[11], however, in this study that the number of inflammatorty cells in mice treated with ISS-ODN still more than normal and this can be explained in the basis of switching T-lymphocytes from Th2 to Th1 with the remaining of total number of T-lymphocytes without great change.

We agree with many studies that our results show various changes are observed including increase the thickness of epithelium and peri-bronchial fibrosis after sensitization and challenge with OVA in both acute and chronic cases. After quantitative analysis and comparison between ovalbumin challenged mice group and ISS-ODN treated mice group in both acute and chronic models of asthma, ISS-ODN treatment decreased features of airway remodeling[20].

In opposite to the study that demonstrated that the decrease of Th2 cytokines as a result of ISS-

ODN drug was related to the decrease in airway remodeling and not the increase in Th1 cytokines[10] our study demonstrated that in acute model of asthma ISS-ODN decrease features of airways remodeling despite the high level of IL-4 which concurrent with a high level of Th1 cytokines IFN-γ. While in chronic model of asthma the decrease in airway remodeling was related to both decreases in Th2 cytokines and increase in Th1 cytokines and as conclusion this underlies a suggestive direct role of IFN-γ against airway remodeling.

In this study, ISS-ODN decreased Th2 cytokines IL-4 in chronic model of asthma only, while in acute model of asthma the level of IL-4 in mice treated with ISS-ODN was still more than normal significantly. It could be explained that one only dose of ISS-ODN in acute asthma could not affect the highly proliferated inflammatory cells while more than one dose of ISS-ODN (in chronic model of asthma) could affect inflammatory cells activation and Th2 cytokines production. Also, ISS-ODN increased Th1 cytokines IFN-γ in acute model asthma which means that one only dose (acute case) can give effect on resting naïve T lymphocytes (Th 0) to be activated to Th1. Also, several doses of ISS-ODN increase IFN-γ level than normal in chronic model of asthma. We agree with the results that explained that it is evident that ISS both induces Th1 responses and inhibits Th2 responses in chronic model of asthma[11].

Summary

We can conclude our results that ISS-ODN can restrict inflammatory cell infiltration, decrease airway remodeling, increase Th-1 cytokines production (IFN-γ) in both acute and chronic models of asthma while more than one dose of ISS-ODN can decrease Th-2 cytokines production (IL-4) so more studies are needed to explain the suggestive direct role of IFN-γ in treatment of airway inflammation and remodeling.

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EFFECT OF ISS-ODN-INDUCED TLR9 STIMULATION ON EXPERIMENTALLY INDUCED AIRWAY INFLAMMATION IN MICE

Mona M. El-Haroun M.Sc., Ali M. Yousef MD and Tarek M. Ibrahim Ph.D

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PROGNOSTIC AND PREDICTIVE VALUES OF KI67 PROLIFERATIVE INDEX IN DIFFUSE LARGE B -CELL LYMPHOMA

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Abstract

Purpose: *To evaluate role and prognostic significance of Ki-67 proliferation index (PI) in diffuse large B cell lymphoma. Methods: Expression of Ki-67 protein was examined immunohistochemically in 34 tumor specimens from patients newly diagnosed with DLBCL and treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen. Results: Univariate analysis showed that a high Ki-67 PI (≥60%) was found in 11 patients (42.3%) whose age was ≤ 60 years compared with 62.5% of older patients (age > 60 years) (P=0.025), and in 46.2% of patients with PS=0-1 (0–1) compared with 71.4% of patients with PS=2 (P=0.03). Three-year survival was 52.8%±7% in the patients with a high index (mean OS 48.3%±2.8 months, median 76 months) and 76%±4.7% in those with a low index (mean OS 76.8±3.6, median not reached, P=0.013). Disease free survival was higher in patients with Ki67<60% than those with Ki67≥60%, and the difference was statistically significant (p=0.042). Multivariate analysis performed by Cox model revealed that IPI≥3 and high Ki-67PI had a significant independent prognostic value concerning overall survival (p<0.05).*

Conclusion: *Initial high Ki 67≥60% associated with high IPI score could represent possible predictive factors of poor prognosis, which would help to identify a high risk subgroup of newly diagnosed DLBCL.*

Keywords: *Diffuse large B cell lymphoma, IPI, prognostic factors, Ki67, immunohistochemistry.*

Introduction

Diffuse Large B-cell Lymphoma (DLBCL) is the most common subtype of Non-Hodgkin's Lymphoma (NHL), comprising about 30% of all NHL cases in all epidemiological reports[1,2] and it accounts for 80% of aggressive lymphomas 3.

The CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen alone or with rituximab (CHOP/R) is standard therapeutic approach for the most patients who have DLBCL. Although DLBCL can be cured with the current chemotherapy regimens, the long-term survival is estimated to be only 50% for high-risk patients 3, a substantial minority of patients (about 30%) are not cured[4,5].

While International Prognostic Index (IPI) was developed to provide a model system for predicting the outcome of patients with aggressive NHL depending on some clinical parameters (age, stage, performance status, number of extranodal sites and serum level of lactic acid dehydrogenase)[6], asubstantial variability in outcome has been observed despite IPI subgroups[7]. Thus, identifying new prognostic parameters might contribute towards better prediction of outcome and the development of effective risk-adaptive strategies[8].

Patients with similar DLBCL diagnoses can have varied molecular profiles, heterogeneous clinical presentations, and clinical outcomes. Several immunohistochemical algorithms and gene profiling sets have been developed to identify DLBCL subgroups with unfavorable prognosis[9,10]. The hallmark features of the tumor cell phenotype, which contribute to aggressive tumor behavior,are: its capacity for sustained proliferation,disregard of signals to stop proliferation and differentiation and the capacity to invade and promote angiogenesis[11]. Despite the sustained research in recent years, risk-adapted therapies based on DLBCL phenotype are still in the development stage.

The Ki67 antigen plays a pivotal role in maintaining cell proliferation and is expressed in all phases of the cell cycle except G0 and has been extensively used as a marker of cell proliferation in a variety of neoplastic[12] and non-neoplastic disorders[13]. The monoclonal antibody anti Ki67 was the only available till the early 90s and it has the inherited drawback that it could be applied only to sections from fresh frozen tissues. Recently using a recombinant parts of Ki67 protein as an immu-

nogen, an equivalent monoclonal antibody, MIB-1 has been developed[12] and can be utilized as a routine stain on paraffin -embedded sections from fixed tissues through microwave process[14] providing an interest in the prognostic validation of MIB-1in different human tumors including NHL.

The aim of this study was to assay Ki-67 immunohistochemically in patients with newly diagnosed diffuse large B cell lymphoma, correlated with IPI regarding to clinical course and outcome.

Material and Methods

Thirty four patients were enrolled in the current studyat the Clinical Oncology and Nuclear Medicine Department, and Department of Pathology, Mansoura University Hospital in a period betweenJanuary 2004 and December 2008. The eligibility criteria for the patients in this study were as follows: age between 18 and 70 years, Eastern Cooperative Oncology Group (ECOG) performance status 0-2, histologically confirmed diffuse large B cell Lymphoma.Adequate hematologic profile and organ function were required for study entry: a neutrophil count greater than 2000/ mm^3, platelet count greater than 100 000/mm^3, serum bilirubin, SGOT, SGPT, serum creatinine within normal limit and normal electrocardiogram. No other history of active malignancy and no other serious medical disease.

Central nervous system lymphoma, post-transplant lymphoproliferative disorders, cutaneous lymphomas, T-cell/histiocyte-rich large B-cell lymphoma and Burkitt lymphoma were excluded.

Patients included in the study had an initial diagnosis of DLBCL according to the World Health Organization (WHO) classification[15]. In all patients; standard clinical, radiological and laboratory data were collected: age, gender, Ann Arbor stage, extranodal sites, bulky disease, clinical stage, B symptoms and LDH[13]. Bulky disease was defined as a mediastinal mass larger than one third of the maximum thoracic diameter and/ or any node over 10cm. Nodal organ was assigned to those cases with a clinical presentation in lymph nodes, waldeyer's ring,

spleen or bone marrow by the criteria of Kramer et al.[16]. Extranodal organ was defined as presentation in other sites with or without local lymph node involvement. The IPI was calculated according to the five high risk features: age >60 years, performance status (PS) >2, Ann Arbor tumor stage 3 or 4, LDH >460 IU/μL, and number of extranodal sites >1 while patients were divided into a low risk group (0-2 factors) and a high-risk group (3 or 4 factors)[17].

All patients treated with CHOP (cyclophosphamide, doxorubicine, vincristine, and prednisone) regimen. Complete re-staging was scheduled at the end of the treatment program and periodic follow up examinations were performed every 3 to 4 months for the first 2 years after completion of therapy and every 6 months thereafter.

Tissue Microarray Construction:

Tissue microarrays (TMAs) were prepared at the Department of Pathology,Mansoura University Hospital from 34 representative patient samples with DLBCL with adequate archival formalin-fixed and paraffin-embedded material.

Five-μm thick paraffin sections from the samples were mounted onto poly-L-lysine coated slides (Sigma, Milan, Italy) and dried over-night at 37°C. Subsequently, sections were de-waxed in xylene, rehydrated according to histopathological standards. Slides were immersed in antigen re-trieval solution (BioGenex Cat. No. HK 090-5K) at a dilution of 1:4 with deionized water. The slides were processed for two cycles of 5 minutes at maximum power (1000w) in a mi-crowave oven (Goldstar, USA). Sections were never allowed to dry. The sections were incubated with the monoclonal antibody MIB-1 (Bi-oGenex ready-to-use) at room temperature overnight in a humid air and successively with a biotinylated rabbit anti-mouse IgG serum. Then, treated with an avidin-biotin system (No-vostain super ABC kit, NovocastraLaborato-ries, Newcastle, UK) for 30 minutes followed by rinsing with buffer for 10 minutes. The anti- gen-antibody complex was visualized using DAB (3, 3`-diaminobenzidine tetrahydrocloride) and then counterstained with haematoxylin. A reac-

tive LN with follicular hyperplasia was used as positive control; negative controls were obtained by omission of the primary monoclonal antibody. The cell Image analyzer system was used for Ki-67quantitation. A cell was considered positive when it exhibits a weak, moderate or strong nuclear and/or nuclear staining. At least 1000 cells from the most representative areas of each tumor were scored and the MIB-1 index was evaluated as the ratio between positive cells and total tumor cells. The percentage of tumor cells with Ki-67+ nuclear staining on 10 different high power microscopy fields (HPF, 400x) was determined.

Statistical analysis:

SPSS version 10.0 was used for data analysis. Chi-square and Fisher exact test were used for testing proportions independence. Disease-free survival (DFS) was measured from the start of treatment to the date of primary treatment failure, relapse, or the date of last follow-up. Overall survival (OS) was measured from the beginning of treatment to the time of last follow-up (censored patients) or time of death. Both were studied by the Kaplan Meier method and the survival curves were compared by the log-rank test[18]. Cox backward proportional hazard model was performed for multivariate analysis of the factors that might be of independence significance in influencing the overall survival. Factors included in the maximum model were IPI [low risk group (0-2 factors) and a high-risk group (3 or 4 factors)]. A Ki-67 PI cutoff of 60% distinguished the tumors with a favorable or poor prognosis. The response rates were analyzed according to widely accepted international Cheson criteria[19]. Complete remission (CR) was defined as the resolution of clinical and radiological evidence of disease for a minimum of 4 weeks. For the purpose of statistical analysis, partial remission, non-response and progressive disease were considered as treatment failures. All p values were always two tailed and values of 0.05 or less were considered statistically significant.

Results

The cell proliferation marker Ki-67 was observed as nuclear

staining in tumor cells and in lymphocytes within the tumor tissues in all 34 cases. The staining intensity and number of tumor cells positive to Ki-67 varied from case to case, ranging from 30% to 95%. None of the patients had more than 95% Ki-67 positive tumor cells.

On ROC curve analysis (Receiver Operating Characteristic curve), a Ki-67 PI of 60% was found to significantly discriminate patients with DLBCL who had a good or bad prognosis (Area under curve = 0.64, P = 0.003). Univariate analysis showed a significant association between the Ki-67 PI (≤60% vs. >60%) and patient age and ECOG PS. A high Ki-67 PI was found in the 11patients (42.3%) whose agewas ≤60 years compared with 62.5% of the older patients (age >60 years) (P=0.025), and in 46.2% of the patients with a good PS (0-1) compared with 71.4% of the patients with a poor PS (2) (P=0.03).

The value of Ki-67 positive tumor cells follows the values of IPI scores, meaning that patients with a high proliferation rate also had high IPI value (IPI > 3). We found statistically significant positive correlation between low and high Ki-67+ and IPI scores (χ^2, $p< 0.05$).

None of the other factors examined yielded a significant association with Ki-67 PI: stage (I, II vs. III, IV) (P=0.6); number of disease-involved extranodal sites (≤1 vs. ≥2) (P=0.57); b2 microglobulin level (P=0.3); B symptoms (present vs. absent) (P=0.56) and LDH (high vs. normal) (P=0.45).

After the first line therapy, there were significant difference in complete remission (CR) for patients with high and low Ki67 (table 2).

Using the cut-off value of 60%, we found a significant association between Ki-67 PI and overall survival in patients with DLBCL (Fig. 1). Three-year survival was 52.8%±7% in the patients with a high index (mean overall survival 48.3%±2.8 months, median 76 months) and 76%±4.7% in those with a low index (mean overall survival 76.8±3.6, median not reached, P=0.013). Disease free

survival was higher in patients with Ki67<60% than those with Ki67≥60%, and the difference was statistically significant (p=0.042) (Figure 2). Multivariate analysis performed by Cox model revealed that IPI≥3 and high Ki-67PI had a significant independent prognostic value concerning overall survival (p<0.05).

Table (1): Patient' scharacteristics. (34)

Total	n	%
Age ≤60	26	76
>60	8	24
Sex Male	14	41
Female	20	59
PS(ECOG)		
0-1	13	38
2	21	62
B-symptoms	11	32
Stage		
I-II	15	44
III-IV	19	56
Extranodal		
0 – 1	20	59
≥ 2	14	41
LDH normal	19	56
high	15	44
IPI		
Low risk (0-2)	16	47
high risk (3-4)	18	53
Ki67< 60%	15	44
≥ 60%	19	56
Bulky		
Yes	10	29
No	24	71

PS (performance status), ECOG (European Cooperative Oncology Group), LDH (Lactic Dehyrogenase).

Table (2): Correlation between treatment response and Ki67 PI.

	Ki67<60 (N=15)%	**Ki67≥60 (N=19)%**	**P- value**
CR%	(13) 87	(11) 58	0.004
Failed%	(2) 13	(8) 42	<0.001

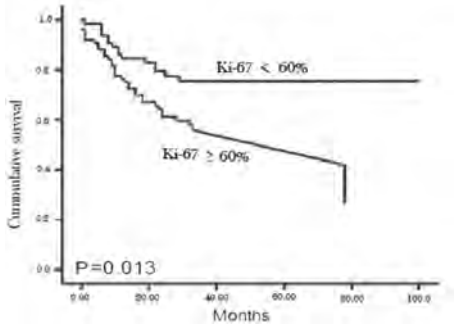

Fig.1: Overall survival in patients with DLBCL and a high versus low Ki-67 PI.

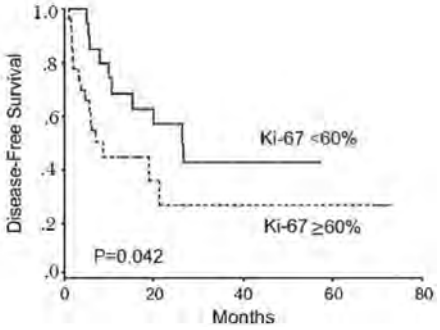

Fig.2: Disease free survival in patients with DLBCL and a high versus low Ki-67 PI.

Discussion

The prognostic significance of Ki-67 expression in DLBCL is controversial 7 and has reported a wide range of expression. The proliferative fraction in DLBCL as detected by Ki-67 staining is usually high (>40%) and may be greater than 90% in some cases[20].

In our study, 34 patients represented to have DLBCL were immunostained for Ki 67 PI. The staining intensity and number of tumor cells positive to Ki-67 varied from case to case, ranging from 30% to 95%. None of the patients had more than 95% Ki-67 positive tumor cells. Ki-67 PI of 60% was found to significantly discriminate patients with DLBCL who had a good or bad prognosis (AUC=0.64, P=0.003). Univariate analysis showed a significant association between the Ki-67 PI (≤60% vs. >60%) and patient age and ECOG PS. A high Ki-67 PI was found in 11 patients (42.3%) aged ≤60 years compared with 62.5% of the older patients (age >60 years) (P=0.025), and in 46.2% of the patients with a good PS (0-1) compared with 71.4% of the patients with a poor PS (2) (P=0.03). Multivariate analysis performed by Cox model revealed that IPI≥3 and high Ki-67+ had a significant independent prognostic value concerning overall survival ($p<0.05$). Three-year survival was 52.8%±7% in the patients with a high index (mean overall survival 48.3%±2.8 months, median 76 months) and 76%±4.7% in those with a low in-

dex (mean overall survival 76.8±3.6, median not reached, P=0.013). Disease free survival was higher in patients with Ki67<60% than those with Ki67≥60%, and the difference was statistically significant (p=0.042) (Figure 2).

On the other hand, Miller et al.[21] analyzed the prognostic significance of Ki-67 staining in 60 representative DLBCL patients from the Intergroup 0067 study that compared four different antracycline-based regimens, and found that the 3-year OS was significantly shorter in patients with Ki-67 nuclear expression in 80% or more malignant cells. However In a subsequent study on 105 DLBCL patients, the same authors demonstrated that high proliferative activity, defined in this study as nuclear Ki-67 expression in greater than 60% of malignant cells, was a strong predictor of poor survival (log rank, p=0.003,)[20]. The Nordic Lymphoma Group Study defined low expresion of Ki-67 as less than 60% of tumor cells, and found that expression of Ki-67 was not associated with significant differences in a 5-year OS[20,22].

Broyde et al[23], in his study of 169 biopsy samples derived from 169 patients with DLBCL at diagnosis and stained for Ki-67. Rates of Ki-67 expression ranged from 15% to 100%, with a mean of 67.5±23%. A Ki-67 PI cutoff of 70% distinguished the tumors with a favorable or poor prognosis, and it correlated with patient age and PS. Similar to our results, three-year survival was 55.9±6% in the patients with a high index (mean overall survival 49.3±3.9 months, median78 months) and 75±5.6% in those with a low index (mean overall survival 77.9±4.7 months, median not reached, P=0.015). Although not significant on multivariate analysis, the Ki-67 PI added significantly to the prognosis of patients with a low IPI. Among the patients with a low IPI, the 3-year survival was 94±4.1% in those who also had a low Ki-67 PI (≤70%) (Mean survival 77.2±2.5 months, median not reached) compared with 64.1±8.1% in those with a high Ki-67 PI (mean survival 56.4±5 months, median 78 months) (P=0.002). In the patients with a high IPI (>2), there was no difference in overall survival between those with a low or a high

Ki-67 PI. The Ki-67 PI also had prognostic value in patients with bulky disease (diameter >10 cm). Three-year survival was significantly better in patients with bulky disease who had a low Ki-67 PI (100%) than in patients with a high Ki-67 PI (25±12%; mean survival 14.5 months, median 9 months) (P=0.012). These data suggest that tumors that reach bulkiness with a low Ki-67 PI still have a very good prognosis.

In the largest study, which was performed by Jerkeman et al[20], included 185 cases. Ki-67 expression was found to be low (<60%) in 116 tumors (63%), moderate (60-90%) in 59 (32%), and high (>90%) in 10 (5%), and it correlated with performance status (PS) (P=0.0005). However, a low Ki-67 PI (<60%) was associated with a low failure-free survival (RR 1.7, 95% CI 1.1-2.6%) compared with a moderate or high Ki- 67 PI. Moreover, patients with either low or high Ki-67 expression demonstrated a trend toward lower overall survival than patients with moderate expression. On multivariate analysis, high bcl-2 and low Ki-67 levels added prognostic information to the clinical International Prognostic Index (IPI)[24] for aggressive lymphoma in terms of failure-free survival. However in later series performed byHasselbladet al[25] no correlation was observed between low Ki 67 PI and subgroups of DLBCL.

Others analyzed DLBCL patients treated by R-CHOP as Yoon DH et al[26] analyzed Ki-67 expression and its correlation with prognosis in 144 patients with DLBCL treated with rituximab plus CHOP (R-CHOP). They found the complete response (CR) rates following R-CHOP administration were not significantly different, based on Ki-67 expression status (P=0.104). However, higher rates of relapse were observed in the high Ki-67 expression group (Ki-67 ≥85%, n=46) with 25.0%, compared to 10.0% in the low Ki-67 expression group (Ki-67<85%, n=88) (P=0.040). The 2-yr event-free survival (EFS) rates were 44.3% and 74.1% in the high and low Ki-67 expression groups, respectively (P=0.011). The 2-yr overall survival (OS) rate was 66.4% in the high Ki-67 expression group and 82.2% in the low Ki-67 expression group (P=0.016).

In multivariate analysis, Ki-67 expression was a significant prognostic factor for EFS [hazard ratio (HR)=2.909; 95% confidence interval (CI) 1.261-6.708; P=0.012]. Ki-67 was associated with higher OS rate but with borderline significance (HR=2.876; 95% CI, 0.972-8.508; P=0.056).

Li ZM et al[27] in their study, evaluated whether Ki-67 expression is an indicator of outcome in DLBCL patients (especially non-GCB DLBCL patients) treated with standard chemotherapy combined with rituximab. They foundoverall survival (OS) and progression-free survival (PFS) were lower in patients with high Ki-67 expression than in those with low Ki-67 expression (3-year OS: 65.2% vs. 81.7%, P=0.030; 3-year PFS: 56.4% vs. 73.3%, P=0.020), similar in patients with GCB subtype and those with the non-GCB subtype (OS: P=0.330; PFS: P=0.287). According to Ki-67 expression status by immunophenotype subgroups, patients with high Ki-67 expression in non-GCB subgroup had the most unfavorable PFS and OS, comparing with the other three subgroups (P=0.004 and P=0.002, respectively). In multivariate analysis, non-GCB with high Ki-67 expression was an independent prognostic predictor of inferior survival in DLBCL patients treated with R-CHOP.

As we see Ki-67 immunostaining has several advantages: it can be performed on routinely processed tissues, permitting the simultaneous evaluation of the morphology and tumor cell kinetics; it stains the sections homogeneously; and there is no background interference, making it suitable for automated image analysis. Furthermore, it can be applied even on small biopsy specimens and core needle biopsies. There are several possible explanations for the discrepancies in the prognostic significance of Ki-67 expression in DLBCL among the studies. First, the cutoff value used to define a high Ki-67 PI ranged from 20% to 80%; some of the studies applied the median value. Second, some studies used manual techniques to assess the Ki-67 PI, whereas others used quantitative image analysis with Cell Analysis Systems (CAS), which are more objective and reproducible[28].

Third, a recent study from the international Lunenburg Lymphoma Biomarker Consortium[29] on technical and inter-observer variations in scoring different immunohistochemical prognostic markers in DLBCL specimens found poor reproducibility among laboratories for Ki-67 PI staining. They concluded that clinical decisions based on immunohistochemical stratification should be performed only in the context of clinical trials with centralized consensus review. Lossos IS et al.[7] support this opinion as they stated that tumors with low Ki-67 index may exhibits resistance to chemotherapy, because the majority of the malignant cells are in G0/G1 and thus are resistant to cycle specific cytotoxic chemotherapy. Furthermore, G0/G1 arrested cells have time to repair DNA damage induced by the chemotherapy and thus survive[7].

Conclusion

Initial high Ki 67≥60% associated with high IPI score could represent possible predictive factors of poor prognosis, which would help to identify a high risk subgroup of newly diagnosed DLBCL.

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PROGNOSTIC AND PREDICTIVE VALUES OF KI67 PROLIFERATIVE INDEX IN DIFFUSE LARGE B -CELL LYMPHOMA

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PROTECTIVE EFFECT OF QUERCETIN ON LIVER DAMAGE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

The negative impact of diabetes on the liver is well recognized. This study was designed to evaluate the hepatoprotective properties of Quercetin in streptozotocin-induced diabetes in rats. Male Wistar rats were made diabetic with a single injection of STZ (40 mg/kg i.p.). Rats were randomly divided into four groups 8 animals each: Group 1, healthy control rats; Group 2 non-diabetic rats treated with 15 mg/kg/day i.p. injection of Quercetin; Group 3, diabetic rats; Group 4, diabetic rats treated with Quercetin (15 mg/kg/day, i.p.) for 8 weeks. Finally, serum ALT, AST, ALP and albumin levels as well as liver MDA contents and activities of GSH-Px, were measured to assess hepatic injury. Liver tissues of Rat in whole groups were removed then prepared for Apoptosis analysis. Liver MDA content and serum ALT, AST, ALP and bilirubin levels in Groups 3 were found to be significantly increased as compared to Group 1 ($P<0.001$) and these parameters in Group 4 were significantly decreased as compared to Group 3 ($P<0.001$). Liver GSH-Px contents and serum albumin level in Group 3 was significantly decreased as compared to Group 1 ($P<0.001$) and were found to be significantly increased in Group 4 as compared to Groups 3 ($P<0.001$). Histopathological examination revealed that diabetes increased apoptotic index in liver tissue while the treatment of diabetic rats with Quercetin was shown to have anti-apoptosis effect. This study showed that Quercetin have hepatoprotective effects in experimentally induced diabetic rats.

Keywords: *Apoptosis, Diabetic, Quercetin, Streptozotocin, Liver, Rat.*

Introduction

Diabetes mellitus is one of the most common endocrine metabolic disorders. It is a chronic disease that characterized by hyperglycaemia[1]. Hyperglycemic in long time have side effect in other tissues especially in liver. Liver dysfunc-

tional has seen Indirectly or directly, the liver is a major target of insulin action. The onset of diabetes is accompanied by development of major biochemical and functional abnormalities in the liver, including alterations in carbohydrate, lipid, and protein metabolism, and changes in antioxidant status[2]. On the other hand, it was established that hyperglycemia increases mitochondrial reactive oxygen species (ROS) production, which could represent a key event in the development of diabetes complications[3,4]. The initial cellular response to high glucose challenge is the generation of ROS, which rapidly induces apoptotic cell death[5]. The balance of ROS and antioxidant is a major mechanism in preventing damage by oxidative stress. However, although it may not be possible to completely reverse diabetic complications, antioxidants could be useful in preventing or attenuating the adverse effects of chronic hyperglycemia[6]. Therefore, the dietary supplement of antioxidants such as vitamins, flavonoids has been used to prevent the occurrence of many chronic diseases[7,8]. Flavonoids are a large group of natural polyphenolic substances widely distributed in the plant kingdom[9]. They are important constituents of the non energetic part of the human diet and are thought to promote optimal health, via their antioxidant effects in protecting cellular components against ROS[10]. Quercetin (3,5,7,3'4'-pentahydroxy flavon) is one of the most widely distributed flavonoids, present in fruit, vegetables, tea olive oil and many other dietary sources[11]. It is a strong antioxidant and it has been shown to reduce oxidative stress [12,13]. It has been demonstrated that quercetin exhibits its therapeutic potential against many diseases, including ischemic heart diseases, atherosclerosis, liver fibrosis, renal injury, and chronic biliary obstruction[14,15,16].

Because liver is subjected to ROS-mediated injury in diabetes [17], our experiments were performed to investigate the potential protective effects of quercetin treatment on liver oxidative stress.

Material and Methods

Animals:

This study was conducted on

32 adult Wistar albino male rats 6-8 weeks old, weighing between 170 and 200g. Animals were housed in the animal laboratory at the medical research center at Benha faculty of medicine. They were housed at room temperature (25℃) and 12h/12h light/ dark cycle. All Rats were fed a standard diet and water.

Groups of the experiment:

The animals were randomly divided into 4 groups each consisted of 8 rats as follow:

Group (I): Control group injected with citrate buffer daily, intraperitoneal (IP).

Group (II): Quercetin (QR) group that received 15 mg/kg QR (IP).

Group (III): Diabetic group that received 40 mg/kg streptozotocin (IP).

Group (IV): Treatment group received 40mg/kg (IP) STZ plus 15mg/kg QR (IP).

Induction and diagnosis of diabetes mellitus:

Diabetes was induced by intraperitoneal (ip) injection of a single dose of STZ (40 mg/kg in freshly prepared citrate buffer pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Control rats were injected by the buffer alone[18].

Diabetes was verified 72 hours later by measuring blood glucose levels (after an overnight fasting) with the use of glucose oxidase reagent strips. Rats having blood glucose level of ≥250 mg/dl were considered to be diabetic.

Quercetin administration:

Quercetin (QR) treatment was initiated 5 days after the administration of streptozotocin. Quercetin (QR) injections of 15mg/kg intraperitoneal (IP)[19] were continued daily to the end of the study (for 8 weeks)[20].

Chemicals used:

***Streptzotocin drug:**

It was purchased from Sigma-Aldrich Company (USA). It is presented in powder form, purity more than 99% to be dissolved in freshly prepared sodium citrate buffer pH 4.5.

*** Sodium citrate buffer pH 4.5:**

Preparation of 0.1MCitrate Buffer:

Weigh accurately citric acid 10.5 gm and sodium citrate 14.7 gm. Mix it with 500 ml water. Make up volume to 1000 ml with distilled water. Adjust pH 4.5 by sodium hydroxide[21].

*** Quercetin drug:**

Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved and diluted with 20% glycerol in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4℃. The quercetin solution was freshly prepared each week[19].

Procedure of the experiments:

At the end of the treatment period, the animals were anesthetized after 12 hour fasting by inhalation of diethyl ether. The animals were fixed on operating table and the blood samples were taken as follow:

Blood sample collection:

A craniocaudal incision of about 2 cm is made, parallel and with slightly to the left of the sternum through the skin and pectoral muscles to expose the ribs. A blunt curved forceps is then binged between the 5th and 6th ribs, through the intercostals muscles. The gap is widened so that the rapidly beating heart becomes visible, then the blood sample were taken from the right ventricle.

Biochemical assessment:

Plasma activities of Alanine Transaminase (ALT), Aspartate Transaminase (AST), alkaline phosphatase (ALP) and concentration of glucose, albumin and total bilirubin were determined by a standard automated technique using Hitachi Analyzer Model 911 and adequate kits from Roche Company (Switzerland)[22]. These were investigated in Banha faculty of medicine at biochemistry analysis unit.

Tissue preparation:

A midline laparotomy was performed to remove the liver. The liver was dissected and fixed in 10% formalin solution at room temperature. Slices of liver tissue were processed for histopathological & immunohistochemical studies.

Immunohistochemical analysis:

Paraffin embedded tissue sections of 5Mm were prepared on

positively- charged slides to be stained with antiBCL-X antibody using Biotin streptavidin immune-peroxidase technique.

Interpretation for immunostaining:

BCL-X was detected as cytoplasmic brown staining in examined tissue.

Stained sections were classified as; Mild intensity for weak brown cytoplasmic stain. Moderate intensity for moderate brown cytoplasmic stain. Strong intensity for strong brown cytoplasmic stain[23].

Measurement of antioxidant activity:

The rat's Liver were removed immediately and washed in normal saline and homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000 x g for 10 minutes at 4°C and supernatant were used for measurement of oxidative stress by determination of malondialdehyde (MDA) as well as estimation of antioxidant enzymes such as glutathione peroxidase (GSH-Px) [24]. Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer[25]. Glutathione peroxidase (GSH-Px) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide . One unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of NADPH to NADP per minute. Results are expressed as units/mg protein[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

Results of the effect of daily treatment of Quercetin at a dose of 15mg/kg for 8 weeks on blood glucose levels of experimental rats are presented in Table 1. The Quercetin treatment produced hypoglycemic effect in both normal and diabetic rats after 8 weeks of

administration,but this hypoglycemic effect is significant in diabetic group (P<0.001). Table 1 shows the effects of Quercetin treatment on the serum levels of markers of liver injury (ALT, AST, ALP and bilirubin) in diabetic rats. ALT, AST, ALP and bilirubin serum contents in Groups 3 was found to be significantly increased as compared to Group 1 (P<0.001) and these parameters in Group 4 were significantly decreased as compared to Group 3 (P<0.001). The albumin serum level in Group 3 was significantly decreased as compared to Groups 1 (P<0.001) and this parameter was significantly increased in Group 4 as compared to Group 3 (P<0.001).

Table 2 shows the effects of Quercetin treatment on antioxidative activity in liver tissue of diabetic rats. MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared to Group 1 (P<0.001) and liver MDA level in Group 4 were significantly decreased as compared to Group 3 (P<0.001). The GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 (P<0.001) and GSH-Px activity were increased in Group 4 as compared to Group 3 (P<0.001).

Pathologically, liver histological structure was normal in healthy control group (Fig. 1, A). In Group 2 also there were no pathological changes so that hepatic lobular structure seemed quite normal (Fig. 1, B). In group 3, Diabetic rats showed liver tissue damage & apoptosis (Fig. 1, C). Finally in group 4, Quercetin treatment of diabetic rats prevented the pathologic changes in the liver (Fig. 1, D).

Table (1): Comparison of the effect of Quercetin on blood glucose levels and serum markers of liver tissue injury among the experimental groups (mean±SD).

Groups	Biochemical parameters					
	Blood glucose level (mg/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)	Total serum bilirubin (Mg/dl)	Albumin (g/dl)
Control group rats	119±2.49	86±1.55	123±1.83	156±1.45	0.83±0.01	4.37±0.02
Nondiabetic rats+ Quercetin (QR) treatment	113±3.12	86±1.28	124±1.04	157±1.41	0.84±0.02	4.36±0.02
Diabetic rats	304±5.99[a]	181±1.30[a]	272±1.67[a]	245±2.03[a]	1.26±0.1[a]	3.14±0.01[a]
Diabetic rats+ Quercetin (QR) treatment	127±2.06[b]	87±1.60[b]	135 ±1.58[b]	181±1.48[b]	0.88±0.01[b]	4.35±0.02[b]

[a]p<0.001; [a]compared to Group 1, [b]compared to Group 3.

Table (2): Comparison of the effect of Quercetin treatment on liver MDA and antioxidant enzymes activities (GSH-Px) among the experimental groups (mean ± SD).

Groups	Biochemical parameters	
	MDA (nmol/g protein)	GSH-Px (U/mg protein)
Control group rats	3.24±0.08	22.76±0.04
Nondiabetic rats+ Quercetin (QR) treatment	3.23±0.01	22.44±0.22
Diabetic rats	5.37±0.03[a]	17.51±0.14[a]
Diabetic rats+ Quercetin (QR) treatment	4.35±0.02[b]	20.65±0.13[b]

*p<0.001; [a]compared to Group 1, [b]compared to Group 3.

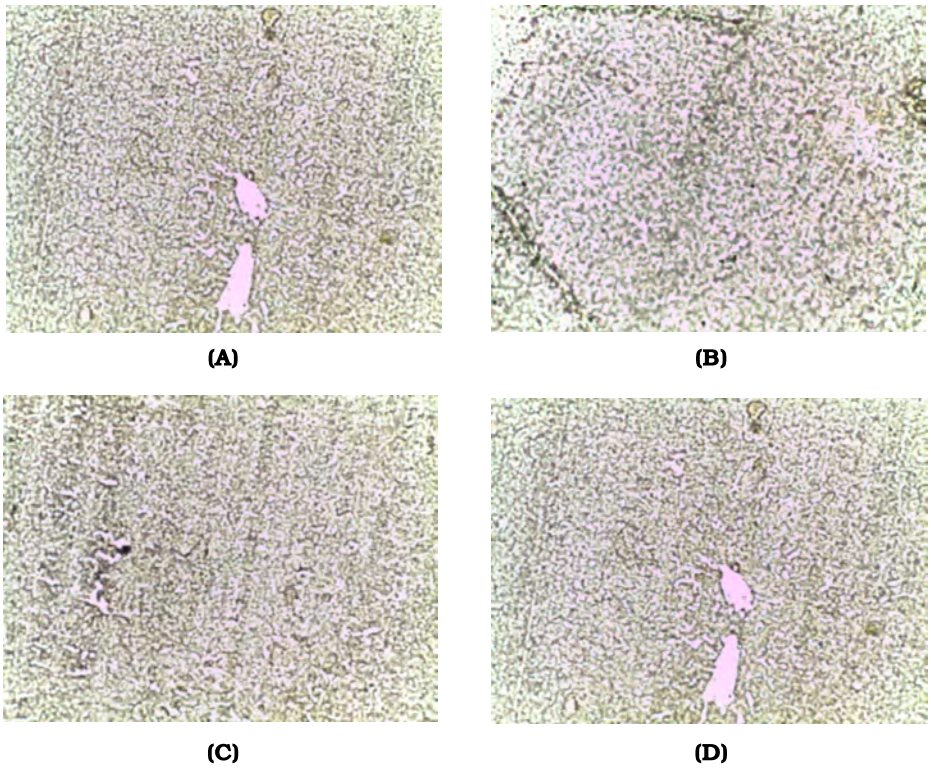

Fig. 1: Microscopic appearance from liver tissues of the experimental rats (strept - avidin - biotin) x200. **(A)** Healthy control rat liver showing weak BCL-X expression in cytoplasm of hepatocytes. **(B)** non-diabetic+ Quercetin treated rat liver shows weak BCL-X expression in cytoplasm of hepatocytes. **(C)** Dabetic rat liver showing strong BCL-X expression in cytoplasm of hepatocytes. **(D)** Quercetin treatment of diabetic rats prevented the pathologic changes in the liver.

Discussion

Worldwide studies have been done to make use of herbal medicine in different fields of medicine. Base on ancient Persians traditional books Use of herbal medicine has positive effect on treatment of different diseases especially on diabetes mellitus[27]. Quercetin as an important and main flavonoids found in human meals[28] has an useful effect in human health involves prevention of diabetes induced cataract, reduced blood vessels fragility, anti microbial, anti viral, anti allergy, and anti inflammatory effects and prevention of platelet aggregation [28,29,30]. In both type 1 and type 2 diabetes mellitus the late diabetic pathological complications are mostly due to excessive elevated production of reactive oxygen species over the capacity of their removal by internal enzymatic and non-enzymatic mechanisms[31]. Therefore, additional numerous dietary artificial or natural antioxidants may be of great importance in such cases[32]. Various natural products have long been used in traditional medical systems for treating diabetes[33]. Most of them contain a wide scale of antioxidants with a potent scavenging activity for reactive oxygen species. Therefore, it might be assumed that these products or isolated natural compounds could play a very important role in adjuvant therapy. In current study, Intraperitoneal injection of Quercetin caused significant hypoglycemic effect in diabetic rats. This results coincides with results of Mahesh and Menom[34] or Coskum et al. [35], who found a hypoglycemic effect of quercetin when given to streptozotocin-diabetic rats. It has been shown that, hypoglycemic effect of Quercetin is mediated through stimulation of synthesis and/or release of insulin[20]. In the current study, significant decline in serum albumin level and elevations in markers of liver injury (ALT, AST, ALP, and bilirubin) reflects the hepatocytes injury in experimental diabetes. These results are consistent with the findings reported by Ramesh et al[36]. The data of our study also revealed that daily treatment with Quercetin markedly improves biochemical parameters of rats with streptozotocin induced diabetes. Liver function tests (LFTs) are commonly used in clinical practice

to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, and albumin. Hepatocellular damage causes release of these enzymes into circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction, and muscular damages. ALT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are an indicator of cellular infiltration and functional disturbance of liver cell membranes[37]. In addition, ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites[38]. On the other hand, bilirubin and albumin values are associated with the function of hepatic cells[39]. Return of the above enzymes to normal serum values following Quercetin treatment may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration [40]. Effective control of bilirubin and albumin shows early improvement of functional and secretory mechanism of hepatic cells. In this study, histopathological evaluation of liver tissues showed liver tissue damage and apoptosis induced by diabetes mellitus of the livers in diabetic rats. With Quercetin treatment in diabetic rats no considerable pathological changes were observed demonstrating the protective effect of Quercetin against hepatic complications of diabetes. In this study, significant reduction of antioxidant enzymes (GSH-Px) activity as well as significant increase in MDA reflects oxidative stress of the liver in experimental diabetes. These results are in line with the findings reported by Khaki et al.[19] Increased oxidative stress in the tissues of streptozotocin diabetic rats was similarly reported. This was said to be a contributory factor in the development of the complications of diabetes[41,42]. The data of our study also revealed that daily treatment of Quercetin markedly improves antioxidant status of liver tissue of rats with streptozotocin-induced diabetes as GSH-Px

significantly increased and MDA level markedly decreased. This indicates that in the presence of Quercetin, there is an improvement in the oxidative stress. This finding is completely in agreement with those of Dias et al[20] who demonstrated antioxidant activity of Quercetin in streptozotocin induced diabetic mice. Liver is one of the most important organs that maintains blood glucose levels within normal limits thus enhancement of blood glucose leads to imbalance of oxidation-reduction reactions in hepatocytes, so that, hyperglycemia through increasing in advanced glycation end products (AGEs) facilities free radicals production through disturbance in ROS production[43]. Therefore, it reveals that diabetic hepatic damage is not controllable only by inhibition of hyperglycemia[44]. In other words, in early stages of diabetes, tissues injuries are in association with hyperglycemia but its progress is not related to hyperglycemia. Therefore, monitoring of blood glucose levels solely is not sufficient in retarding diabetes complications. Thus, a suitable drug must have both antioxidant and blood glucose decreasing properties[45]. One of the Quercetin anti oxidant mechanism is removal of free radical such as xanthine super oxide and xanthine oxidase[46]. Therefore suggested, increased use of herbal medicine, fruit, vegetables, onion, tea and black burgundy grape which are full of flavonoids and Quercetin can decrease side effects of diabetes mellitus on liver tissue in diabetic patient complicated with hepatic diseases.

Conclusion

We observed that Quercetin improved serum biomarkers of liver tissue injury and histopathologic properties of this organ. It is presumed that Quercetin prevents diabetic complications and ameliorates diabetic hepatopathy through its antioxidant potential.

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PROTECTIVE EFFECT OF QUERCETIN ON LIVER DAMAGE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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PROTECTIVE EFFECT OF QUERCETIN AGAINST INDOMETHACIN INDUCED GASTRIC ULCER IN RATS

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Abstract

Aim: *This study was designed to study the protective effects of Quercetin against indomethacin induced gastric damage in rats.*

Materials and Methods: *Adult male albino rats weighing 180 – 200 gm were divided into 3 groups: group 1 (control group), group 2 received an ulcerogenic dose of indomethacin (200 mg/kg body weight) in drinking water. Group 3: receives quercetin orally at a dose of 50mg/kg body weight for one week before induction of gastric ulcer with indomethacin.*

Results: *A significant reduction in number and the mean area of gastric ulcer, gastric tissue malondialdehyde (MDA) and the plasma levels of tumor necrotic factor alpha (TNF-α) and interleukin 1-B (IL-1B) and a significant increases in the PH, gastric tissue superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were observed in quercetin treated rats compared to indomethacin ulcerated rats.*

Conclusion: *Quercetin exerts a potent anti-inflammatory gastroprotective effect besides its clear antioxidant effect against indomethacin induced gastric ulcer.*

Introduction

Peptic ulcers are a common disorder of the entire gastrointestinal tract that occurs mainly in the stomach and the proximal duodenum. This disease is multifactorial and its treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs. The use of natural products for the prevention and treatment of different pathologies is continuously expanding throughout the world[1]. It is

caused by many factors like stress, drugs, alcohol, etc. and is reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding[2].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed drugs worldwide which are useful as analgesic and anti-inflammatory agents. Indomethacin (IND) is one of the most popular NSAIDs which prescribed extensively for to treatment of rheumatoid arthritis, osteoarthritis, cervical spondylitis, ankylosing spondylitis and acute musculoskeletal disorders and infective inflammation. A long-term use of NSAIDs among patients is associated with a range of oesophagho-gastroduodenal changes with a very high morbidity and mortality rates[3]. It accounts for gastro duodenal mucosal erosions in approximately 35-60% of patients, gastric or duodenal ulceration in 10-25% of patients and severe complications, such as gastrointestinal hemorrhage or perforation in 1% of patients[4].

The toxicity of NSAIDs is mainly attributed to inhibition of prostaglandin synthase activity that inhibits prostaglandin production in the GI tract resulting in accumulation of intracellular arachidonic acid[5], induction of mitochondrial injury[6] and production of reactive metabolites that covalently bind to critical cellular Proteins[7].

Flavonoids are a group of naturally occurring compounds widely distributed as secondary metabolites in the plant kingdom found mainly in fruits, vegetables, leaves and grains. They have been recognized for having interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities[8]. They are able to inhibit a series of enzymes which are activated in inflammatory process. Many studies support the idea that reactive oxygen species (ROS) generating in a situation of oxidative stress plays an important role in inflammation[9]. Quercetin (3,5,7,3',4'-pentahydro- xyflavone) is one of these plant derived flavonoids. Which is founding black and green tea, apples, onion, red grapes, citrus

fruits, tomato and leafy green vegetables[10].

Based on the previous data, the present study was directed towards assessment of the gastroprotective efficacy of quercetin against NSAID (indomethacin) induced gastric ulcer and to clarify the possible mechanisms underlying that effect.

Materials and Methods

I-Chemicals used:

1- Indomethacin provided in tablets, each one containing 25mg and manufactured by Misr CO., Egypt. It was dissolved in drinking water.

2- Quercetin provided as powder manufactured by Sigma CO., USA.

3- Tissue MDA, SOD GSH-Px kits (Ransod and Ransel and Randox Laboratories GmbH, Netherland).

4- IL-1 and TNF- were determined by ELISA according to the manufacturer's instructions (Assay Designs, Ann Arbor, MI; Bender MedSystems, SanDiego, CA).

5- Diethyl ether: available in the form of solvent ether from laboratory Rasayan (1 L. M.W. 74.12).

II- Animals used:

Experimental protocol for the study was approved by the ethics committee on animal experiments in Benha University.

Thirty healthy adult male albino rats weighting 180-200 g. averaging 16 weeks old were brought from Experimental Animal Breeding Farm, Helwan - Cairo to be utilized in this study. They were housed in cages (5 rats/cage) under standard laboratory conditions (12h light/dark cycle, 20-25℃, relative humidity 55%).The animals were given commercial standard caloric diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and tap water ad libitum. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences. After acclimatization for 1 week, the rats were randomly classified into 3 equal experimental groups: Group I (Control group): received no medication and given free access to food and water. Group II (Indomethacin ul-

cerated group): received an ulcerogenic dose of indomethacin (200 mg/kg body weight) in drinking water. Group III (Quercetin and indomethacin): receives quercetin orally at a dose of 50mg/kg body weight for one week before induction of gastric ulcer with indomethacin.

IV-Procedure of the experiment:

At the end of the experiment, the rats were anaesthized by ether and both chest and abdominal wall were opened. Intracardiac blood samples were collected then put in the incubator till it is clotted and the plasma was taken and kept at -20°C till the time of measurement of plasma TNF-α and IL-1B. The stomach of each rat was removed after the lower oesophageal[11] and the pyloric ends have been ligated[12].

1- Measurement of the number and area of gastric ulcer as well as gastric PH:

• The removed stomach was cut open along the greater curvature and the contents were collected in centrifuge tubes and centrifuged at 200 x g for 10 min. The resultant supernatant fluid is transported to a test tube where PH was determined by a PH-meter [13].

• The stomach was then washed with warm saline, and the inner surface was photographed and the area of gastric ulcers in mm^2 was calculated. Next, the gastric mucosal tissues were removed, frozen in liquid nitrogen and stored at -80°C[14].

• Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The specimens were then embedded in paraffin, sectioned and stained with hematoxylin and eosin (H & E) before being evaluated by light microscopy[15].

2- Measurement of gastric tissue MDA, SOD and GSH-Px levels:

• Tissue malondialdehyde (MDA) (mmol/l) was determined by the double heating method of Draper and Hadley[16].

• Tissue SOD and GSH-Px activities were measured by using Ransod and Ransel and Randox Laboratories GmbH commercial kits, respectively with the Shimadzu UV-1601 spectrophotometer [17].

3- Determination of plasma TNF-α and IL-1B levels:

Blood samples in EDTA-containing vials were centrifuged at 1000 x g for 10 min at 4°C. The levels of IL-1 and TNF- were determined by ELISA according to the manufacturer's instructions from Assay Designs, Ann Arbor, MI, Bender Med Systems[19,20].

Results

Table (1) and figures (1a) clarify that the number of gastric ulcer increases from 0 in the control group to 10±1.49 in the indomethacin ulcerated group (P<0.001) while coadminstration of quercetin with indomethacin decreases this number to 1.4±0.84 (P<0.001). The mean area of ulcers (mm2) increases from 0 in the control group to 28.8 mm2±2.53 in the indomethacin group (P<0.001). Indomethacin-induced mean ulcer area was decreased by coadminstration of Quercetin with indomethacin to 5.14 mm2±1.75 (P<0.001). PH decreases from 3.6±0.62 in the control group to 1.53±0.36 in the indomethacin group (P<0.001). Treatment with quercetin together with indomethacin increases it to 3.66±0.57 (P<0.001).

The gastric damage was also confirmed by macroscopic and histological examination (figures 1b and 1c). The gastric mucosa was normal in control group. In indomethacin group, it was extensively damaged involving the cells lining the gastric pits or into the gland area but this was partly protected by administration of quercetin.

Table (2) and figure (2) clarify that the gastric tissue malondialdehyde was increased from 78.9±4.95 μmol/g protein in the control group to 152.9±5.57 in the indomethacin ulcerated group (P<0.001), while coadminstration of quercetin with indomethacin decreases it to 83.7±5.4 (P<0.001). Superoxide dismutase decreases from 28.95±2.29 U/mg protein in the control group to 9.68±1.33 in the indomethacin group (P<0.001). Coadminstration of quercetin with indomethacin increases it to 27.88±2.71 (P<0.001). Glutathione peroxidase decreases from 0.412±0.043 U/mg protein in the control group to 0.185±0.032 in the indomethacin ulcerated group (P<0.001), while coadminstration of quercetin with indomethacin increas-

es it to 0.385±0.044 (P<0.001). Plasma TNF-α increases from 38.2±3.86 pg/ml in the control group to 103.5±6.77 in the Indomethacin ulcerated group (P<0.001). Treatment with quercetin decreases it to 49.3±8.52 (P<0.001). Plasma IL-1B increases from 119.3±3.74 pg/ml in the control group to 291.5±9.04 in the indomethacin ulcerated group (P<0.001) and this was decreased to 135.25±8.65 by coadminstration of Quercetin with indomethacin (P<0.001).

Table (1): Effect of pretreatment with quercetin (50mg/kg) for one week on the number of gastric ulcers, the mean area of ulcers (mm^2) and gastric PH. All values are expressed as Mean ± Standard deviation (SD for 10 rats in each group; control group (group 1), indomethacin (group 2) and indomethacin + Quercetin (group 3).

	Control	IND	IND + QUE
No. of gastric ulcers	0	10 ± 1.49	1.4 ± 0.84
Mean area of ulcer (mm^2)	0	28.8 ± 2.53	5.14 ± 1.75
PH	3.6 ± 0.62	1.53 ± 0.36	3.66 ± 0.57

Table (2): Effect of pretreatment with quercetin (50mg/kg) for one week on gastric tissue malondialdehyde (MDA) in µmol/g protein, superoxide dismutase (SOD) in U/mg protein, glutathione peroxidase (GSH-Px) in U/mg protein and the plasma levels of tumor necrotic factor alpha (TNF-α) in pg/ml and interleukin 1B (IL-1B) pg/ml. All values are expressed as Mean ± Standard deviation (SD) for 10 rats in each group; control group (group 1), indomethacin (group 2) and indomethacin + Quercetin (group 3).

	Control	IND	IND + QUE
Gastric tissue MDA	78.9 ± 4.95	152.9 ± 5.57	83.7 ± 5.4
Gastric tissue SOD	28.95 ± 2.29	9.68 ± 1.33	27.88 ± 2.71
Gastric tissue GSH-Px	0.412 ± 0.043	0.185 ± 0.032	0.385 ± 0.044
Plasma TNF-α	38.2 ± 3.86	103.5 ± 6.77	49.3 ± 8.52
Plasma IL-1B	119.3 ± 3.74	291.5 ± 9.04	135.25 ± 8.65

Figure (1a):

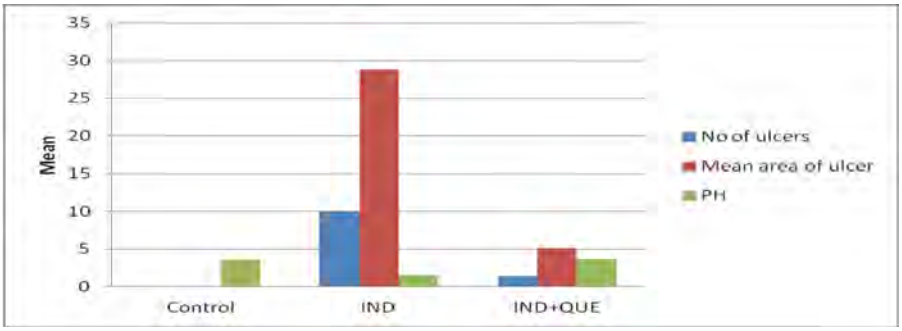
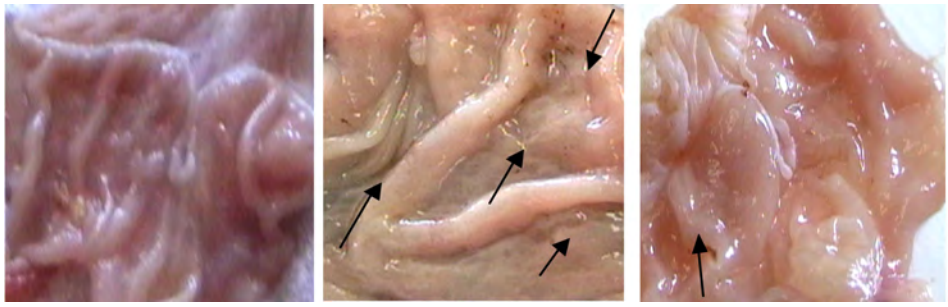
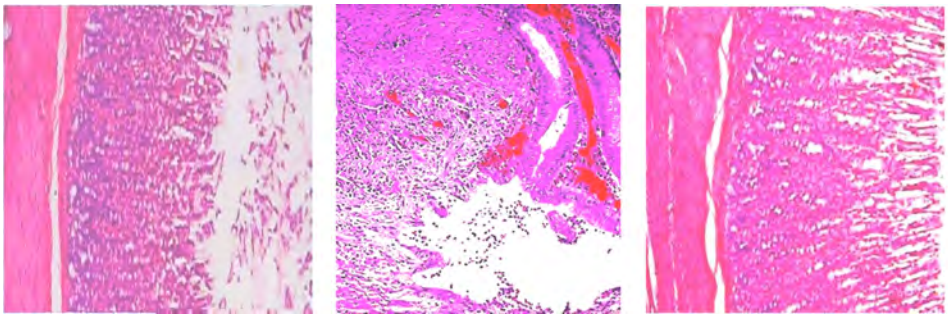


Figure (1b) macroscopic picture:



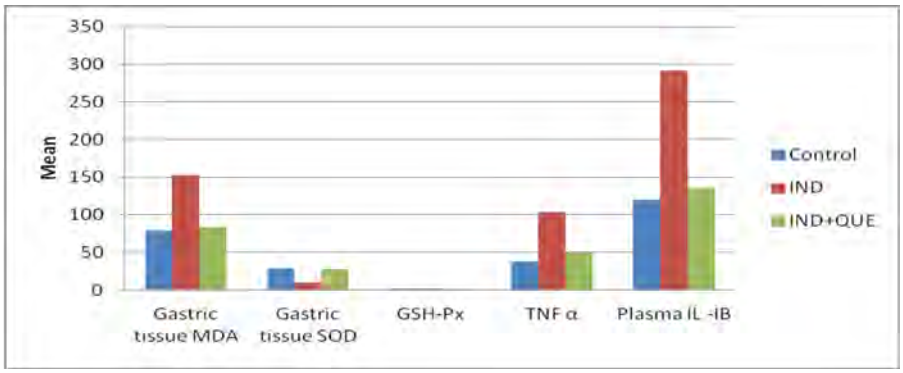
Normal gastric mucosa | INDO ulcerated group | Quercetin treated group

Figure (1c) microscopic picture:



Normal gastric mucosa | INDO ulcerated group | Quercetin treated group

Figure (2):



Discussion

Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric ulceration in human and experimental animals [21].

Most of the available drugs are thought to act on the offensive factors which neutralize acid secretion like antacids, H2 receptor blockers or interfere with acid secretion as proton pump blockers [22].

Quercetin is a phenolic compound widely distributed in the plant kingdom. It is found in frequently consumed foods as apples, berries, onions, tea and vegetables. Quercetin is reported to have many beneficial effects on human health including cardiovascular protection, anticancer activity, antiulcer effects, antiallergic activity, cataract prevention, antiviral activity and anti-inflammatory effects[23].

The present study was designed to demonstrate gastric ulcer protective activity of quercetin in rats and the possible mechanisms implicated in it.

In the present study, the gastro protective effectiveness of the quercetin was evident from significant reduction in the number and mean area of the gastric ulcer in quercetin treated rats as compared with indomethacin ulcerated rats. The protective effect of quercetin in preventing ulcer development can be due to their protein precipitation as Quercetin helps in precipitating microproteins on the ulcer site. Therefore forming a protective layer over the lining mucosa thus protects the underlying mucosa from gastric acid secretion, toxins and other irritants[24].

Gastric acid plays an important permissible role in NSAIDs associated mucosal injury[25]. In our study, there was a significant decrease in gastric PH indicating increased gastric acidity, and in ulcerated animals and gastric PH was increased by treating the rats with Quercetin which is a highly desirable for gastroprotection and antiulcer effect. This protective effect may be due to its direct inhibitory action on the acid producing

cells. Quercetin also has antihistaminic properties preventing the release of histamine from gastric mast cells and inhibiting the gastric H+ - K+ proton pump, diminishing acid gastric secretion. Moreover, it increases prostaglandins E2 and I2 synthesis by gastric mucosa. They inhibit the secretion of gastric acid and stimulate mucus and hydrophobic surfactant like phospholipids secretion in gastric epithelial cells [26,27].

Also it was reported that quercetin exhibit ulcer healing effect in indomethacin induced gastric ulcerated rats by several mechanisms such as increasing mucous secretion evidenced by higher hexosamine and carbohydrate over protein ratio, increasing mucosal resistance, increasing mucosal blood flow and epithelialization rates. These actions were referred to the triterpenoid saponins and flavonoids components of quercetin. Quercetin intake also reduces cell shedding and increase DNA content of gastric mucosal cells indicating gastric mucosal renewal ability[28].

In our study we tried to explore the antioxidant effect of quercetin in indomethacin ulcerated rats by measuring gastric tissue content of malondialdehyde (MDA) concentration which is considered as an indicator of lipid peroxidation which is a well known example of oxidative damage that affects cell membranes, lipoproteins and other lipid containing structures under conditions of oxidative stress and gastric antioxidant enzyme activity by measuring superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Our results showed that there was significant increase in MDA concentration in indomethacin ulcerated group with reduction in the levels of antioxidant enzyme (SOD, GSHPx). We also found that pretreatment with quercetin in indometheacin ulcerated rats significantly decreased the elevated MDA and increased the reduced antioxidant enzyme activities. Our results were agreed with other findings that revealed that the preventive effects of quercetin is due to inhibition of lipid peroxidation by its antioxidant nature indicating that quercetin has an antioxidant effect[26,28,29].

NSAIDs induces gastric ulcer

by inhibiting cyclooxygenase enzyme complex that convert free eicosapolyenoic acids like arachidonic acid to cyclic endoperoxides which is the key intermediate in prostaglandin synthesis[29]. In animal experiments, quercetin inhibits production of inflammation producing enzymes (cyclooxygenase (COX)[30] and lipoxygenase (LOX)[31] and decreasing NF-kappaB activation, NOS (nitric oxide synthase) overexpression[32,33,34] and TNF-α[35,36].

So in our study we aimed to find out the anti-inflammatory efficacy of quercetin against gastric ulcer induced by indomethacin that was assessed by measuring plasma levels of TNF-α and IL-1B. Our results showed that there was a significant increase in both TNF-α and IL-1B levels in the plasma in indomethacin ulcerated group and this was significantly decreased in quercetin group.

Conclusion

Flavonoids as quercetin represent a highly diverse class of secondary metabolites with potentially beneficial effects on human health. These compounds protect the gastrointestinal mucosa from lesions produced by various experimental ulcer models and against different necrotic agents. Several mechanisms of action may be involved in this protective effect including antioxidant, anti-secretory and anti-inflamatory effects. Quercetin may represent an attractive therapeutic option for preventing and healing NSAIDs induced gastric ulcers but we recommend for further studies on its anti-inflammatory effects against gastric ulcer.

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PROTECTIVE EFFECT OF QUERCETIN AGAINST INDOMETHACIN INDUCED GASTRIC ULCER IN RATS

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CAUDAL BUPIVACAINE, KETAMINE AND THEIR COMBINATION FOR PEDIATRIC POSTOPERATIVE ANALGESIA

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Abstract

Background: *The aim of this study was to compare the analgesic efficacy of ketamine either alone or in combination with bupivacaine for caudal blockade in pediatric surgery.* ***Methods:*** *After the induction of general anesthesia without premedication sixty children, were allocated randomly into 3 groups to receive single shot caudal blockade by bupivacaine 0.25% 1 ml/kg (group B, n=20), ketamine 1 mg/kg (group K, n 20), or a mixture of bupivacaine 0.25% 1 ml/kg and preservative-free s-ketamine 0.5 mg/kg (group BK n=20). Postoperative pain was assessed using children's and infant's postoperative pain scale (CHIPPS), 4-points sedation scores, analgesic requirements and associated side effects were recorded for the first 24 hours after operation.* ***Results:*** *The recovery time from general anesthesia in group-K was significantly longer (P>0.05). Sevoflurane concentration was significantly lower in group-K (P<0.001). The duration of absolute analgesia and the time to first request for analgesic was significantly prolonged in Group-K. Patients in Group-K had significantly less pain scale especially at the time to first request for analgesic. The 4-points sedation score was significantly higher in Group-K than the other two groups during the first three hours postoperative. Patients in Group-B were more wakeful with less sedation score and the difference was statistically significant (P<0.05). Supplemental analgesia requirements with intravenous paracetamol were significantly less in group-K (2 subjects). Five Patients in group-B, 3 in group-BK and only one in group-K experienced postoperative nausea and vomiting Postoperative urinary retention was noted in 10% in group-B and 5% in group-BK, while 0% in group-K, while Nystagmus occurred in two patients in group-K and one*

patient in group-BK ***Conclusions:*** *Caudal administration of ketamine alone (1 mg/kg) provided adequate postoperative analgesia of similar quality and slightly longer duration than caudal injection of 0.25% bupivacaine (1 ml/kg) with (ketamine 0.5 mg/kg), whereas it is proved to be superior from the caudal administration of 1 ml/kg of bupivacaine 0.25% alone in pediatric surgery without producing many side effects.*

Keywords: *caudal analgesia, buvivacaine, s-ketamine.*

Introduction

Regional anesthetic techniques have gained considerable popularity for use with pediatric patients. The primary advantage of regional supplementation are lowering general anesthetic requirements intraoperatively and providing good postoperative pain relief[1], Caudal epidural analgesia is one of the most popular and commonly performed regional blocks in pediatric anesthesia. It is a reliable and safe technique that can be used with.general anesthesia for intra-¬and postoperative analgesia in patients undergoing abdominal and lower-limb surgery[2].

Many anesthetic agents have been used for caudal analgesia in pediatric patients, with lignocaine and bupivacaine being most common[1], Bupivacaine has been in clinical use for more than 30 years and is widely used for caudal epidural analgesia in children because of its long duration of action and beneficial ratio of sensory to motor blocks[3], The main disadvantage of caudal anesthesia is the short duration of action after a single injection of local anesthetic solution. Prolongation of caudal analgesia using a 'single-shot' technique has also been achieved by the addition of various adjuvants[4].

Ketamine is an anesthetic and analgesic agent with a wide range of applications in pediatric anesthesia[5], It exerts its effects by binding non-competitively to a subset of glutamate receptors stimulated by the excitatory amine N-methyl D-aspartate (NMDA), blockade of which leads to a decrease in the activation of dorsal horn neurons. These receptors are located throughout the CNS as well as 'in the substantia gelatino-

sa in the spinal cord and play an important role in central pain processing and in neural plasticity in the spinal cord[6].

The aim of this study was to compare the analgesic efficacy and side effects of preservative free ketamine either alone or in combination with bupivacaine for caudal blockade in pediatric patients after hypospadias surgery.

Materials and Methods

Unpremedicated 60 male children aged 2-5 years of ASA (American Society of Anesthesiologists classification) physical status I and II; undergoing hypospadias surgery in Mansoura University Hospital in plastic surgery unit, were included in this study. Children with history of allergic reaction to bupivacaine or ketamine and those with any contraindication for central neuroaxial blockade (either absolute or relative), were excluded from the study. After receiving informed consent from parents, the children were randomly allocated to receive one of the three solutions for caudal epidural injection after induction of general anesthesia.

Group-B: were given 1 m1/kg of 0.25% bupivacaine.

Group-K: were given 1 mg/kg of ketamine diluted with 0.9% saline using weight-related volumes 1 ml/kg.

Group-BK: were given a mixture of 0.25% bupivacaine and 0.5 mg/kg of ketamine at a volume of 1 ml/kg.

No, premedication was given and all operation was performed under general anesthesia. One surgeon was performed all operations. IV cannula 22 guage was inserted in the dorsm of hand with inhalation of oxygen and Sevoflurane, tracheal intubation with suitable size endotracheal tube. Anesthesia was maintained using oxygen 50% and Sevoflurane spontaneous breathing. Intraoperative Sevoflurane concentration was adjusted to maintain adequate depth of anaesthesia.

Caudal analgesia technique: After induction of general anesthesia, patients were given caudal injections in left lateral position using a 23-gauge needle. The area

was carefully and thoroughly cleaned with an antiseptic solution, sterile drapes were placed around the site. The technique was performed in the simplest way to penetrate the sacrococcygeal membrane by introducing a 23-gauge hypodermic needle perpendicular to it with the bevel parallel to the long fibers of the membrane. The needle was advanced until there is loss of resistance as it pierced the sacrococcygeal membrane. Once the needle crossed the membrane, it was directed upwards so that it made an angle of 20-30 degrees with the skin. The needle was then advanced for 2 to 3 mm so as to ensure that the entire bevel was within the sacral canal. The entire volume of injection was made over a period of 60 to 90 seconds and after completion of the injection the child was placed supine after placing a small Elastoplast dressing over the site of the sacral hiatus.

No analgesic drugs used intraoperative. Standard monitoring was used during anesthesia and surgery. The concentration of volatile agent will be reduced towards the end of surgery in order to achieve rapid awakening before return to the recovery ward. All patients were admitted to the recovery ward for at least one hour and, when filly awake and pain free, were returned to the ward.

Heart rate, mean arterial pressure, respiratory rate and arterial oxygen saturation were recorded before operation and after caudal block, these variables were recorded every 5 mm till the end of surgery. The time from induction of anesthesia to the end of surgery when the anesthetic agent was discontinued (Anesthesia duration) was recorded, and the time from stoppage of anesthesia to opening the eyes on calling the patient's name or on tactile stimulus (Recovery time) was noted. The duration of caudal analgesia was defined from the time of caudal injection to the time the child first complains of pain or time of first postoperative analgesic requirement.

During the first 24 hours after operation, the following variables were recorded: heart rate, arterial pressure, respiratory rate and arterial oxygen saturation every

hour. Sedation Was assessed using a four-point sedation score (0= eyes open spontaneously; 1= eyes open to speech; 2= eyes open when shaken; 3= unrousable).

Postoperatively, a blinded Post-Anesthesia Care Unit (PACU) nurse assessed the quality of analgesia with children's and infant's postoperative pain scale (CHIPPS) was recorded every 2 hours in the first 6 hours then every 3 hours till 24 hours postoperatively[7] after recovery from anesthesia at rest and on movement. Significant pain is defined as total score above 3 and consequence required a supplementary dose of analgesia. Intra-venous paracetamol 15 mg/kg.

The duration of absolute analgesia was measured from the time of caudal administration of the drug to the time when the VAS pain score was more than 0 (Absolute analgesia). Number of painful episodes, number of children requiring analgesic and time to first request for analgesic were also recorded. Motor block was assessed by using a modified Bromage scale 4-points [0= flexion of knees and feet, 1= flexion of knees, 2= little movement of feet only, 3= no movement of knees or feet]. The incidence of adverse effects such as nausea, vomiting, urinary retention, nystagmus, behavior changes (hallucination or agitation), and any side effects related to injection of ketamine or bupivacaine were recorded.

Data analysis: All statistical analyses were carried out using SPSS statistical package (SPSS 10.0 for Windows). ANOVA with multiple comparisons was used for comparisons between the groups. Using Chi squared test compared the non-parametric data. $P<0.05$ was regarded as statistically significant.

Results

The demographic data in the three groups were comparable in age, height and weight (table 1).

There were no significant differences among groups for incidence of hemodynamic changes (HR and MABP) and SaO2 during the study period. None of the children suffered from hypotension or bradycardia and arteriel O2 saturation (97%-100%).

As shown in table 3 the operative data revealed that the duration of general anesthesia and the duration of surgical procedure were nearly similar in the three groups, while the longest recovery time from general anesthesia was for Ketamine group (10.5±0.25 mm), which was significantly longer (P<0.05) than both Bupivacaine and Bupivacaine with Ketamine groups. Sevoflurane concentration required was significantly lower in group K (P<0.05) than other two groups (B and BK).

According to the quality of postoperative analgesia as shown in table 4, both the duration of absolute analgesia and the time to first request for analgesic was significantly prolonged in Group K (210±30; 7.0±3.2 respectively) compared with Group B (115±20 min; 4.20±3.1 hours respectively) and Group BK (155±23 min; 6.50±2.11 hours respectively). Patients in Group K had significantly less pain scale especially at the time to first request for analgesic. The 4-points sedation score (figure 1) was significantly higher in Group K (0.200±0.41/4) than the other two groups (Group B 0.02±0.22/4 and Group BK 0.150±0.36/4) during the first three hours postoperative and more patients in Group B were more wakeful with less sedation score as compared with Group K and Group BK and ~the difference was statistically significant (P<0.05).

Supplemental analgesia requirements with intravenous paracetamol within 24 hours postoperative were significantly less in caudal Ketamine group (2 subjects 1 received once + 1 received twice) when compared with Ketamine plus Bupivacaine group (8 subjects 5 received once, 3 received twice), and Bupivacaine group (16 Subjects = 9 received once, 7 received twice) (Table 5).

The incidence of postoperative side effects was compared between the groups. Five patients in the caudal bupivacaine group, three patients in the caudal bupivacaine-ketamine group and only one patient in the caudal ketamine group experienced postoperative nausea and vomiting. Postoperative urinary retention was noted in two patients in group B and one patient in group BK.

Table (1): Demographic data (Mean ± SD).

Variable	Group B (n = 20)	Group K (n = 20)	Group BK (n = 20)
Age (yr) Range	4.3 ± 1.40 2 – 5	4.35 ± 1.1 2 – 5	4.05 ± 1.2 2 – 5
Weight (kg) Range	16.3 ± 2.6 11 – 22	16.1 ± 3.2 13 – 22	16.5 ± 3.6 11 – 24
Height (cm) Range	100.1 ± 1032 82 – 120	101.0 ± 9.61 82 – 120	99.85 ± 9.21 85 – 114

Table (2): Hemodynamic and Respiratory data (Mean ± SD).

Variable	Group B (n = 20)	Group K (n = 20)	Group BK (n = 20)
MABP (mmHg)	90.5±2.1	93.3±1.2	89.6±1.4
HR (beat/min)	106±12	111±12	112±9
SaO2 (%)	98	97	99

Table (3): Operative data (Mean ± SD).

Variable	Group B (n = 20)	Group K (n = 20)	Group BK (n = 20)
Surgery duration (min)	43.5±7.3	45.2±8.1	42.5±7.6
Anesthesia duration (min)	52.5±9	50.9±8.6	53.9±9.4
Recovery time (min)	4.8±1.05	10.5±0.25*	4.01±4.2
Sevoflurane concentration (%)	1.80±0.86	1.11±0.31*	1.61±0.80

* Significant when compared with group B and group BK.

Table (4): Quality of Postoperative analgesia (Mean ± SD).

Variable	Group B (n = 20)	Group K (n = 20)	Group BK (n = 20)
Time to first request for analgesic (hours)	4.20±3.1	7±3.02*	6.50±2.11
CHIPPS (at the time to first request for analgesic)	5.35±1.56	3.45±1.46*	4.55±1.73
Duration of absolute analgesia (min)	115±20	210±30*	155±23
4-points sedation score (at 3-hours postoperative)	0.02±0.22	0.200±0.41	0.150±0.36

* Significant when compared with group B and group BK.

Table (5): Number (%) of subjects required analgesia and Number of doses analgesic.

Group-B (n = 20)		Group-K (n = 20)		Group-BK (n = 20)	
No (%) of patients	Parace-tamol (No of Doses)	No (%) of patients	Parace-tamol (No of Doses)	No (%) of patients	Parace-tamol (No of Doses)
9 (45%)	Once	1 (5%)	Once	5 (25%)	Once
7 (35%)	Twice	1 (5%)	Twice	3 (15%)	Twice
4 (20%)	–	18 (90%)	–	12 (60%)	–

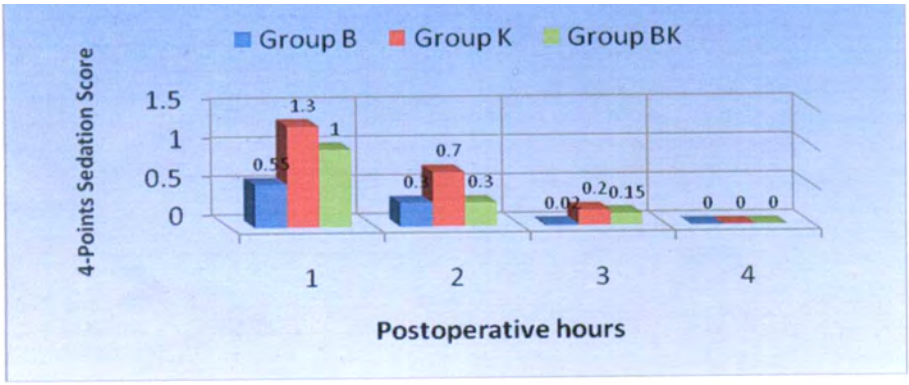

Fig. (1): 4-Points Sedation Sore.

Discussion

This study was designed to compare whether the addition of preservative free ketamine to bupivacaine, when administered caudally, would prolong the duration of postoperative analgesia more than caudal ketamine or caudal bupivacaine alone in children undergoing surgery. Our results revealed that caudal administration of 1 mg/kg ketamine without bupivacaine significantly prolonged the duration of postoperative analgesia when compared with 1 ml/kg of 0.25% caudal bupivacaine alone but nearly comparable to that associated with caudal injection of a mixture of 0.125% bupivacaine and 0.5 mg/kg of ketamine at a volume of 1 ml/kg. Also, our results demonstrated that, patients in group ketamine had significantly decreased visual analogue scale score especially at the time to first request for analgesic.

Caudal administration of bupivacaine alone can provide adequate analgesia in the early postoperative period~ but a single caudal injection provides analgesia only for the duration of the local anesthetic[8]. Bupivacaine 2-2.5mg/kg has duration of action of only 2-4 hours[9]. As a result, systemic analgesia is usually required as the block wears off. In this study no patients in any of the three groups was found not requiring additional analgesic. But the number of doses of postsurgical intravenous paracetamol (within the first 24-h) in caudal bupivacaine group (16 patients) was

significantly higher than that of bupivacaine-ketamine group (8 patients) or that of caudal ketamine only 2 patients. This revealed that caudal administration of ketamine (1 mg/kg) alone significantly reduced the need for subsequent postoperative analgesia.

Oluklola and his colleagues (2002) found that addition of 0.5 mg/kg of ketainine to caudal 0.25% plain bupivacaine significantly decreased the need for rescue analgesics in the first 24 h postoperative period in pediatric patients undergoing elective inguinal and lower abdominal operations, and revealed that caudal administration of ketamine alone provided analgesia of similar quality and slightly longer duration than 0.25% bupivacaine but this was not statistically significant[10] Another study done by Frank Weber and Hinnerk Wulf (2003) and they concluded that addition of preservative free s- ketamine 0.5% mg/kg to caudal bupivacaine 0.25% 1 ml/kg provides significant prolongation of analgesia without producing negative side effects[11].

In our study, we increased the dose of ketamine to 1 mg/kg, while using it as a sole agent, which perhaps increased the potency of analgesia. One other study using the same dose has shown a median duration of 16.5 hours (in our study 7.05 hours)[9].

Ketamine, a phencyclidine derivative, has structural similarities to bupivacaine and has some local anesthetic effects[12]. The primary mechanism of action is through the blockade of N-methyl-d-aspartate receptors situated in the substantia gelatinosa of the spinal cord[13]. Ketamine also binds to the opioid receptors, with a preference for the μ receptors[14]. Although different doses of ketamine (0.25%-1.0%) have been reported in combination with local anesthetics to increase the duration of analgesia, the optimal dose is probably 0.5 mg/kg[15].

A potential advantage of central neuraxial ketamine is that it may counteract local anesthetic-induced hypotension by an inhibitory effect on the sympathetic nerve activity and tends to increase the respiratory rate[16]. In our study, the observed periopera-

tive hemodynamic stability with the use of caudal bupivacaine plus ketamine and caudal ketamine only supports this contention.

Kumar et al (2005) showed that the incidence of vomiting was not significantly different among groups, two patients each in group BK and B[17], but in our study, 5 in group B, 3 in group K, and only one in group K. Others did not observe any negative side effects attributable to the caudal block of s-ketamine[11].

In this study, the patients who received caudal epidural block with ketamine had decreased Intraoperative requirements for Sevoflurane. This is in accordance with the study of Gunduz et al (2006) who found that Sevoflurane concentration required was significantly lower in group receiving caudal ketamine[18]. Also Joseph Toblas (1996) concluded that preincisional caudal epidural block with ketamine had decreased Intraoperative inhalational anesthetic requirements during umbilical herniorrhaphy in children[19].

In conclusion this study demonstrates that caudal administration of preservative-free ketarnine alone (1 mg/kg) provided adequate postoperative analgesia of similar quality and slightly longer duration than caudal injection of both 0.25% bupivacaine 1 ml.kg-1 and ketarnine 0.5 mg/kg whereas it is proved to be superior in those respects from the caudal administration of 1 ml/kg of bupivacaine 0.25% alone in pediatric patients without producing many side effects.

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CAUDAL BUPIVACAINE, KETAMINE AND THEIR COMBINATION FOR PEDIATRIC POSTOPERATIVE ANALGESIA

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EFFECT OF INTRAPERITONEAL INSTILLATION OF LORNOXICAM, AND BUPIVACAINE COMBINATION ON PATIENTS OUTCOME AFTER LAPAROSCOPIC CHOLECYSTECTOMY

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Abstract

Laparoscopic Cholecystectomy(LC) is the gold stander technique for gall bladder surgery. ***Aim:*** *Evaluate the effect of intraperitoneal instillation of Lornoxicame combined with bupivacaine on postoperative pain and pulmonary function after laparoscopic cholecystectomy.* ***Patients and Methods:*** *forty four ASA 1 and 2 patients of either sex,aged between 20-50 years, undergoing laparoscopic cholecystectomy The patients were randomly assigned to either of the two groups: (group P) (n=22) received 25 ml of normal saline intraperitoneally or (group LB) received 20 ml of bupivacaine 0.5%combined with 5ml of Lornoxicame (16mg). All patients received general anesthesia. Intraoperative monitoring consisted of ECG, NIBP, end tidal CO2, pulse oximetry and intraabdominal pressure. Arterial blood gases (Ph, Pao2, paco2) and Spirometric values includes FEV1, FVC, FEVI/FVC were assessed. Postoperative pain was assessed by utilizing visual analogue scale (VAS) and Visual Rating Prince Henry Scale Score (VRPHSS) and duration of analgesia were recorded by determine the first request of analgesia .* ***Results:*** *Both groups were comparable as regarded age, weight, height, sex, duration of surgery and days of hospital stay. There was insignificant differences in between both groups intraoperative hemodynamic parameters and arterial blood gases preoperatively and postoperatively. There was significant increased in pulmonary function tests includes FEV1, FVC and FEV1/FVC ratio at 4 hours postoperatively in group (LB) in compared with group (P).*

Moreover, there was a highly significant increase in the analgesic requirements in group (P) more than (LB) group. VASS was significantly decreased in (LB) group more than (P) group. VRPHSS was significantly lower in (LB) group than group (P). There was two case of postoperative vomiting in (P)group. No recorded cases of dizziness postoperatively in both groups. ***Conclusion:*** *Iintraperitoneal instillation of Lornoxicam in combination with bupivacaine after laparoscopic cholecystectomy had more better postoperative analgesic effect with less analgesic requirements and less affection of the pulmonary function(FEV1, FVC and FEV1/FVC ratio)*

Keywords: *Laparoscopic Cholecystectomy, Lornoxicam, Intraperitoneal instillation, postoperative complications.*

Introduction

Laparoscopic Cholecystectomy (LC) is more preferable technique than open surgery. As LC strategies is minimally invasive technique with less tissue trauma than open surgery, leading to less postoperative pain with early home discharge[1]. However, prolonged hospital stay was recorded in some patient after (LC) due to pain or postoperative pulmonary complications after laparoscopic surgery [2,3]. Intraperitoneal instillation of bupivacaine associated with good pain relief after laparoscopic cholecystectomy[4]. Lornoxicam is a new NSAID of the oxicam class with analgesic, anti-inflammatory and antipyretic properties[5], it has non-selective inhibiting effect to cyclooxygenases enzyme (COX2) [6]. Lornoxicam has an analgesic effect as morphine[6] meperidin,[7] and tramadole.[8] but better tolerated than morphine when administered intravenously by patient-controlled analgesia in the treatment of moderate postoperative pain.[9] Furthermore, The intraarticular injection of ropivacaine, morphine, and xefocam combination was superior to control or to a combination of ropivacaine and morphine alone in postoperative pain control and in decreaseing the need for opioid suggesting a local effect.[10] Un fourintely, there was no previous study to assess the effect of intraperitoneal analgesia on postoperative pulmonary functions. Current study was designed to assess the effect of intraperitoneal instillation of Lornoxicam combined with bupivacaine on postoperative pain and

pulmonary function after laparoscopic cholecystectom.

Patients and Methods

The current study was conducted in a double blinded randomized manner (closed envelope technique) on forty four ASA 1 and 2 patients of either sex, the studied patients aged between 20-50 years, undergoing laparoscopic cholecystectomy at Mansoura Main University Hospital from October 2012- to April 2013 under general anesthesia. Informed consent was taken and the study was approved by the hospital ethics committee. The exclusion criteria included patients with acute cholecystitis, who did not give consent and who had any contraindications to NSAID. The patients were randomly assigned to either of the two groups according to the intraperitonal instilled solution which prepared by the pharmacists in coated suringes: Group (P) (n=22) received 25 ml of normal saline or group(LB) (n=22) received 20 ml of bupivacaine 0.5% combined with 5ml of Lornoxicame (Xefo) (Necomed) (16mg) at the end of surgery in the trendlenberg position. The observer anesthetist and surgeon who instilled the solutions intraperitoneal were blinded to the solution. All patients were premedicated with diazepam 5mg per orally at the morning of the surgery. Induction was carried out with 5 mg kg-1. thiopental sodium and intubation achieved with 1.5 mg kg-1 of suxamethonium using suitable size of endotracheal tube. Intraoperative muscle relaxation was achieved by atracuroium 0.05 mg kg-1. The anesthesia was maintained by 02, intermittent doses of atracroium, whereas intraoperative analgesia was achieved with morphine sulphate 0.15 mg kg-1.Ventilation was adjusted to maintain end tidal C02 between 35-40 mmHg, whereas intra abdominal pressure was maintained between 10-12mmHg. The muscle relaxation was reversed at the end of surgery with neostigmine 0.05 mg kg-1 and atropine 0.025 mg kg-1. Intraoperative monitoring consisted of ECG, NIBP, end tidal CO2, pulse oximetry and intraabdominal pressure. Either the study or placebo solutions was sprayed on the upper surface of the liver and on right subdiaphragmatic space, to allow it to diffuse into the hepatodiaphragmatic space,

near and above the hepatoduodenal ligament and above gall bladder by the surgeon. This was done using a catheter inserted into the subcostal trocar under direct laparoscopic control. Arterial blood gases (Ph, Pao2, paco2) are assessed preoperatively, 2hours,12 hours and 24 hours postoperatively. Spirometric values includes FEV1, FVC, FEVI/FVC are recorded by (Smart PFT, Medical Equipment Europe (MEE Spirometry) Gmbh and vitalograph Copd-6 TM) preoperatively, 4 hours and 24 hours postoperative. Postoperative pain was assessed at immediate, 4 hours, 12hours and 24 hours by utilizing visual analogue scale (VAS), Visual rating Prince Henry scale (VRS)[10] for shoulder pain assessment The VAS consisted of a 10 cm scale representing varying intensity of pain from 0 cm (no pain) to 10 cm (worst imaginable pain). The visual rating Prince Henry pain scale[10] consisted of 0-4 grades with 0. No. pain no cough, 1-pain on cough but not on deep breathing 2-pain on deep breathing but not on rest, 3-pain on rest slight and 4-pain on rest-severe. Duration of analgesia were recorded by determine the first request of analgesia. Rescue analgesic consisted of intra muscular Diclofenac 75 mg at VAS more than 6 and VRS more than3. Number of patient request Rescue analgesic consisted were recorded. Dizziness and postoperative nausea and vomiting also were assessed

Statistical analysis:

Sample size was calculated by using t test for mean in G *power 3.1 (Faul, Erdfelder, Lang, and Buchner (2007) in Germany) program. According to pilot study (5 patients in each group) we calculated that 19 patients per group were give $p<0.05$ significant with confidence interval of 95% with a actual power of 95% when mean value of FEV1/FVC ratio at the 4 hours postoperative in group(P) was 0.74 and in group (LB) was 0.79. we added 3 cases for each group to ensure more accuracy of the statistical results.

Statistical analysis was carried out using the Statistical Package for Social Sciences 16 (SPSS Inc., Chicago, IL, USA). Data was presented as number, percentage, means and standard deviations. Parametric data were analyzed us-

ing Student unpaired- samples t test. Non parametric data were analyzed by Mann-Whitney test. chi-square test was used for comparison between percentages and frequencies. Significance level was established at a P value ≤0.05

Results

The study groups were comparable in the patients characteristics included age, weight, height, sex, duration of surgery and days of hospital stay (table 1).

There was insignificant differences in between both groups as regared hemodynamic parameters (HR and Mean arterial blood pressure, end tidal CO2 and O2 saturation) and arterial blood gases (Ph, Pao2, and PaCo2) preoperatively, intraoperatively and postoperatively ($p>0.05$).

There was a statistically significant increased in pulmonary function tests includes FEV1($p<0.001$), FVC ($p<0.008$) and FEV1/FVC ratio($p<0.02$) at 4 hours postoperatively in group (LB) in compared with group (P) (table2).

As regarded pain, there was highly significant increase in the duration of postoperative analgesia in (LB) group more than (P) group ($p<0.001$) (table2). Moreover, there was significant increase in the analgesic requirements in group (P) in compared with (LB) group ($p<0.001$) (table 3).

Visual Analogue Scale Sore was significantly decreased in (LB) group more than (P) group immediately ($p<0.001$), 4 hours ($p<0.001$), and 12 hours (p 0.006) postoperative (Figure1).Visual Rating Prince Henry Scale Score (VRPHSS) for postoperative shoulder pain assessment was significantly lower in (LB)group than group (P) immediately (p 0.03) and 4hours (0.04) postoperatively (Figure 2).

There was two case of postoperative vomiting were recorded in (P) group. No recorded cases of dizziness.

Table (1): Patients characteristics (data were presented as mean ± SD / number.

	Group (p) (n=22)	**Group (p) (n=22)**
Age (year)	36.2±2.7	35.2±3.1
Weight (kg)	76.6±6.3	77.5±6.7
Sex male/female	4/18	6/16
Height (cm)	152±6.9	150±7.7
Operative time (min)	53±4.3	55.3±4
Hospital stay days	2.5±0.7	2.3±0.6

Group (p): placebo group Group (LB): lornoxicame bupivacaine group

Table (2): Pulmonry function tests (FEV1, FVC AND FEV1/FVC ratio) preoperative and postoperative for 24 hours (data were presented as mean±SD).

	Group (p) (n=22)	**Group (LB) (n=22)**	**P value**
FEV1 (liters/second)			
preop	2.6±0.2	2.7±0.2	
4 hours postop	2±0.3	2.4±0.1*	0.001
24 hours postop	2.2±0.4	2.3±1.2	
FVC (liters)			
preop	3.2±0.2	3.3±0.3	
4hours postop	2.6±0.3	2.9±0.2*	0.008
24hours postop	2.7±0.4	2.8± 0.2	
FEV1/FVC (percent)			
preop	0.81±0.07	0.82±0.06	
4 hours postop	0.77±0.09	0.83±0.05*	0.02
24 hours postop	0.81±0.06	0.82±0.03	

Group (p): placebo group Group (LB): lornoxicame bupivacaine group
* significant in (LB) group compared with group(P)when p≤0.05

Table (3): Duration of postoperative analgesia (hours) and Distribution of postoperative request of rescue analgesic (numbers) (data were presented as mean ± SD/number (percent).

	Group (p) (n=22)	**Group (LB) (n=22)**	**P value**
Duration of postoperative analgesia (hours)	1.95±0.68	23.3±0.9*	<0.001
Distribution of Postoperative request of rescue analgesic (numbers)			
no	0	19(86%)*	<0.001
Once	1(5%)	2(9%)	0.3
Twice	13(59%)	1(5%)*	0.001
Trice	8(36%)	0(0%)*	0.002

Group (p): placebo group Group (LB): lornoxicame bupivacaine group
* significant in (LB) group compared with group(P)when p≤0.05

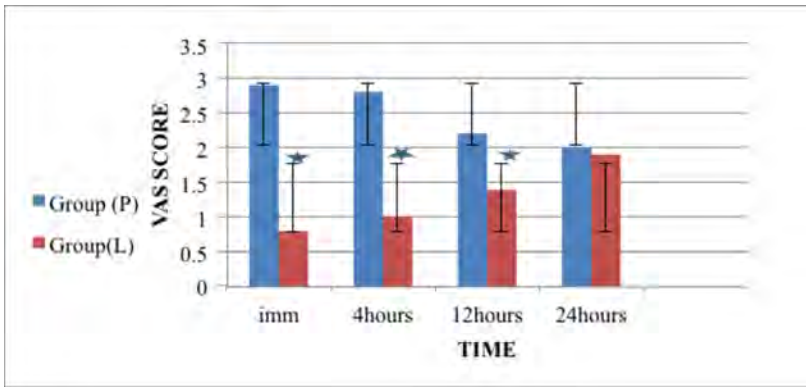

Fig.1: Post operative visual analogue scale score (Data expressed as Mean (SD).
Group (p): placebo group Group (LB): lornoxicame bupivacaine group
* significant in (LB) group compared with group(P) when p≤ 0.05.

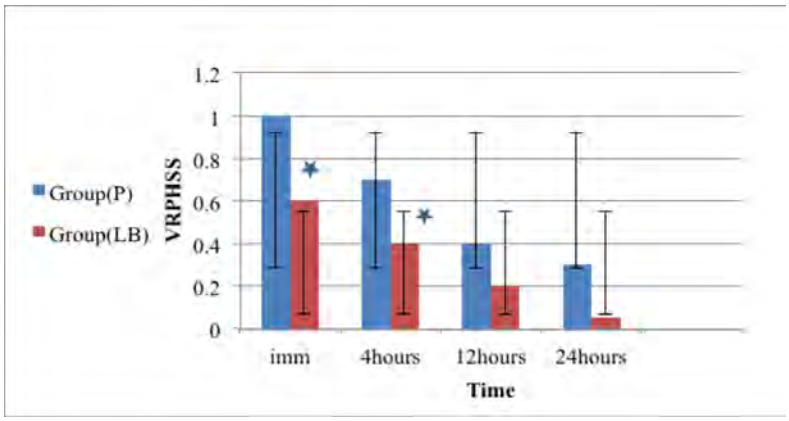

Fig.2: Postoperative visual rating Prince Henry pain scale score(VRPHSS)(Data expressed as Mean (SD).
Group (p): placebo group Group (LB): lornoxicame bupivacaine group
* Significant in (LB) group compared with group (P) when p≤0.05

Discussion

Postoperative pain and pulmonary complications are the major causes of morbidity after laparoscopic Cholecystectomy (LC)[11]. Previous studies reported that the postoperative pain and diaphragmatic dysfunction were considered the major contributing factors of postoperative pulmonary complications[2,12]. The possible cause of postoperative shoulder pain af-

ter (LC) is the diaphragmatic irritation by insufflated CO2. Carbon dioxide may be transformed to irritative carbon dioxide by combination with the fluid in the peritoneal cavity[13]. Another possible cause of shoulder pain after LC may be due to overstretching of the diaphragmatic muscle fibers and phrenic nerve neuropraxia a owing to high rate of insufflations[14]. In present study shoulder pain was low in all treatment groups due to carful removal of residual intraperitoneal CO2 by the surgeon. Moreover postoperative pulmonary complications may be attributed to manipulation and local stimulation of the gallbladder and its bed during laparoscopic Cholecystectomy which may stimulate reflex inhibition of the diaphragm as shown in animal study[12].

Present study recorded that there was significant increase in the duration of postoperative analgesia, reduction of the pain scores and analgesic requirements with significant improvements of the pulmonary functions including FEV1, FVC and FEV1/FVC postoperatively in the group that receive intra peritoneal instillation of (NSAID) lornoxicame combined with bupivacaine than that receive intra peritoneal instillation of saline.

In agreement with current study Memedov et al[15] found that intraperitoneal instillation and port site infiltration of ropivacaine and lornoxicam during laparoscopic cholecystectomy reduces the postoperative pain.

Lornoxicam has been successfully used in prevention and treatmentof postoperative pain in patients undergoing laparoscopic gynecological surgeries[16]. Sen et al[17] reported that lornoxicam and lidocaine combination during intravenous regional anesthesia reduce sensory and motor block onset times, prolongs sensory and motor block recovery times, reduce tourniquet pain and increase duration of analgesia with decreasing total amount of analgesic requirements.

Lornoxicam is not a local anesthetic agent but it has a COX2 inhibitor effect leading to reduction of secretion the of the pain mediators in the areas of surgical ma-

nipulation and besides its analgesic effect, in addition to local antinociceptive effect[6]. NSAIDs may induce predominant peripheral antinociceptive effect by prevent conduction of C Fibres and opening of the K+ channels located in the primary afferent nerve endings[18]. Also, Lornoxicam have a peripheral analgesic effect via activiation of NO-c GMP pathway and the opening of K+ channels [17]. Surprisingly, Lornoxicam has antioxidative effects in rats decrease the dose of analgesics and prevent the negative impact of reactive oxygen species on nociception[19]. In correlation to present study results of the effect of pain management on the pulmonary function, Spence and Smith[20] documented that the continuous extradural nerve block in patients undergoing vagotomy with gastroentrostomy or pyloroplasty had less effect on the postoperative pulmonary function (FEV1/FVC ratio) and arterial oxygenation than intravenous morphine as the result of better pain control.

The conclusion of current study is the intraperitoneal instillation of Lornoxicam combined-with bupivacaine after laparoscopic cholecystectomy had more better postoperative analgesic effect with less analgesic requirements and less affection of the pulmonary function (FEV1, FVC and FEV1/FVC ratio).

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EFFECT OF INTRAPERITONEAL INSTILLATION OF LORNOXICAM, AND BUPIVACAINE COMBINATION ON PATIENTS OUTCOME AFTER LAPAROSCOPIC CHOLECYSTECTOMY

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COLOR DOPPLER ECHOCARDIOGRAPHIC CHANGES 2 YEARS AFTER 2D PLANNED RADIATION THERAPY FOR LEFT BREAST CANCER, ITS RELATION TO CARDIAC BIOMARKERS

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Abstract

Introduction: *Radiation treatment has been associated with radiation induced cardiotoxicity, especially with older, long-outdated, techniques. Such complications include pericarditis, myocardial fibrosis, valvular injury, ischemic heart disease, and myocardial infarction. Some of these complications may be associated with changes in cardiac biomarkers.*

Aim of the study: *The aim of this study is to assess the effect of 2D planned radiation therapy (RT) after 6 months and 2 years on cardiac function using color Doppler echocardiography as well as to assess the relation of these changes to cardiac biomarkers in order to early detect and treat these changes in the proper time.*

Patients and methods: *80 women under 65 years of age with stage III left breast cancer who received postoperative radiotherapy were included during the time from January 2009 to January 2010. Each patient were subjected to thorough history taking with special concentration upon cardiovascular symptomatology, full cardiac examination, color Doppler echocardiographic examination as well as assessment of serum high sensitivity C-reactive protein (hs-CRP), cardiac troponin (cTn) and brain natriuretic polypeptide (BNP) before radiation therapy (T1), 6 months after RT (T2) and 2 years after radiation therapy (T3) for stage III left breast cancer patients in Mansoura University Hospital, Egypt.*

Results: *There was no significant change in color Doppler echocardiographic parameters after 6 months of radiation therapy, however, there was*

decrease in L.V. ejection fraction at 2 years but the result was statistically insignificant (P=0.095). Serum levels of hs-CRP, cTn and BNP were normal at baseline. There was no significant change in levels of cTn and BNP during the 2 years follow-up. However, serum level of hs-CRP was normal at 6 month and significantly increased at 2 years follow-up (P=0.003) which was associated with insignificant decrease in left ventricular ejection fraction (LVEF).

Conclusion: *There is limited value of color Doppler echocardiographic examination in prediction of cardiac toxicity after RT for stage III left breast cancer except for LVEF assessment after 2 years which had insignificant value. There was statistically significant role of hs-CRP in prediction of cardiac toxicity after RT for left breast cancer.*

Recommendations: *We recommend future studies including larger numbers of patients, different stages of breast cancer, different radiation therapeutic modalities as well as different tools for assessment of cardiac function and prediction of cardiac toxicity of RT.*

Keywords: *Echocardiography, Left breast cancer and cardiac biomarkers.*

Introduction

Radiation therapy (RT) in patients with breast cancer significantly reduces locoregional recurrence in up to 60% of cases[1]. The most recent systematic overview, conducted by the Early Breast Cancer Trialists Collaborative Group (EBCTCG), indicated that post-mastectomy RT reduces absolute breast cancer mortality by an average of 5% at 10 years[2]. The relative benefits are similar after-mastectomy or breast conservation surgery in the presence or absence of adjuvant systemic therapies as well as in axillary node-negative and -positive patients.

However, RT has been associated with radiation-induced cardiotoxicity, especially with older, long-outdated techniques[1,3,4]. Such complications include pericarditis, myocardial fibrosis, valvular injury, ischemic heart disease, and myocardial infarction, and have been observed particularly in the literature on photon

radiation of post-mastectomy chest wall and direct internal mammary lymph nodes[5,6].

At present, there is a controversy as to whether modern RT techniques are also cardiotoxic, especially in cases of left breast cancer. Gustaysson et al.[7] found that women younger than 5o years at the time of adjuvant RT following mastectomy for early breast cancer had no serious cardiac sequelae 13 years on. On the contrary, Paszat et al.[8] suggested that adjuvant RT for left-sided breast cancer diagnosed in women younger than 6o is associated with a higher risk of fatal myocardial infarction 10 to 15 years later compared with adjuvant RT for right-sided cases. Moreover, Harris et al.[9] proposed that irradiation to the left breast is not associated with a higher risk of cardiac death up to 20 years after treatment, but is associated with an increased rate of diagnoses of coronary artery disease and myocardial infarction compared with right breast treatment.

Cardiac biomarkers are protein molecules that are widely used in the early detection of heart failure. Serum levels of cardiac troponin (cTn), C-reactive protein (hs-CRP) and brain natriuretic polypeptide (BNP) have been shown to be sensitive markers for left ventricular dysfunction and powerful markers for morbidity and mortality in the heart failure setting. All these biomarkers have been evaluated in prediction of early cardiac dysfunction after different chemotherapeutic agents. However, their ability to detect early cardiac dysfunction after radiation therapy for stage III left breast cancer is not still obvious[10,5,11].

The aim of this study is to asses the color Doppler echocardiographic changes after 6 months and after 2 years of 2D planned radiation therapy for women with stage III left breast cancer and to assess the relation to serum levels of some cardiac biomarkers as hsCRP, cardiac troponin (cTn) and brain natriuretic polypeptide (BNP) in order to evaluate the role of these biomarkers for early prediction of radiation induced cardiac toxicity.

Patients and Methods

Eighty women under 65 years

of age with stage III left breast cancer who received postoperative radiation therapy in Clinical Oncology and Nuclear Medicine Department, Mansoura University Hospital, Egypt, were included during the time from January 2009 to January 2010. Patients with previous cardiovascular disease, thyroid disorder, significant anemia with serum hemoglobin <8.0 gm/dl, previous ECG abnormalities and previous echocardiographic abnormalities were excluded from the study. 15 patients (18.75%) were hypertensive and 10 patients (12.5%) were diabetic type II. Each patient was subjected to thorough history taking with special concentration upon cardiovascular symptomatology, full clinical cardiac examination, color Doppler echocardiographic examination as well as assessment of serum cardiac biomarkers (serum hs-CRP, cardiac troponin cTn and BNP) before start of radiation therapy (T1), 6 months after radiation therapy (T2) and 2 years after radiation therapy (T3).

Color Doppler echocardiographic examination was performed using Vivid-5 ultrasound (GE-vingmed ultrasound AS, Horten Norway) with 2.5-3.5 MHz transducer in left lateral decubitus position. Parasternal and apical projections were obtained according to the recommendations of American Society of Echocardiography[12].

Standard two-dimensional echocardiographic evaluation for left and right ventricular size and function was performed. Left and right ventricular diameters as well as left atrial diameter were measured from a parasternal long-axis view by M-mode examination at the speed of 50 mm/s[13]. Left ventricular ejection fraction (EF) was measured from the apical four chamber view using biplane Simpson's method[12].

hs-CRP, cTn, and BNP were evaluated before, 6 months after and 2 years after postoperative radiation therapy. Quantitative determinations of cTn levels were performed with a third-generation Roche Elecsys assay (Roche Diagnostics, Inc., Indianapolis, Indiana). The CRP levels were measured with the Immage 800 (Beckman Coulter, Brea, California) antigen-antibody precipitant

rate reaction. The N-terminal pro-BNP levels were measured with an electrochemiluminescence sandwich immunoassay (Elecsys ProBNP, Roche Diagnostics) with the Roche 2010 system.

As regards radiotherapy technique, accurate patient-specific anatomic information of the breast is a prerequisite for planning and implementing the delivery of radiation to the entire breast while minimising exposure to critical structures such as lungs, ribs and heart. The image data from the patients' breasts were taken by a diagnostic multi-slice CT scanner with a flat couch, which could be restrictive for setting up patients with immobilization devices in the treatment position. During RT all patients were placed supine with left hand up and attached to immobilization devices in precise reproducible position of treatment. The anterior and lateral isometric lines and other landmarks were marked on the patient's skin by radio-opaque markers or lead-beads.

From all diagnostic CT slices, the central one was chosen as the one in where the disease was most extensive. Thereafter, we delineated the target area as well as the organs at risk, and these data were input to a 2-dimensional (2D) computer treatment planning. The entire breast was included in the planning target volume (PTV) with a 1 cm margin around palpable breast tissue. The physician and the radiotherapist could then select the treatment beam directions. The objective was to treat the PTV disease tissue plus a 1 cm margin to a tumoricidal dose while limiting the dose to the surrounding normal tissues. If critical tissues were located nearby, the aim was to keep the dose to these organs to a level within the acceptable limit of complication. The reference point (100% dose) was located in the centre of the PTV and in the junction of the axes of the tangential fields. The dose variation permitted inside the PTV was between -5% and +l0%. In order to further adjust doses and to prevent radiation of sensitive healthy tissues such as lungs and pericardium, axes with a deviation larger than 180° as well as wedges of different angles (15°, 30°, 45°, 60°) were-employed.

Radiotherapy was delivered with a LA linear accelerator treatment unit using a pair of opposed tangential beams (medial and lateral). The daily dose was 200 cGy, the total dose on the PTV was 50 Gy in 25 fractions over 5 weeks without boost, while in some patients a boost dose of 5 to 6 Gy was given as necessary. None underwent RT to the internal mammary chain.

Statistical analysis:

Data, analysis was performed by using Statistical Package for Social Sciences (SPSS) version 11.5 software (SPSS Inc., Chicago. IL. USA). For the continuous variables, parametric test conditions were tested. Descriptive statistics were shown as mean ± standard deviation or median (maximum-minimum) where appropriate. While the mean differences between measurement times were compared by repeated measures of ANOVA. Degrees of association between continuous variables were calculated by Spearman's correlation analysis. A P=value <0.05 was considered statistically significant.

Results

Fiteen patients were hypertensive (18.75%) and 10 patients were having type II diabetes (12.5%) before start of radiation therapy (RT). No new patients developed hypertension nor diabetes during the 2 years follow-up. Mean age of patients was 47±8 years. Mean BMI was 26±5. No history of smoking and only 3 patients (3.75%) have family history of CAD.

Table I summarizes echocardiographic data of the studied patients. No significant changes in echocardiographic parameters were detected after 6 months and after 2 years of RT apart from mild decrease in LVEF after 2 years and the results were statistically insignificant (P=0.095).

Table II summarizes serum serial levels of cardiac biomarkers in the studied patients before start of RT (T1), 6 months after RT (T2) and 2 years after RT (T3). This table showed that there was no significant changes regarding serum levels of cardiac troponin and BNP after 6 months and after 2 years of RT. However, there was increase in hs-CRP after 2 years and the result was statistically significant (P=0.003).

Table (I): Echocardiographic findings:

	T1	T2	T3	P-value T1 vs T2, T2 vs T3 & T1 vs T3
Pericardial fluid	0	1	0	
EDV (mL)	111.5±16.8	112.5±12.9	116.6±16.9	0.112, 0.117, 0.116
ESV (mL)	43.8±7.5	43.9±8.4	45.8±9.5	0.121, 0.113, 0.114
EF (%)	64.8±7.6	63.3±7.1	60.4±7.1	0.112, 0.101, **0.095**
LVIDd (cm)	5.2±0.4	5.3±0.8	5.4±0.5	0.116, 0.112, 0.111
LVIDs (cm)	3.7±0.7	3.8±0.9	3.9±0.6	0.113, 0.114, 0.118
LVPwd (cm)	0.82±0.2	0.83±0.2	0.86±0.1	0.119, 0.111, 0.103
IVSd (cm)	0.75±0.3	0.76±0.1	0.77±0.1	0.112, 0.109, 0.107
IVSs (cm)	1.11±0.2	1.12±0.3	1.14±0.2	0.121, 0.111, 0.102
LAD (cm)	2.8±0.6	2.9±0.7	3.0±0.5	0.121, 0.118, 0.119
RVD (cm)	1.8±0.2	1.8±0.3	1.9±0.2	0.181, 0.123, 0.128
RWMA	0	0	0	

Abbreviations: ***EDV****, end diastolic volume;* ***ESV****, end systolic volume;* ***EF****, ejection fraction;* ***LVIDd****, left ventricular internal dimension in diastole;* ***LVIDs****, left ventricular internal dimension in systole;* ***IVSd****, interventricular septum in diastole;* ***IVSs****, interventricular septum in systole;* ***LAD****, left atrial diameter;* ***RVD****, right ventricular diameter;* ***RWMA****, resting wall motion abnormalities, P<0.05 is significant.*

Table (II): Summary of serial cardiac biomarkers.

Cardiac biomarkers	At baseline T1	6 months after RT T2	2 years after RT T3	P-value T1 vs T2, T2 vs T3 & T1 vs T3
Cardiac troponin (ugm/l)	<0.01	<0.01	<0.01	1.99, 1.99, 1.99
hs-CRP (mg/dl)	5.5±1.2	6.1±1.1	15.2±2.1	0.921, 0.061, **0.003**
BNP (pmol/l)	27.5±2.4	28.2±2.1	29.6±1.9	0.121, 0.122, 0.101

Discussion

Cancer therapy has shown great progress leading to important reduction of morbidity and mortality of several kinds of cancer. The therapeutic management of oncologic patients includes combinations of drugs, radiation therapy and surgery. Many of these therapies produce adverse cardiovascular complications which may negatively affect both the quality of life and the prognosis. For several years the most common noninvasive method of monitoring cardiotoxicity has been represented by radionuclide ventriculography while other tests as effort EKG and stress myocardial perfusion imaging may detect ischemic complications, and 24-hour Holter monitoring unmask

suspected arrhythmias[10]. Also biomarkers such as troponin I and T and B-type natriuretic peptide may be useful in early detection of cardiotoxicity. The widely used non-invasive method of monitoring cardiotoxicity of cancer therapy is, however, represented by Doppler-echocardiography which allows to identify the main forms of cardiac complications of cancer therapy: left ventricular (systolic and diastolic) dysfunction, valvular heart disease, pericarditis, pericardial effusion and carotid artery lesions[17].

A meta-analysis by Cuzick[14] showed a 62% increase in cardiac death in women receiving RT. Similarly, EBCTCG found a 30% increase in vascular mortality in women receiving RT for breast cancer[15]. Some authors reported no increased risk for patients with left breast disease treated with techniques used approximately since 1975[16,11], while others claimed a 2-fold risk of fatal myocardial infarction for left-sided treatment compared with right-sided[8].

A lot of data are available about the cardiotoxic effect of different chemotherapeutic agents. However, the data about the cardiotoxic effect of radiation therapy (RT) is lacking.

Our study was designed to evaluate the cardiotoxic effects of postoperative radiation therapy at 6 months and 2 years in patients with stage III left breast cancer and its relation to serum levels of some cardiac biomarkers as hs-CRP, cTn and BNP that can allow us for early detection and prediction of cardiac toxic effects of RT.

Reported data from studies on the comprehensive examination of the long-term cardiac mortality and morbidity after left breast irradiation using contemporary RT techniques indicate a significant association with an increased incidence of coronary artery disease and myocardial infarction 20 years after RT treatment. However, our study shows non significant reduction of LVEF 2 years after RT. Furthermore, none of the patients developed myocardial infarction or coronary artery disease. All other echocardiographic parameters remained practically

unchanged, showing that RT did not affect them. Pericardial fluid was present in only 1 case at 6 months after RT, an effect that was temporary.

These results are contradictory with those in most other publications. In the most recent population-based case-cohort study[10], the authors concluded that in addition to risk factors such as increasing age at diagnosis, smoking history, and history of acute myocardial infarction before postoperative RT, anatomic characteristics of RT such as RT for left-sided tumors, the use of an anterior internal mammary field, and increased area of an anterior left breast boost field are associated with increased risk of acute myocardial infarction[10]. The discrepancy could most likely be explained by the small total number of participants, the short follow-up period, the absence of internal mammary irradiation, and, especially, the younger mean age of patients at the time of diagnosis.

At baseline, cardiac biomarkers including hs-CRP, cTn and BNP were within normal limits for the entire patient population studied. No changes in serum level of biomarkers after 6 months. However, there was increase in serum level of hs-CRP after 2 years and the result was statistically significant (P=0.003). Our data goes in contrast with the data reported by Nazanin et al.[17] who studied the utility of cardiac biomarkers in predicting early L.V. dysfunction in patients with Human Epidermal Growth Factor Receptor II breast cancer treated with Trastuzumab therapy with or without RT who reported no change in serum biomarkers in one year follow-up of their patients[17]. The difference in results may be attributed to different number of patients, different ages included, different geographic patient characteristics as well as longer follow-up duration in our study.

In conclusion, color Doppler echocardiography could be valuable for detection of pre-clinical cardiac toxicity in patients with stage III left breast cancer after RT. However, follow-up of serum hs-CRP levels may be of more significant value before patients can go through symptomatic cardiac toxicity and HF. We recommend future studies

with larger number of patients, different patient characteristics, different RT technique and doses and different serum cardiac biomarkers to be conducted to help cardiologists and radiotherapists for early detection and prediction of cardiac toxicity following RT in left breast cancer patients.

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METRONOMIC LOW DOSE CARBOPLATIN AFTER RADIOTHERAPY IN STAGE III TESTICULAR SEMINOMA

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Abstract

Background: *Testicular germ cell tumor (TGCT) is the most common cancer in men between age of 15 and 35 years, it represents 5% of urologic tunmors. Extended field radiotherapy is a standard of care for stage II testicular seminoma, while stage III seminoma, neoadjuvant 3-4 cycles of Cisplatin based chemotherapy followed by radiotherapy of residue (if residue is more than 3 cms) but if residue less than 3 cm follow up by PET CT every 6 months was recommended.*

Patient and methods: *51 patients with testicular seminoma stage IIIa and IIIb were treated between May 2009 to May 2010 with orchidectomy followed by 3 to 4 cycles of Carboplatin AUC-7 then by radiotherapy on residue, radiation dose ranged from 30-35 Gy. All patients received metronomic low dose Carboplatin (not exceed 150 mg) weekly for 12 weeks after 3 weeks from end of radiotherapy.*

Results: *After follow-up of 36 months with 74% of patients having been followed for >1.5 years. There have been no metastatic nor local relapses (95% CI of relapse free survival of 93%). Toxicity has been low with grade 3 toxicity limited to four patients with grade 3 haematological toxicity with (no clinical sequelae) and one patient with grade 3 nausea (during radiotherapy). No patients experienced grade 4 toxicity.*

Conclusions: *The results of this study suggest that a metronomic low dose of Carboplatin after radiotherapy may reduce relapse rate compared with those who received radiotherapy alone and this approach is proposed for further investigation.*

Keywords: *seminoma, Carboplatin, radiotherapy*

Introduction

Traditionally, stage II testicular seminoma has been treated with radiotherapy alone with an extended field including both paraaortic and ipsilateral pelvic lymph nodes. With this policy, there is a relapse risk of 5%-11%[1,2]. Though these recurrences can then be usually treated successfully with combination chemotherapy[1], there is a concern over long-term complications from the intensity of their treatment, such as cardiovascular disease or second cancers[3]. A single dose of Carboplatin (Hospira, UK) can reduce recurrence in stage I seminoma[4], and in-field recurrence is rare after radiotherapy. While, in stage III testicular seminoma, new adjuvant 3-4 cycles of Cisplatin based chemotherapy followed by radiotherapy of residual lesion if residual lesion is more than 3cm, but if residue is smaller than 3 cm, follow up by PET CT every 6 months is recommended. We hypothesized that a similar reduction in metastatic relapse could be achieved in stage III seminoma if metronomic low dose of Carboplatin after radiotherapy was taken. We used a metronomic low dose of Carboplatin to achieve a blood concentration x time of Carboplatin (150 mg) weekly. This report is on the 51 patients of testicular seminoma treated in this way.

The initial strategy was to reduce the risk of recurrence and thus there is a need for salvage therapies. Subsequently, we also reduce the risk of metastatic relapse. The hypothesis was that the addition of metronomic Carboplatin would be worthwhile to achieve these goals.

Methods

51 patients with testicular seminoma stage IIIa and IIIb were collected from Clinical Oncology and Nuclear Medicine Department and Surgical Oncology Department, Oncology Center, Mansoura University in period between May 2009 to May 2010. All patients had a diagnosis of classical seminoma of the testis. Staging was by sequential analyses of blood levels of tumour markers alphafetoprotein, and beta human chorionic gonadotrophin (HCG), and by computed tomography (CT) scan of the thorax, abdomen and pelvis and classified by the maximum axial diameter using The Royal Marsden Hospital

Classification such that stage III= infradiaphragmatic and supradiaphragmatic lymph nodes, which classified into IIIa abdominal nodes <2 cm, IIIb abdominal nodes 2-5 cm and IIIc abdominal nodes >5 cm.

This analysis represents a retrospective audit of the standard treatment practice in our institution. Eligibility was generally that men had stage III seminoma with node metastasis up to 5 cm in diameter. If node involvement was equivocal, the scan was repeated 4-6 weeks later or was repeated with the associated PET analysis of fluorodeoxyglucose uptake (PET-CT scan). Ultimately classification as stage III disease was determined by collective judgement at a radiological nodes, supported in equivocal cases by repeat CT (n=5) or PET scans (n=5) or in one case by excision biopsy. Once classified as stage III, the number of nodes >1 cm in diameter was recorded as 'involved'. Orchidectomy was done then three to four cycles of Carboplatin was administered via a 1h infusion at a dose to achieve an area under the concentration x time curve of 7 mg. min/ml (dose (mg) = (GFR + 25) x 7, where GFR = glomerular filtration rate measured, by EDTA clearance). Oral antiemetics were prescribed before chemotherapy and during radiotherapy. Radiotherapy was initiated 3-6 weeks following Carboplatin cycles, patients were treated with anterior and posterior parallel-opposed portals shaped on virtual simulator CT planning. Initially, the fields encompassed both para-aortic and ipsilateral pelvic lymph nodes (dogleg field) extending superiorly to the lower border of the D10 vertebral body and inferiorly to the obturator fossa. This field was treated to a dose of 30-35 Gy in 15 fractions following which the para aortic nodes (D10-L5) were boosted by a further 5 Gy in 3 fractions for 12 cases while involved field was used for 39 patients. The follow-up was a 36 months. All patients received metronomic low dose of Carboplatin (total 150 mg weekly for 12 weeks) started three weeks after end of radiotherapy.

Toxicity was recorded qualitatively and classified according to its grade. Following treatment, patients were followed and confidence intervals for relapse risks were calculated[5,6].

Results

The age of 51 patients (Table 1) were 18-73 years (median 33 years) at the time of orchidectomy for seminoma. Only three were more than 50 years of age. All patients were presented with stage III disease with nodes varying from 1.1 to 5.0 cm in diameter. At the time of treatment, 19 patients had stage IIIa and 32 stage IIIb seminoma. Two further patients were treated as stage IIIb though strictly classified as IIIc with masses 6.2 and 7.0 cm in transverse diameter (para-aortic and pelvic, respectively), and this was because the masses were positioned such that they could be irradiated without causing renal damage. Of the 19 patients with stage IIIa disease the largest involved node in each individual ranged from 1.1-1.9 cm, (mean 1.5 cm); radiotherapy was to a dogleg field in 12 and para-aortic lymph node involved failed in 39. Three had 35 Gy and the rest had 30 Gy. Of the 32 patients treated as stage IIIb disease, the largest involved node in each individual ranged from >2.0 to 5.0 cm (mean 3.3 cm and 17 cases had node size >3 cm). Twenty-two had para-aortic rather than dogleg fields and 24 had a dose of 30 Gy rather than 35 Gy. While, in stage IIIa a patient had para-aortic involved failed and 16 patients received 30 Gy of radiotherapy. At the time of diagnosis of stage III disease, there was an elevated blood level of lactic dehydrogenase (LDH) in 12 of 48 patients and of HCG in 13 of 51 patients.

Follow-up was 36 months from date of start of Carboplatin. There have been no germ cell cancer recurrences. The 95% confidence intervals for relapse-free survival in all 51 patients are 93%-100%.

Toxicity has been mild and only short-term and is shown in Table 2. In summary, there were four patients out of 51 assessed who developed haematological toxicity grade 3 (and none >3) (platelet counts less than 50/cmm and total white blood count less than 2/cmm and none of whom suffered any clinical consequences. Platelet nadirs were 1-3 weeks after Carboplatin. White blood count nadir was 2-6 weeks after Carboplatin. Mild nausea occurred mainly during radiotherapy and was grade 3 in only 1 patient. Seven patients suffered grade 1-2 fatigue during radiotherapy.

Table (1): Patient details.

	N=51
Age: Median (range)	33 (18-73) years
Stage at protocol: IIIA/B	n = 19/32
RT: Field (dogleg/involved field) Dose (30 Gy/35 Gy)	n = 12/39 n = 48/3
Stage: IIIA (30 Gy/involved field) IIIB (30 Gy/involved field)	n = 16/9 n = 24/22
Elevated LDH	12
Elevated HCG	13

Two patients with node masses slightly >5 cm diameter have been included as IIB in the table.

Table (2): Peak acute toxicities.

Toxicity (number assessed)	Grade	No. of patients
Nausea (31)	0	16
	1	10
	2	24
	3	I
Diarrhoea (51)	0	38
	1	9
	2	4
WBC (50)*	>1	12
Platelets (50)*	>1	6
Fatigue	1 + 2	7

*One patient needle phobic and did not have follow-up blood counts.

Table (3): Radiotherapy for stage II and III seminoma.

Series	Patient cohort	Number	Stages[a]	% Relapse	5 years % CSS
Patterson et al. [2001]	1970-1997	80	IIA	13	95
			IIB	27	
Zagars et al. *[13]*	1960-1999	44	IIA	0	100
			IIB[b]	13	
Classen et al. [2003]	1991-1994	87	IIA	5	100
			IIB	11	
Chung et al. [2004]	1981-1999	95	IIA	8	95
			IIIB	10	
			IIIa	55	
Detti et al. [2009]	1965-2005	83	IIAb	10	99
Domont et al. [2011]	1980-2001	33	IIA	0	94
			IIIB	29	
			IIIA	75	

[a] Staging by RMH classification.
[b] Many had supradiaphragmatic fields also. CSS, cause-specific survival.

Table (4): Chemotherapy for seminoma.

Series	Stages	Regimen	Number	% percentage	% overall survival
Mencel et al. (1994)	BC-IV	EP	60	8	97
		EC	35	17	86
		VAB-6	45	21	78
		All		14	88
Fossa et al. (1997)	II-IV	Various	148	7	85
		Various	279	17	
Horwich et al. (2000)	II-IV	E[a] P	66	19	89
		C[b]	64	33	84
Clemm et al. (2000)	II-IV	IPE	251	5	95
		C[b]		26	87
Chung et al. (2004)	II	EP	31	7	97[c]
Krege et al. (2006)	IIA IIB	C[d]	108	18	99
Garcia-del-Muro et al.(2008)	IIA IIB	E[e] P or BEP	72	8	95

Staging by RMH classification.
[a] Etoposide 360 mg/m2, [b] 400 mg/m2, [c] Cause-specific survival. d At AUC 7, [d] Etoposide 400 mg/m2, [e] Etoposide; P, cisplatin; C, carboplatin; VAB-6, vinblastine, actinomycin and [d] Bleomycin, cisplatin, cyclophosphamide.

Discussion

This study has demonstrated a high progression-free survival in 51 patients with stage IIIa and stage IIIb testicular seminoma treated with a metronomic low dose of Carboplatin after radiotherapy confined to the involved nodal region to a dose of 30 Gy in 15 fractions. In Patterson[7], 31 patients with stage IIIa and IIIb seminoma were treated with a protocol with Carboplatin at 400 Mg/M2 and a radiation dose of 35 Gy and 2 relapsed cases were detecting. A low recurrence risk minimizes the need for combination chemotherapy salvage, and the associated risks of myelosuppression, pneumonitis, neuropathy, hearing loss, renal damage, cardiac events and infertility, as well as second cancers.

Our current regimen has very modest short-term toxicity. Long-term toxicity risks such as of second cancers are unlikely ever to be determined reliably given the rarity of this substage of disease, so will need to be estimated by extrapolation from larger series of patients who have had analogous treatments. In a recent national Dutch cohort of 2700 testicular cancer survivors, followed for a

median of 17 years, the risk of second malignancy was increased by 2.6x after infradiaphragmatic radiotherapy and by 2.1x after combination chemotherapy[3]. This is consistent with the multinational Cancer Registry study of 567 testicular cancer survivors reported by Travis et al.[8]. However, the radiation carcinogenicity risks were based on an extended field which included pelvis as well as abdomen. Radiation risks can be reduced by using a smaller radiation field and an estimate of the reduced risk after para-aortic field compared with a full 'dogleg' field is a reduction of 48%-63%[9].

Historical studies showing increased risk in those treated with both modalities may reflect a high total treatment burden such as for relapse. Carcinogenesis by chemotherapy has been linked to drug dose and class[10,11]. Powles et al.[12] found no excess of second cancer after treatment with Carboplatin but confidence intervals were wide and there have been insufficient long-term analyses to be confident of the lack of Carcinogenesis by Carboplatin or that this drug will not enhance radiation carcinogenesis.

Stage III seminoma has traditionally been treated with neo-adjuvant chemotherapy then radiotherapy (Table 3), but in early years staging was by lymphography. The standard radiation field was. extended to treat both para-aortic and ipsilateral upper pelvic nodes and doses ranged from 25 to 40 Gy. In the past, many centres also treated mediastinal or supraclavicular nodes. In a report from Toronto[2] on 79 patients, 8 (10%) relapsed; 4 were originally stage IIa and 4 stage IIb. A prospective registration study from 30 German centres[1] included 94 patients with stage IIb and IIIa seminoma. After a median of 70 months, the 5-year relapse-free rate was 95% for stage IIb patients and 89% for stage IIIa patients.

Platinum-based chemotherapy is also very effective for seminoma [14,15,16]. Most series (Table 4) indicate long-term progression-free survivals of >80% and survivals of >90%. Trials have shown that single agent Carboplatin is less effective than Cisplatin-based combination chemotherapy since it

achieved only an —70% disease-free survival[17,6]. The results of Carboplatin in 108 patients with low-volume stage II showed 80% DFS[17]. In contrast, a trial of more standard EP chemotherapy in 72 patients showed a 90% 5-year PFS[18].

Some centres have adopted a selective approach employing radiotherapy for stage IIIa disease and recommending chemotherapy for those with more bulky nodes. For example, a retrospective analysis of 106 cases from Florence[1] included 89 treated with radiotherapy and the 5-year relapse-free survival was 94% for IIIa and 72.5% for IIIb. However, the radiotherapy included pelvis, abdomen, mediastinum and supraclavicular fossa in 42 patients. The Institute Gustave Roussy treated most stage IIa and IIB patients with radiotherapy and most stage IIc and IIIa with chemotherapy[19].

A number of treatment guideline publications have addressed management of stage II-III seminoma[20,21]. The European Association of Urology guidelines[16] recommend that radiotherapy is the standard treatment with para-aortic and ipsilateral iliac nodes to be treated to a dose of 30 Gy for IIa and 36 Gy for IIb stages, while neo-adjuvant 4 cycles of Cisplatin based chemotherapy, followed by radiotherapy of residual lesion more than 3 cm in greatest dimension is advised.

Conclusion

The case for recommending our approach for stage IIIa and IIIb seminoma is not only the evidence for efficacy, but also the expectation that long-term toxicity risks will be low. Carboplatin is a low toxicity drug with high level of activity against seminoma[22,23] and is used with a low total dose. Though the radiotherapy component has both short-term gastrointestinal toxicity and a long-term risk of carcinogenesis, the risk should be higher with more extended radiation fields and with higher radiation doses. Also, the approach may allow in the future evaluation of even further reduction of the extent of radiotherapy to less than a standard para-aortic field if post-radiotherapy low dose of Carboplatin is used. A recent node mapping study[24,25]

has for example demonstrated the rarity of node involvement above the renal vessels and if this region was not included there would be less irradiation of stomach and pancreas. The results presented here demonstrate that our regimen is effective and has modest toxicity so is worthy of additional evaluation and development.

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METRONOMIC LOW DOSE CARBOPLATIN AFTER RADIOTHERAPY IN STAGE III TESTICULAR SEMINOMA

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COMPARISON OF NERVE STIMULATOR VERSUS ULTRASOUND GUIDED BRACHIAL PLEXUS BLOCK FOR UPPER EXTREMITY SURGERY

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Abstract

Objectives: *The aim of this prospective study is to compare ultrasound guided and nerve stimulator guided supraclavicular block as regard the block performance times, the spread of sensory block, the intensity and duration of motor block and the safety of both approaches.*

Methods: *After obtaining the research ethics board approval and written informed consent from 80 patients of either sex aged 20-40 years, ASA I & II, scheduled for surgery of the distal arm, forearm, or hand were included in this study and they were randomized into two equal groups. Group 1: ultrasound guided supraclavicular block and Group 2: nerve stimulator guided supraclavicular block.*

Results: *Patients in ultrasound guided block showed rapid onset of sensory and motor block, more easier, less needle puncture and less complication than nerve stimulator guided block.*

Conclusion: *Ultrasound guided block confers confidence and accuracy of needle placement for nerve localization and examines the pattern of local anesthetic spread.*

Keywords: *ultrasound, nerve stimulator, brachial plexus block, lidocaine, bupivacaine.*

Introduction

Brachial plexus block is one of the most commonly used peripheral nerve blocks in clinical practice. It can be used as the sole anaesthetic technique or in combination with general anaesthesia for intraoperative and postoperative analgesia.[1] The supraclavicular approach to the brachial plexus is anesthetically efficient; a small volume of solution can be delivered at a point in which the three trunks are compactly arranged, resulting in rapid onset of reliable blockage of the brachial plexus.[2]

The key steps in any successful regional anesthetic technique involve identifying the exact position of the nerve, reaching it with a precisely placed needle (without damage to any adjacent structures), and carefully injecting right dose of the right drug.[3]

The success of the classical supraclavicular block depends on a good understanding of anatomy. Because of the blind nature of this technique, unpredictable block failure, inadvertent puncture of adjacent structures (blood vessels, pleura and nerves) may occur leading to complications or, frustrating and time-consuming trial, and error attempts.[4] The introduction of the peripheral nerves-timulator into clinical practice was a major advance. Unfortunately, performance is still far from perfect because of the blind nature of this technique.[5]

Ultrasound-facilitated nerve blocks were first reported in 1978, and interest has increased owing to pogress in transducer technology, image processing, portability and cheaper equipments.[6] High frequency ultrasound can be used to identify the brachial plexus before the block, guide the block needle, and visualize the pattern of local anesthetic spread.[7-8]

Patients and Methods

After approval of Local Ethics Committee and the patient's written informed consent was obtained, the study was carried out over eighty patients of either sexes aged from 20-40 years who were presented for surgery of the distal arm, forearm, or hand. The patients were randomly allocated into two equal groups (40 patients

each) according to the used method of nerve localization by using a computer-generated sequence of random numbers. Group 1, Ultrasound guided Supraclavicular block (US group), in which nerve localization was detected by ultrasound. Group 2, Nerve Stimulator guided supraclavicular block (NS group), in which nerve localization was detected by nerve stimulator. Exclusion criteria were patients with clinically significant coagulopathy, infection at the injection site, allergy to local anesthetics, evident pulmonary pathology, mental incapacity, a body mass index more than 35, and preexisting motor or sensory deficit in the operative limb.

Preoperative investigation includes coagulation profile, complete blood picture, liver enzymes (SGOT and SGPT), creatinine level, serum electrolytes, plain chest X-ray preoperatively and postoperatively.

Basal monitoring of ECG, blood pressure in the contralateral limb, pulse oxymetry. At the night before surgery, patients had been visited for evaluation (history of any exclusion criteria, medical illness, the current drugs, clinical examination of various body systems and laboratory investigations, explain the technique of the desired block and Visual Analogue Scale (VAS) for patients, take an informed written consent and give instructions about the fasting period (at least 6 hours before surgery).

After arrival to anaesthetic room, 18 gauge IV cannula was inserted at the contralateral upper limb to the operated one and blood pressure. Cuff applied to this side also, ECG leads and pulse oximetry probe applied to the chest and bigtoe of one of the lower limb respectively for monitoring throughout procedure.

Midazolam 0.5 -2 mg and fentanyl 25-50µg will be provided as needed before performance of the block. No other sedation will be administered until the evaluation of the block will be completed. All patients placed supine with the head turned to the side away from the side to be blocked, the arm adducted and the hand extended with the head of the patient slight-

ly raised from the operating table. The block was performed using 40 ml of mixture of lidocaine (2%) and bupivacaine (0.5%) in equal volume. In ultrasound group (US group) nerve location was done using 38 mm, 7-12 MHz linear probe and a mindry digital ultrasonic diagnostic imaging system (DP-1100Plus China). The probe surface was covered by a sterile transparent sheath and a sterile gel was applied prior to scanning. After skin and transducer preparation, the probe was placed firmly over the supraclavicular fossa to obtain the transverse view of the subclavian artery as a landmark where the subclavian vein lies more medially and the anterior scalene muscle inserts onto the first rib between these 2 vessels. The hyperechoic first rib lying deep to the vessels, where the brachial plexus trunks toward lateral and posterior to the subclavian artery and above the first rib. In-Plane (IP) approach is highly recommended for this block to track the needle tip in real time to avoid inadvertent pleural puncture. The insulated needle was inserted on the lateral end of ultrasound transducer after skin local anesthetic infiltration, where the needle will be advanced along the long axis of the transducer. To optimize visualization of the needle shaft and tip, the needle was inserted at the skin on the outer end of the ultrasound probe (craniolateral to the probe) so that the path of needle advancement would be in-line with and in the same plane as the ultrasound beam. Once the needle was judged in satisfactory position (in contact with the brachial plexus in the supraclavicular location, the local anesthetic was injected under direct visualization and its distribution was confirmed when encircling the target nerves. The injection was over 1 min, with repeated aspirations every 5 ml.

In nerve Stimulating group (NS group) the blocks were performed with peripheral nerve stimulator and 22 G x $^{1}/_{4}$ insulated short beveled electric needle (Stimuplex B Braun NEL Singen, Germany.

The operator stand lateral to the patient at the level of the patient's upper arm and feel the interscalene groove between the posterior border of the sternomastoid

muscle and the anterior scalene muscle and then feel the groove between the anterior scalene muscle and the middle scalene muscle at the level of cricoid cartilage (C6) where the external jugular vein is located. The subclavian artery is felt above the mid-point of the clavicle and lateral to the artery 22 G x 2 inch insulated needle inserted caudally aiming the ipsilateral nipple or big toe. If the first rib was encountered, walking the needle antero-posterior on the rib until the plexus was encountered, taking care from air aspiration due to pleural puncture, nerve stimulation was set as follows: 1 mA initial current intensity, 2 Hz frequency and 0.1 millisecond pulse duration till the desired motor response achieved (contraction of deltoid and biceps muscles), then the current intensity decreased to reach the least current achieving visible motor response (0.5 mA) or less than 1 ml of local anesthetic was injected (Raj test) if the desired motor response stopped, then the target nerve or nerve location considered adequate and the remaining local anesthetic volume was injected. Patient showed discomfort and irritability were given 1μg/kg fentanyl.

The following parameters were detected and recorded in each group:

(1) Block performance time: Time between the start of needle insertion to the end of local anesthetic injection.

(2) Number of needle punctures and redirection.

(3) Ease of block by doctor, score consistes of: 1= not diffcult, 2=moderate diffculty, 3= extremly diffcult.

(4) The onset of sensory block: It was defined as the time to diminished response to pinch in all dermatomes.

(5) The onset of motor block: The time of motor weakness of three joints.

(6) Complete sensory block onset: It is the time from the moment of local anesthetic injection to the moment of complete loss of cold sensation (in minutes), it was graded as follow (normal sensa-

tion= 0, reduced sensation= 1 and no sensation= 2).(9)

(7) Complete motor block:

It is the time from the moment of local anesthetic injection to the moment of complete paralysis of shoulder, elbow and or wrest.

(8) Assessment of quality of block:

It done after 30 minutes of the block,was made on a three point scale: (0= complete failure, 1= unsatisfactory block inadequate analgesia, inadequate relaxation or patient required general anesthesia because of agitation or restlessness, 2= satisfactory block).(9)

(9) Patient satisfaction:

It was assessed using, Visual analog score of 0-10 (0= not satisfied, 10=entirely satisfied) after 24 hours postoperatively.(10)

(10) Incidence of complication: Painful paraesthesia, vascular puncture, local anesthetic toxicity., subcutaneous haematoma and bruises, postoperative neuropathy (neurological examination was performed in the first day after surgery to assess new transient or permanent nerve damage), cardiovascualr changes (HR-MAP) (baseline, skin incision, intraoperative and skin closure), pneumothorax, horner's syndrome.

Statistical analysis:

Statistical analysis was done by using statistical package for social scientists (SPSS) program version 16. Data was proved parametric by using kolmogro-Smimov test. The quantitative data was presented in the form of mean and standard Deviation, median and range and frequncies. Independent sample T test was used to compare between quantitative data of the two groups. Visual analog score and quality of block were represented by median and range and were analyzed by Chi-square test to compare between the two groups. Significance was considered when P-value is less than 0.05.

Results

Patient's demographic data, there were no significant differences between the study groups with respect to age, weight, sex (table 1).

Characteristics of supraclavicular block in the studied groups

including the time to perform block was significantly shorter in US group than nerve stimulator group (5.13±0.36 vs 7.43±1.32 minutes respectively) (p.value <0.001) (table 2), the onset of sensory block was significantly shorter in US group than NS group. (8.84±0.75 vs 10.35±0.47 minutes respectively) (p value <0.001) (table 2), the time of complete sensory block was significant shorter in US group than NS group (18.38±1.00 vs 22.25±1.01 respectively) (p.value <0.001) (table 2).

The onset of motor block wad significantly shorter in US group than NS group (11.09±0.60 vs 12.85±1.05 respectively) (p.value <0.001) (table 2). The time of complete motor block was significantly shorter in US group than NS group (20.23±1.14 vs 23.28±0.78) (p.value <0.001) (table 2).

There was no statistically significance difference between the 2 groups as regard Patient satisfaction score, in US group the score ranging from 5-10 (median 9.5) while in NS group ranging from 5-10 (median 9) (table 2).

At 30 minutes after injection of local anaesthetic solution in US group there was 3 cases failure of block, while in nerve stimulator group there were 5 cases failure of the block. There were 4 cases in US group showed incomplete block while 33 cases showed complete block, in NS group 5 cases showed incomplete block while 30 cases showed complete block, statistically there were significant difference between the 2 groups as regard quality of block (P value less than 0.05). (table 3)

As regard ease of block by the doctor it was significantly easier in US than NS group (P value less than 0.001) (table 4).

As regard number of needle puncture and needle passen ,there was significant difference between the two groups, in US group all the 40 cases showed one needle puncture while in NS group 28 cases showed double needle puncture and 12 cases showed 3 needle puncture (P value less than 0.001). (table 5)

Regarding heart rate, mean arterial blood pressure, and pulse oximetry. There were no signifi-

cant differences between the studied groupes. Total incidence of complication was significantly lower in US group than nerve stimulator group 8 cases (20 %) vs 18 cases (45%) respectively, the incidence of each complication is mentioned separatly in (table 6).

Table (1): The demographic characteristics (age, sex and Body Weight) of the studied groups. Data expressed as mean ± standared deviation and frequency.

	US group	NS group
Age	32 ± 8	33± 7
Sex:		
Male	30 (75%)	26 (65%)
Female	10 (25%)	14 (35%)
Body Weight	67.60 ± 5.98	68.55 ± 6.21

There was no significant difference between the two groups.

Table (2): Characteristics of supraclavicular block (Time performance, Onset sens block, Onset motor block, Time complet sens block, Time complt motor block and Satisfaction score). Data expressed as mean ± standared deviation, median and range.

	US group	NS group	P value
Time performance	5.13 ± 0.36	7.43 ± 1.32	< 0.001*
Onset sens block	8.84 ± 0.75	10.35 ± 0.47	< 0.001*
Onset motor block	11.09 ± 0.60	12.85 ± 1.05	< 0.001*
Time complet sens block	18.38 ± 1.00	22.25 ± 1.01	< 0.001*
Time complt motor block	20.23 ± 1.14	23.28 ± 0.78	< 0.001*
Satisfact score	9.50 (5-10)	9 (5-10)	0.47

* Significant difference between both groups.

Table (3): Quality_block_score, Data expressed as frequencies.

Quality_block_score	US group	NS group	P value.
0	3	5	
1	4	5	0.017*
2	33	30	

* There was significant difference between both groups.

Table (4): Ease of block score, Data expressed as frequencies.

score	US group (n = 40)	NS group (n = 40)	P value
1	40	0	< 0.001*
2	0	30	
3	0	10	

* There was significant difference between the two groups.

Table (5): number of needle puncture, Data expressed as frequencies.

No.	US group (n = 40)	NS group (n = 40)	P value
1	40	0	< 0.001*
2	0	28	
3	0	12	

* There was significant difference between the two groups.

Table (6): Incidence of Complications: Data expressed as number and percentage.

	US group		NS group		P value
	No	%	No	%	
Pneumothorax	0	0	2	5	0.018*
Vascular puncture	0	0	2	5	
Postoperative neuropathy	1	2.5	2	5	
Pherenic paresis	3	12.5	6	15	
Horner's syndrome	4	10	6	15	
Total incidence of complications	8	25	18	45	

* There was significant difference between the two group.

Discussion

In the present study we compared US guided versus NS guided the brachial plexus blocks as an alternative to blind block. We find that US guided block reduce the number of needle puncture and needle repositioning. All the 40 cases in US group block done from the first needle puncture but in NS group 28 cases showed double needle puncture and 12 cases showed three needle puncture. Also time to perform block, onset of sensory and motor block, time of complete sensory and motor block were significantly shorter in US group than NS group, this can be explained by possibility of observing the nerves of brachial

plexus and distribution of local anaesthic liquid and whether the consequently applied local anaesthic liquid had completely reached the targeted tissues or not can also be monitored.

Marhofer et al.[11] stated that ultrasound visualization of anatomical structures is the only method offering safe blocks of superior quality of optimal needle positioning. In addition the amount of local anesthetic needed for effective nerve block can be minimized by directly monitoring its distribution. They also demonstrated that ultrasound guidance significantly improved the puncture-to-onset interval and the quality of sensory and motor block while avoiding complications, because local anesthetics could be applied more accurately with ultrasound guidance compared with the blind classical technique and these results were parallel to the present study.

Also in parallel to the present study Soeding et al.[12] detected that ultrasonography application significantly reduced the starting time of sensory and motor block and that it significantly increased the block quality. Kefalianakis et al.[13] stated that ultrasonography application decreases the starting of block. In the present study, we have identified that sensory block start was earlier in the ultrasonography-applied group.

According to Liu et al.[14] ultrasonography application provides more accomplished sensory and motor blocks. Also William et al.[15] compared the same technique with nerve stimulation guidance and found that ultrasound guidance was superior, they concluded that, while inexperienced users may prefer to use both nerve stimulation and ultrasound to verify the position of the needle, it is generally better to avoid nerve stimulation, thereby sparing the patient the painful muscle contractions associated with this approach.

In the present study there was no difference between US group and NS group as regard satisfaction of patients. A successful block may be defined as one providing complete anesthesia of all target nerves. In the present study

3 cases in US group and 5 cases in NS group showed complete failure of block, 4 cases in US group and 5 cases in NS group showed in complete block and theses cases needed analgesia or sedation only and the operation was completed.

Sandhu and Capan[5], found that the use of ultrasound guided brachial plexus block, appears to permit accurate deposition of the local anesthetic perineurally and to be associated with a high success rate, short onset time, easy placement of catheter, low complication rate, and excellent analgesia.

As regard complications; in the present study there were no reported cases of pneumothorax or vascular injury in US group while there was two cases of pneumothorax and two cases of vascular injury in NS group, these cases were observed clinically and by postoperative radiology and these cases not need any treatment.

Fanelli et al.[16], reported a rate of 1.7% transient neurological complications using a multiple injection technique for peripheral nerve blockade. Liu et al.[17], observed that ultrasound guidance for interscalene block does not appear to offer major advantages over nerve stimulator guidance. However, the exact pathophysiology of postoperative neurological symptoms is unclear. Other proposed etiologies for postoperative nerve injury after shoulder arthroscopy include patient position [18], compression due to fluid extravasation.[19] amount of traction[20], selection of arthroscopy ports.[21] or toxic effects of local anesthetics.[22]

In the present study overall incidence of complications in US group were 8 cases (20%), but in NS group were 18 cases (45%) so US showed less incidence of complications than NS guided block. This can be explained that the ultrasound approach identifies nerves, vessels, muscles, and septa.

Conclusion

We found that ultrasound guidance is clinically useful for supraclavicular brachial plexus block. It confers confidence and accuracy

of needle placement for nerve localization and examines the Pattern of local anesthetic spread.

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COMPARISON OF NERVE STIMULATOR VERSUS ULTRASOUND GUIDED BRACHIAL PLEXUS BLOCK FOR UPPER EXTREMITY SURGERY

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STUDY OF CAUSES OF LOW BACK PAIN AND ITS PREVALENCE IN REPRESENTATIVE COHORT OF EGYPTIAN POPULATION

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Abstract

Aim: *To determine the prevalence and causes of LBP in outpatient clinic in Egyptian population of various age, sex and occupation.*

Methods: *600 patients randomly collected from OPD within a period of 2 years to estimate the prevalence and causes of LBP in both male and female with variable occupations and age ranged from 17 to 70 years old. Radiographs, CT or MRI were done.*

Results: *60 % diagnosed as non specific LBP while the most common diagnosis was herniated disc (15%), spinal canal stenosis found in (6%), ankylosing spondylitis in (4%), visceral disease (3.5%), malignancy (1%), infection (1%), osteoporosis (1.5%), fractures (1%), spondylolisthesis (1.5%), congenital anomalies (2%), fibromyalgia (1.5%) and (3%) others. The point prevalence was 58% while the one year prevalence was 60% in this study.*

Conclusions: *Most cases of LBP diagnosed as non specific LBP. Individual, psychosocial and occupational factors are risk factor for LBP development. LBP is one of the most common problems and one of the most common causes of work absence with high prevalence between population of both sex and variable occupation.*

Keywords: *back pain -prevalence-occupational LBP-MRI spine.*

Introduction

Low back pain (LBP) is one of the most frequent musculoskeletal disorders in daily practice. It is defined as pain between the costal margins and inferior gluteal folds, with or without referred leg pain [1]. Low back pain is a major health and socioeconomic problem and is associated with high costs, work absenteeism and disability worldwide[2]. There is a high prev-

alence of LBP in all western industrial countries[3] as well as in Egypt.

Several classification schemes, each with its own philosophy and categorizing method, subgroup low back pain (LBP) patients with the intent to guide treatment. Previous research suggests that treating patients based on a classification approach results in better clinical outcomes than non classification-based treatment strategies[4].

Non-specific low back pain is defined as low back pain not attributable to a recognisable, known specific pathology (eg, infection, tumour, osteoporosis, fracture, structural deformity, inflammatory disorder, radicular syndrome, or cauda equina syndrome). Disc is the most common aetiology of chronic specific low back pain in adults[5].

Prevalence measures the proportion of the population that experiences low back pain at a given time, which can be at any specified point (point prevalence) or in a past period such as 1 month, 1 year, or a lifetime[6].

Individual, psychosocial and occupational factors are the commonest risk factors of LBP although varies between studies[7].

Diagnostic triage is used to distinguish those patients with non-spinal or serious spinal disorders from those with pain of musculoskeletal origin, by means of history and examination, with particular emphasis on red flags[8]. Once serious disease has been ruled out, the next priority is to identify patients with radicular pain. All other cases are classified as non-specific[9].

Most guidelines advise that all imaging studies should be reserved for patients with progressive neurological deficit, or when serious underlying causes are suspected. MRI of the lumbar spine has become the initial imaging modality of choice in complicated LBP, displacing myelography and CT in recent years[10].

Most people with low back pain do not seek medical care. Many self treat with the counter medica-

tions and lifestyle changes. There is a wide range of treatment possibilities for patients with low back pain, including education behavioural therapy, pharmacotherapy, physical modalities, manual therapy, exercise, spinal injections, surgery and others[11].

Patients and Methods

epidemiological study, based on a prospective analysis of the collected data conducted on 600 patients (male and female) visited the back pain clinic, rheumatology and rehabilitation outpatient clinic in Mansoura University Hospital, from the period of June 2011 till June 2013 with age range from 17-70 years. All patients were assessed according to spine disorder sheet edited in the department including personal, past and present history, local, general and neurological examination, and investigations required. Most patients performed X ray while some underwent CT and others exposed to MRI.

The Statistical Package for Social Sciences (SPSS) version 17 was used for statistical analyses of data. Descriptive statistics including means, standard deviation, frequency and percent were used to describe sociodemographic data. Comparisons between both groups (worker, employee) (analytic measures) were done using chi-square. The P-values ≤0.05 was considered statistically significant.

Exclusion criteria:

Any person younger than (17) years of age as these patients usually referred to pediatric hospital, pregnant ladies and cases of referred and psychological pain.

Results

From the period of June 2011 till June 2013, 350 patients of variables age, gender and occupation were diagnosed to have LBP from the total 600 patients underwent this study.

Sociodemographic data of LBP in workers (including house wives) versus LBP in employee (including computer workers) were more common (71.2%) among workers compared to (52.8%) of employee in younger age group (<40 years). However, higher prevalence (47%) of LBP was among employees compared to (28.8%) among work-

ers in older age group ≥40 years. Age, gender, occupation, smoking and obesity were statistically significant value in prevalence of LBP ($p<0.001$) table (1).

Also, Sociodemographic data of 150 university students visited the clinic with age range (17-24) shown in table (2) and the point prevalence was (40%).

LBP related absence was (42%) in workers, (38%) in employee and (32%) in students.

Point prevalence was (58%) in studied population, while year prevalence was 60% in the last year, table (3).

X ray:

From the 350 patients with LBP, 245 (70%) patients did plain x ray as initial investigation, most of the other 105 improved with initial treatment and some refused to do so. 47% of the performed x rays were within normal with no positive findings. While (53%) showed variable findings as shown in table (4).

CT:

From the 245 patients did x ray, 100 patient underwent lumbosacral CT scan. (24%) of these CT were free from findings while the other (76%) showed different findings as in table (4). Total number of patient did CT was100 (28.5%).

MRI:

145 patients with LBP underwent MRI scan, (23%) of them were normal while (77%) showed the following changes in table (4). Total number of patient did MRI was 145 (41%).

Final diagnosis:

Nearly (60%) diagnosed as non specific LBP while the most common diagnosis was herniated disc (15%) with (30%) of them associated with sciatica, spinal canal stenosis found in (6%) of patients, arthropathies in (4%), nearly half cases are ankylosing spondylitis, visceral disease in (3.5%), other diagnoses are shown as in table (5).

Table (1): LBP between workers and employee.

	LBP in workers n=180		LBP in employee n=110		Test of significance
Age					
<40 YEARS	128	71.2%	58	52.8%	χ2=13.8
≥ 40 YEARS	52	28.8 %	52	47.2%	p=0.001*
Sex					χ2=22 p<0.001*
Male	100	56%	30	27%	OR (95%CI)=3.3
Female	80	44%	80	73%	(1.94-5.76)
Marital status					χ2=0.7 P=0.3
Married	28	16%	13	12%	OR (95%CI)= 1.37
Single	152	84%	97	88%	(0.65-2.96)
Special habits					
Smoker	90	50%	20	18%	χ2=33.6
Ex smoker	10	6%	3	3%	P<0.001*
None smoker	80	44%	87	88%	
BMI					
under 25	100	55%	33	30%	χ2=22.67
over 25	80	45%	77	70%	P<0.001*
LBP related absents					χ2=0.46 P=0.4
work absence	76	42%	42	38%	OR (95%CI)= 1.18
No absence	104	58%	68	62%	(0.71-1.98)
Point prevalence	**(180/ 250)**	**72%**	**(110 / 200)**	**55%**	

Table (2): LBP in university students.

		LBP in students 60 cases	
Sex	Male	18	30%
	Female	42	70%
Marital status	Married	14	24%
	Single	46	76%
Special habits	Smoker	12	20%
	None smoker	48	80%
BMI	under 25	32	53%
	over 25	28	46%
LBP related absence	work absence	19	32%
	No absence	41	68%
Point Prevalence of LBP		**60/ 150**	**40%**

Table (3): prevalence of LBP in studied people.

prevalence LBP	number	%
point prevalence	350/600	**58%**
workers	180/600	30%
employee	110/600	18%
students	60/600	10%
one year prevalence	362/600	**60%**

Table (4): radiological finding in LBP patients.

Diagnosis	X ray 245		CT 100		MRI 145	
Normal	115	47%	24	24%	34	23%
Scoliosis	27	10%				
Narrowing of disc space	61	25%				
Spondylolisthesis	6	2.4%	6	6%		
Scheuermann's	3	1.2%	1	1 %	2	2%
Osteoporosis	6	2.4%				
Fracture	4	1.5%				
DISH	4	1.5%				
Paget's	2	1%				
Sickle cell anaemia	2	1%				
Calve' s disease	2	1%				
Ankylosing spondylitis	4	2%			6	4%
sacroiliac joint	10	4%				
Spondylosis			19	19%	45	31%
Disc prolapsed			16	16%	38	26%
Fracture			4	4%		
Spinal canal stenosis			9	9	12	8%
Congenital anomalies			7	7%		
visceral diseases			14	14%		
infections					4	3%
Malignancy **Metastatic** **1ry**					4 2 2	3%

Table (5): final diagnosis of LBP cases.

diagnosis of 350 LBP	number	%
disc herniation	54	15%
sciatica 30%	16	(4.5%)
seronegative spondyloarthropathies	14	4%
Rieter	2	
Psorisasis	2	
Enteropathic	4	
Ankylosing spondylitis	6	
spinal canal stenosis	21	6%
visceral disease	14	3.5%
malignancy	4	1%
infection	4	1%
osteoporosis	6	1.5%
fractures	4	1%
Spondylolisthesis	6	1.5%
congenital anomalies	7	2%
fibromyalgia	6	1.5
Others (paget, anaemia, Scheuermann's)	13	3%
non specific LBP	208	60%

Discussion

Studies have shown that low back pain occurs most often in those between the ages of 20 to 50; an age group that corresponds to the largest component of workers[12].

The findings in the current study had shown that there was statistically significant (p=.01) association between age and low back pain. Low back pain was predominant at the middle age group between 30 and 49 in agree with [13,14].

Some studies have reported a higher proportion of women reporting back pain and sciatica than men[15,16]. In contrast[17], found the opposite with more males having sciatica. It was found that there was no statistical difference in male: female ratio[18], like in this study.

Obesity or high body mass index (BMI) (>30 BMI) are associated with an increased occurrence of low back pain[19], in agree with this study. The prevalence of LBP was statistically significant higher in overweight and obese patients (BMI >25) (p<.001).

Smoking was associated with LBP and there was significant correlation between disc herniation and smoking[20]. In this study there was none statistically significant correlation between smoking and LBP.

The prevalence and risk of occupational low back pain in the United States with high physical demands are high[21], in agree with our study. The association between low back pain and bending, twisting, lifting and vibration was established[22].

Individuals with sitting or standing jobs occupying most of the workday had an increased risk of low back pain[23], in agree with this study.

In this study, lack of regular exercise and fitness is strong predictor for LBP development (p<0.001), in agree with[24].

Approximately ≥90% of patients who present to primary care has nonspecific LBP[25].

In this study nearly (60%) of

cases are non specific LBP; which is considered lower than most studies; this may be due to that the main sample of study was collected from specialized back pain and rheumatology outpatient clinic.

Osteoporotic fractures of the spine are represented by (1%)[26], this agrees with our study (1.5%). While it was (4%)[2], most of our patients was postmenopausal females in agree with[27].

Frequency of arthropathies was (3-5%)[2]. Like in our study, (4%) were seronegative spondyloarthropathies most of them were Ankylosing spondylitis.

Malignant neoplasm accounts for less than (1%) of episodes of low back pain. However, metastatic cancer should be considered as a potential aetiology in any patient with a previous history of cancer, until proved otherwise[26]. (1%) of our LBP patients were diagnosed as malignancy.

Lumbar disc herniations are one of the most common causes of low back pain. The one year prevalence of low back pain which was diagnosed as herniated disc is (12%-15%) of all adults[28], in agree with our study (15%) and (20%) in[14]. However[3] has described up to (25%) with LBP had herniated disc.

The prevalence of lumbar spinal stenosis (LSS) in association with LBP was varied from (4%-20%), but the actual ratio remains unclear[29]. This was agreed with our study in which LSS prevalence was (6%).

The prevalence of spondylolisthesis in this study was (1.5%). While[30] found incidence of (2.7%). Most studies revealed incidence of visceral disease about (2%)[31]. Visceral diseases were diagnosed in (3.5%) in our patients.

The point prevalence in this study was (58%), this is considered high prevalence, but this is due to that most cases were caught from back pain clinic, in agree with[12] (57%) and[32] (58%). The prevalence was divided into 3 categories: physical worker (include house wives) (30%), employee (static job) (18%) and students

(10%). The one year prevalence in this study was (60%) in agree with[33] (61.3%).

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STUDY OF CAUSES OF LOW BACK PAIN AND ITS PREVALENCE IN REPRESENTATIVE COHORT OF EGYPTIAN POPULATION

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VINORELBINE AND 5 FLUORO-URACIL / FOLINIC ACID VERSUS DOCETAXEL AS FIRST LINE TREATMENT FOR PATIENTS WITH METASTATIC BREAST CANCER

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Abstract

Background: *Vinorelbine (V) and 5-Fluorouracil (5FU) is an effective combination for the treatment of metastatic breast cancer (MBC). Available Phase II clinical data reports response rates ranging from 60-64% in first line MBC. Single agent Docetaxel (D) is also an effective treatment for MBC, demonstrating an objective response rate of 48% in a pivotal phase III trial. We evaluated the efficacy and safety of V + 5FU (Arm A) versus D (Arm B) in patients (pts) with MBC relapsing after adjuvant anthracycline based treatment.*

Materials & Methods: *100 pts (50% Arm A, 50% Arm B) were enrolled between Jul 2003 and March 2005. All pts had measurable MBC after adjuvant anthracycline treatment, WHO PS 1, adequate bone marrow, renal and hepatic functions. Pts were randomized to Arm A: Vinorelbine i.v. 25 mg/m D1, D3 + folinic acid 100 mg/m D1, D2, D3 + 5FU 350 mg/m D1, D2, D3 or Arm B: Docetaxel 100 mg/m D1 with optional prophylactic G-CSF. Cycles were repeated every 3weeks. Pts with PD went off study while those with CR, PR, or SD continued treatment for a maximum of 8 cycles.*

Results: *Median age (Arm A; Arm B): 53 & 50 years; the majority had WHO PS 0 in both arms. Previous adjuvant therapy: anthracycline (100%), hormonotherapy (60% & 46% in Arms A & B respectively). The majority of patients, in both arms, presented with more than one metastatic site and two thirds had visceral disease. Liver was the most commonly involved organ. Total number of cycles delivered (Arm A: 281, Arm B: 282). Median number of cycles per patient: 6 in both arms. An objective tumor response of*

64% & 68% and a complete response of 26% & 22% were achieved in arms A & B respectively. Median time to progression & overall survival: Arm A: 15 & 27 months, Arm B: 15 & 30 months. No WHO grade Gr 3-4 toxicities were noted in Arm A. Gr 3 alopecia (18%) & Gr 3 liver enzymes elevation (2%) were noted in Arm B.

Conclusions: *Our results suggest that Vinorelbine-5FU combination and single agent Docetaxel demonstrate similar efficacy as first line treatment for MBC. Vinorelbine-5FU is however better tolerated besides being a less costly therapeutic option in Egypt. A large prospective randomized trial is needed to confirm these results.*

Introduction

Breast cancer is the most common malignancy among women and is the second cause of cancer deaths in female population[1].

Despite the proven benefit of adjuvant systemic therapy in reducing the risk of recurrence[2,3], a significant number of patients with operable breast carcinoma will eventually develop metastatic disease, and ultimately die of advanced metastatic breast carcinoma (MBC)[4].

Many chemotherapeutic agents have shown antitumor activity in MBC, among which the anthracyclines have been considered standard therapy[5,6].

Vinorelbine, a semisynthetic vinca alkaloid, has been considered one of the most active cytotoxic drugs against MBC, with a low toxicity profile. Vinorelbine has been found to yield response rates of 34-50% as a single agent when used as first-line therapy, and 15-30% as second-line therapy[7-13].

The combination of vinorelbine and infused 5-fluorouracil (5-FU) has been acknowledged as an effective palliative regimen for MBC, especially in Europe, and has been tested in several phase II studies[14-16]. Although this regimen showed high response rates, up to 70%, as first-line therapy, treatment tolerance was not satisfactory.

This regimen has also been employed as front-line treatment for MBC, alone or in combination

with FA, with a 60% overall response rate[17,18].

Docetaxel, an antimitotic agent that blocks cells in the M phase of the cell cycle it is recognised as one of the most active agents currently available for the treatment of breast cancer[19].

In previously untreated patients, single-agent docetaxel provides ORR of 40% to 68%[20,21].

The first schedules of administration of docetaxel employed doses ranging from 75 to 100 mg/m2 as a 1-hour intravenous infusion every three weeks. The 3-week schedule of docetaxel 100 mg/m2, although extremely active, showed an important myelosuppression with more than 90% of cases experiencing grade (G) 3-4 neutropenia[21,22].

Our aim in this study is to compare the clinical efficacy and safety of the VNR plus 5FU/FA regimen versus docetaxel when given as first-line treatment in patients with MBC.

Patients and Methods

This study included 100 female patients with metastatic breast cancer (50 in Arm A and 50 in Arm B) and were enrolled between Jul 2003 and March 2005

Patient population:

Key Eligibility Criteria:

- Female Patients between 18 and 65 years-old.
- Performance Status (WHO) <2.
- Life expectancy ≥12 weeks.
- Histologically confirmed metastatic breast cancer.
- At least one bi-dimensionally measurable lesion.
- Prior neo/adjuvant chemotherapy with anthracycline was allowed.
- Previous hormonal therapy for adjuvant or metastatic disease was also allowed.
- Interval between last course of chemotherapy and the treatment protocol should exceed 3 months.
- No previous treatment with chemotherapy for metastatic disease.
- Adequate bone marrow, hepatic & renal functions. (WBCs ≥3.5*109/L, platelets ≥100*109/L), renal function (creatinine clearalance >60 mL/min) and liver function (serum bilirubin <1.5mg/

dL, transaminases <3 times the upper limit of normal).

- Oral informed consent.

Exclusion criteria:

No symptom or sign of brain metastasis. Patients with known brain or leptomeningial infiltration were excluded. Pregnant or lactating women & male patients. Patients were ineligible if they had local relapse only. Another malignancy within the previous 5 years, except cured basal cell carcinoma of the skin or excised carcinoma in situ of the cervix, were also excluded. Bone metastasis or malignant pleural effusion as only site of metastasis were excluded. The pretreatment evaluation included medical history, physical examination, performance status, pregnancy test if needed, ECG, chest X-rays, tumor measurements by computed tomography (CT), and bone scan if indicated.

Treatment Schedule:

Pts were randomized to Arm A: Vinorelbine i.v. 25 mg/m D1, D3 + folinic acid 100 mg/ m D1, D2, D3 + 5FU 350 mg/m D1, D2, D3 or Arm B: Docetaxel 100 mg/mD1 with optional prophylactic G-CSF. Cycles were repeated every 3weeks.

Vinorelbine was administered in a short infusion over 6 to 10 min, followed by a rapid infusion of 500 ml of normal saline and 5FU 350 mg/m2 diluted in 250 cc of 5% dextrose, folinic acid (FA) 100 mg/m2 diluted in 100 cc of normal saline both administered i.v. on days 1, 2 and 3.

Docetaxel 100 mg/m2 was administered as a 1-hour intravenous infusion. -Dexametazone premedication starting one day before & continued for two days after the administration of docetaxel. Secondary G-CSF prophylaxis was administered when needed.

Dose modifications. The whole treatment regimen was delayed for 1 week in case of grade 3 or more toxicity.

Treatment assessment:

On day 1 of each cycle, physical condition and performance status were assessed and hematological and blood chemistry measurements were established.

Objective responses were as-

sessed every 2 cycles by clinical examination and a CT scan chest, abdomen and pelvis and bone scan if indicated. Treatment response and systemic toxicities were evaluated according to the WHO criterlria[23].

Follow-up duration was calculated from the day of treatment to either the day of death or the day of last follow up.

Patients with PD were withdrawn while those with CR, PR or SD continued treatment for a maximum of 8 cycles.

Study objectives:

Primary objective:

To compare the objective response rate of the combination of Vinorelbine and 5-Fluorouracil versus Docetaxel single agent.

Secondary objective:

To compare the combination of Vinorelbine and 5-Fluorouracil versus. Docetaxel single agent in terms of:

- Safety profile.
- Overall Survival.
- Progression-free survival.

Statistical Methods:

The Kaplan-Meier product limit method was used to calculate TTP [24] Recurrence rates were compared using the chi-square test. The statistical significance of differences among survival curves was analyzed using the log-rank test[25]. Prognostic factors related to PFS were assessed using a Cox proportional hazards regression model. All statistical tests were done using SPSS soft ware version 10.

Results

ARM A:

	Day 1	Day 2	Day 3
Vinorelbine I.V. 25 mg/m	X		X
5-Fluorouracil 350 mg/m2	X	X	X
Folinic Acid 100 mg/m2	X	X	X

ARM B:

	Day 1	Day 2	Day 3
Docetaxel 100 mg/m2	X		

cycles were repeated every 3 weeks.

Table (1-a): Patiens" characteristics.

	Arm A		Arm B	
	No of pts	**%**	**No of pts**	**%**
Number of patients	50		50	
Average Age [range]	53 36-64		50 36-63	
WHO PS 0 1	 38 12	 76 24	 37 13	 74 26
Anthracyclin pretreated: Prior Neoadjuvant Therapy Prior Adjuvant Therapy	 0 50	 0 100	 0 50	 0 100
Prior Hormone Therapy	30	60	23	46

Table (1-b): disease" characteristics.

	Arm A		Arm B	
	No of pts	**%**	**No of pts**	**%**
Number of Metastatic sites 1 2 3 >3	 1 21 25 3	 2 42 50 6	 3 26 19 2	 6 52 38 4
Visceral involvement No Yes	 16 34	 32 68	 16 34	 32 68
Main Metastatic sites Lung Skin Lymph Nodes Bone Liver	 22 22 28 17 28	 44 44 56 34 56	 24 15 28 10 29	 48 30 56 20 58

All patients, whether in Arm A or B, received adjuvant and non received neoadjuvant chemotherapy. The majority of patients, in both arms, presented with more than one metastatic site and two thirds had visceral disease. Liver was the most commonly involved organ.

Table (2): Drug delivery.

	Arm A	Arm B
Total number of cycles	281	282
Median number of cycles per patient [range	6 [3-6]	6 [3-6]

Safety profile:

Table (3-a): WHO Hematologic Toxicity Grading.

	Grade 1		Grade 2		Grade 3	
	Arm A Pts No	Arm B Pts No	Arm A Pts No	Arm B	Arm A Pts No	Arm B Pts No
Anemia	14	6	0	4	0	0
Thrombocytopenia	0	0	0	0	0	0
Neutropenia	21	26	0	8	0	0

Anemia was common in Arm A, while neutropenia was common in Arm B. There was no G3-4 hematologic toxicity.

Table (3-b): Non-Hematological toxicity.

	Grade 1		Grade 2		Grade 3	
	Arm A Pts NO	Arm B Pts No	Arm A Pts No	Arm B Pts No	Arm A Pts No	Arm B Pts No
Nausea-vomiting	0	16	0	6	0	0
Oral Stomatitis	24	28	0	4	0	0
Diarrhea	0	18	0	2	0	0
Alopecia	0	4	0	76	0	18
Peripheral neuropathy	0	0	0	0	0	0
Constipation	20	0	0	0	0	0
Local Phlebitis	8	0	0	0	0	0
Hepatic enzymes	0	0	2	2	0	2
Bilirubin	0	0	0	2	0	2
Creatinine	0	0	4	0	0	0

Alopecia was a common problem in Arm B, while constipation was obvious in Arm A.

Table (4): Treatment Response.

	Arm A		Arm B	
Evaluable patients	50		50	
	No of pts	%	No of pts	%
Complete response(CR)	13	26	11	22
Partial response(PR)	19	38	23	46
Objective response(CR+PR)	32	64	34	68
Stable disease	14	28	14	28
Disease control (OR+SD)	46	92	48	96
Progressive Disease (PD)	4	8	2	4

There was no significant difference in tumor response among the 2 arms.

Median time to tumor progression: in Arm A and Arm B were 15 months.

Median overall survival: in Arm A was 27 months versus 30 months in Arm B.

Discussion

The appropriate management of patients with breast cancer after development of metastases represents a therapeutic problem. With the increasing use of anthracycline-based combinations in the adjuvant setting, more patients with metastatic disease have a prior history of anthracycline exposure, thus limiting the use of this relatively effective group of drugs for palliation.

There is a need, therefore, for the development of non-anthracycline-based combinations for the treatment of patients with metastatic breast cancer.

The availability of several new active chemotherapeutic agents allows for different management options. These drugs can be used in combination regimens or as single agents in sequence[26].

Chemotherapy for metastatic breast cancer is, at best, a palliative measure and the main goal of any treatment chosen for metastatic disease is therefore to obtain complete symptomatic relief, ideally through tumor regression.

Vinorelbine is a semisynthetic vinca alkaloid that has cytocidal effect on a wide range of tumor cell lines[27-29].

It is a mitotic inhibitor that has a higher therapeutic index and less neurotoxicity than other vinca alkaloids related to the lower degree of damage of axonal microtubules [30]. The dose limiting toxicity of vinorelbine is granulocytopenia[31].

Given as single agent in breast cancer the drug yields response rates of 40% to 60% in previously untreated patients[32-34], and of about 30% when used as a second- or third-line therapy[35-36].

The combination of two drugs, such as vinorelbine and 5-FU, without overlapping toxicity profiles seems particularly attractive in this clinical setting.

The combination of vinorelbine with 5-Fluorouracil (5-FU) achieved high response rates of 60 to 64% and a manageable toxicity profile, though the tolerance profile of this regimen depends on the schedule[13,14].

Docetaxel is regarded as the

single most effective cytotoxic agent for advanced breast cancer with substantial objective response rates in previously untreated patients (up to 68% in phase II trials) and anthracycline-pretreated patients (30% to 43% in phase III trials)[37,38,39].

We conducted this trial to compare the efficacy and toxicity profile of those 2 regimens.

In our study, the objective response rate was 64% which is comparable to studies conducted by (Nole et al[18], 1997 and Dieras et al[14], 1996), who reported 62% Objective response rate and slightly better than Kornek et al[39] (1998) who reported 59% objective response.

After a median follow up of 30 months. The overall survival in our study was 27 months and in Dieras et al "s study[14] it was 23 months.

The median time to tumor progression was better in our study when compared to Nole et al[18], (1997) (15 months versus 10 months) and this could be due to inclusion of more patients with multiple metastatic sites in Nole et al study than ours.

In our study G1 neutropenia was the commonest hematologic toxicity (42%), which is less than Nole et al[18] (1997) who reported 77% neutropenia.

In our study and in studies conducted by (Nole et al, 1997 and Kornek et al, 1998), nonhematologic toxicities were mild and manageable.

In our study docetaxel achieved 68% the objective response, MTP 15 months and median OS 30 months, our results are comparable to others who reported objective respone 58-68%, MTP (12-18 months) and median OS 25-30 months[39-43].

In our study grade 1 and grade 2 neutropenia were encountered in 46% and 16 %, compared to Joensuu et al, 2009[40] reported 99% hematologic toxicity.

The lower incidence of hematologic toxicity in our trial could be due to the frequent use of prophy-

lactic granulocyte growth factors.

In our study and in others non hematologic toxicity was mild and manageable except for alopecia[39-43].

We have observed from our study that there is no significant difference between the two regimens in terms of response, while the toxicity profile on navelbine is much better than docetaxel.

Conclusion

Vinorelbine-5FU is better tolerated besides being a less costly therapeutic option in Egypt. A large prospective randomized trial is needed to confirm these results.

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VINORELBINE AND 5 FLUORO-URACIL / FOLINIC ACID VERSUS DOCETAXEL AS FIRST LINE TREATMENT FOR PATIENTS WITH METASTATIC BREAST CANCER

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EFFECT OF PRESSURE VERSUS VOLUME CONTROLLED VENTILATION ON RESPIRATORY MECHANICS, HEMODYNAMICS AND INTRA-ABDOMINAL PRESSURE DURING ABDOMINOPLASTY

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Abstract

Background: *This prospective double blind randomized study was designed to evaluate the effect of pressure versus volume controlled ventilation on lung mechanics, gasometry, hemodynamics variables and intra-abdominal pressure in obese patients underwent abdominoplasty.*

Methods: *The study was carried out in Mansoura university hospital included fifty patients ASA physical status I and II. Approval of the study protocol was obtained from the institutional ethics committee, and all patients gave written informed consent before inclusion. Inclusion criteria were age between twenty and fifty years old and body mass index (BMI) 30 -55 kg mg-2 All patients were randomly allocated in two groups; each one was composed of twenty five patients, according to intra-operative ventilatory strategy. Group I: (Pre_{PCV}-$Post_{VCV}$): Started with pressure controlled ventilation (PCV) during pre-pliclication period until plication of rectus muscle then change into volume controlled ventilation (VCV) through post-pliclication period till the end of surgery. Group II: (Pre_{VCV}-$Post_{PCV}$):Started with volume controlled ventilation (VCV) during pre-pliclication period until plication of rectus muscle then change into pressure controlled ventilation (PCV) through post-pliclication period till the end of surgery. Lung mechanics, gasometry, hemodynamics variables and intra-abdominal pressure were obtained*

and recorded.

Results: *As regards hemodynamics (HR and MBP), gasometry our results revealed that no significant difference between the studied groups. As regards lung mechanics, peak, plateau, mean airway pressures, static and dynamic lung compliance, there was a significant difference when compared both PCV and VCV with tendency to be much better during PCV. We also found insignificant difference between PCV and VCV on IAP*

Conclusion: *Regarding hemodynamics, oxygenation, gasometry and intra-abdominal pressure we believe that VCV or PCV appear to be equally suited in obese patients undergoing abdominoplasty with observation that the PCV has the upper hand in improvement lung mechanics over VCV.*

Keywords: *Obesity, Abdominoplasty, Intra-abdominal pressure, Abdominal compartmental syndrome, Pressure controlled ventilation, Volume controlled ventilation.*

Introduction

Abdominoplasty is a surgical procedure that effectively removes a considerable amount of abdominal skin and fat with tightening the abdominal wall. This is a very popular procedure for obese women who have lost a considerable amount of weight or have had multiple pregnancies and there is a loss of elasticity or looseness of the abdominal wall. Another common reason to perform abdominoplasty is obesity. In some cases, liposuction is an additional procedure that may be performed during or directly following abdominoplasty to remove fat that cannot be removed by diet or exercise[1].

Difficulties in ventilation are frequently encountered problems during anesthesia in obese patients undergoing abdominoplasty. Because of the restrictive ventilatory effects of obesity; these patients often show arterial hypoxemia, hypercapnia and ventilation-perfusion mismatch[2].

During the procedure of abdominoplasty there is an increase in the intra-abdominal pressure (IAP) to variable degree which is an additional factor worsens the ventilation[3]. The effects of elevated (IAP)

have been known since 1863, when Marey of Paris highlighted that "the effects that respiration produces on the thorax are the inverse of those present in the abdomen"[4]. Abdominal compartment syndrome has been reported after full abdominoplasty[5]. Also, elevated (IAP) has multi-systemic deleterious effects[6].

The operation of abdominoplasty is usually carried out under general anesthesia. The use of volume-controlled ventilation (VCV) is common, despite frequently seen high-pressure levels in obese patients. This high airway pressure may lead to volutrauma, barotrauma and biotrauma. The mechanical consequences of reduced lung compliance and chest wall compliance, added to the reduction of functional residual capacity duo to increased intra-abdominal pressure explain impaired alveolar ventilation and the high airway pressures in those patients[7].

Pressure-controlled ventilation (PCV) is a time-cycled mode in which square waves of pressure are applied and released by means of a decelerating flow. The decelerating flow often results in a higher mean inflation pressure when compared with constant flow of VCV. PCV has been proposed as an alternative to VCV in ICU patients with adult respiratory distress syndrome and in obese patients to achieve adequate oxygenation and normocapnia[8]. Evidences have been accumulating that PCV compared with VCV during anesthesia for bariatric surgery improves gas exchanges without increasing ventilation pressures or causing any hemodynamic side-effects[9].

Patients and Methods

This prospective double blind randomized study was carried out in Mansoura university hospital included fifty patients ASA physical status I and II subjected to abdominoplasty. Approval of the study protocol was obtained from the institutional ethics committee, and all patients gave written informed consent before inclusion. Inclusion criteria were age between twenty and fifty years old and body mass index 30-55 kg mg^{-2}. All patients were randomly allocated in two groups by closed

envelope method according to intra-operative ventilatory strategy; each one was composed of twenty five patients.

Exclusion criteria for this study were patient refusal, ASA physical status III and IV, history of OSA and body mass index below 30 and above 55 kg mg^{-2}. Intra-operative exclusion criteria were inability to perform tracheal intubation in conditions of usual practice, inability to maintain stable mechanical ventilation settings for 30 min, inability to maintain an appropriate end tidal CO_2 and inability to remove the tracheal tube in the operating room.

Patients preparation and monitoring:

*** One day before operation:**

• Complete medical history.

• Complete clinical examination

• Routine investigations were done (CBC, ECG, liver function, random blood glucose, serum creatinine and coagulation profile).

• Chest X-ray.

• Echocardiography.

*** In the morning of the operation:**

After an overnight fasting, the patients were transferred to the anesthesia room, 20- gauge IV cannula was placed. Midazolam 0.03 mg/kg I.V to alleviate anxiety. Patient has been attached to the monitor to display NIBP, HR, SpO2 and ECG. An arterial sample was obtained for arterial blood gases analysis as basal values. Epidural catheter was inserted at level L3-L4 for pain control intra-operative and post-operative by injection of 8-12 ml of bupivacaine %0.25 according to patient height.

NIBP (systolic, diastolic and mean), HR and SpO2, were measured automatically and recorded just before induction of general anesthesia as a basal guideline.

Induction of general anesthesia was done as follow:

• After Pre-oxygenation with 100% oxygen for 5 minutes, 1 µg/kg fentanyl followed by 2 mg/kg propofol mixed with 2ml lidocaine 2% was injected to induce unconsciousness.

• Endotracheal tube was inserted using the direct laryngoscope after 1 mg/kg succinylcholine and

its correct position was confirmed with the capneogram.

• Maintenance of anesthesia was done using isoflurane with a concentration titrated for each patient to ensure deep plain of anesthesia. Loading dose then incremental doses of atracurium were used to maintain muscle relaxation. Controlled ventilation through the operation was done as follow:

*** Group I: (Pre_{PCV}-$Post_{VCV}$):**

Started with pressure controlled ventilation (PCV) during pre-pliclication period until plication of rectus muscle then change into volume controlled ventilation (VCV) through post-pliclication period till the end of surgery.

*** Group II: (PreVCV-PostPCV):**

Started with volume controlled ventilation (VCV) during pre-pliclication period until plication of rectus muscle then change into pressure controlled ventilation (PCV) through post-pliclication period till the end of surgery.

(PCV) parameters were as follow:

• Tidal volume: $P_{plateau}$ has been set so that tidal volume =10 ml/kg ideal

Weight: 50+ {0.91x (height in cm-152.4)} for men.

and: 45.5+ {0.91x (height in cm-152.4)} for women.

• Respiratory rate 12/min.

• The ratio of inspiratory-to-expiratory time (I:E) was 1:2.

• FIO_2 was 0.5 and PEEP =5 cm H_2O.

(VCV) parameters were as follow:

• Tidal volume =10 ml/kg ideal weight.

• Respiratory rate 12/min.

• (I:E) was 1:2.

• FIO_2 was 0.5 and PEEP =5 cm H_2O

• Plateau time was 20% of inspiratory time allowing the ventilator to measure plateau pressure.

Continuation of mechanical ventilation has been done according to the following algorithm:[9]

Foley's urinary catheter was inserted just before induction of anesthesia and before the beginning of the surgery. basal values of intra-abdominal pressure were measured through this Foley's

catheter by measuring intra-vesical pressure as shown in the following figure:

Fluid maintenance was done using 4-2-1 rule and according to intraoperative patient need.

Intraoperative monitoring and recorded data:

• NIBP (systolic, diastolic and mean), HR and SpO_2, were measured automatically and recorded every15 minutes.

• Respiratory rate, tidal volume, minute volume, peak pressure, plateau pressure, mean airway pressure, static and dynamic compliance were recorded every 20 minutes.

• Arterial blood gas was done after 40 minutes from induction of anesthesia after establishment of the ventilation mode. Another ABG was done at 120 minutes from beginning of the surgery after the plication of the rectus and switch to the other mode to obtain: PH, PaO_2, $PaCO_2$, PAO_2-PaO_2 gradient and $PaCO_2$-ÈTCO_2 gradient.

• Intra-abdominal pressure was measured through Foley's catheter by measuring intra-vesical pressure using technique described by Kron and colleagues every 20 minutes.

• Abdominal perfusion pressure (APP) will be calculated and recorded every 20 minutes as follow:

APP = MAP - IAP.

MAP: mean arterial pressure.

IAP: intra-abdominal pressure.

• Rectus plication time was marked as a target for switching between the ventilation modes. After termination of the surgery, discontinuation of the anesthesia and reversal of muscle relaxant was prepared and given to the patient in the form of atropine (0.03mg/kg) and neostigmine (0.06 mg/kg). Extubation was done after fulfillment of the extubation criteria. Patient was transferred to the recovery room and the ward after fulfillment of discharge criteria.

Postoperative monitoring:

After 6 hours the following data have been recorded:

• NIBP (systolic, diastolic and mean), HR, IAP and APP.

• Serum creatinine and ABG were ordered for every patient

Statistical Analysis:

Statistical analysis was per-

formed using IBM SPSS Statistics version 22 software. All data were tested for normality using Kolmogorov-Simirnov test. Parameters obtained during PCV and VCV were collected and calculated as mean values. Independent-Sample T test was performed to compare non-parametric values between the two studied groups (inter-group comparison). Paired-Sample T test was used to compare values within the two studied groups (intra-group comparison). All data were expressed as mean ± SD. A value of p- value < 0.05 was considered to be statistically significant.

Results

There were no significant differences between the 2 groups regarding demographic data (table 1).

The heart rate values were recorded as basal, means of intra-operative values through pressure controlled ventilation (PCV) or volume controlled ventilation (VCV) according to the studied group and 6 hours post-operative. Analysis of data showed insignificant inter-group and intra-group differences. (table 2).

Mean arterial blood pressure values were recorded as basal, means of intra-operative values through (PCV) and (VCV) ventilation according to the studied group and 6 hours post-operative. Analysis of data showed insignificant inter-group and intra-group differences. (table 3).

PH (tables 4), $PaCO_2$ (tables 5) and HCO_3 (tables 6) values from arterial blood gases were recorded as basal, intra-operative during PCV and VCV according to the studied group and 6 hours post-operative. Arterial-end tidal CO_2 gradient values (tables 5) were recorded intra-operative during PCV and VCV according to the studied group. Analysis of data showed insignificant inter-group and intra-group differences.

Values of peripheral arterial oxygen saturation (SPO_2) (tables 7), arterial oxygen tension (PaO_2) (tables 8) and alveolar-arterial oxygen gradient (A-a O_2 g) (tables 9) were recorded basal, intra-operative during PCV and VCV according to the studied group and

6 hours post-operative. Analysis of data showed the following:

a) Inter-group analysis: showed insignificant differences regarding comparison between the basal values.

b) Intra-group analysis:

I. Group I: Concerning basal SPO_2, PaO_2 and A-aO_2g there was a significant difference when compared with both Pre_{PCV} and $Post_{VCV}$ values and insignificant difference when compared with of post-operative values. There was also a significant difference of A-aO_2g between both Pre_{PCV} and $Post_{VCV}$.

II. Group II: Concerning basal SPO2, PaO2 and A-aO2g there was a significant difference when compared with both PreVCV and PostPCV values and insignificant difference when compared with of post-operative values. There was also a significant difference of A-aO2g between both PreVCV and PostPCV.

As regards respiratory mechanics; peak, plateau, mean airway pressures, static lung compliance (St. comp) and dynamic lung compliance (Dyn. comp) were monitored and the mean values through PCV and VCV were recorded according to the studied group. Analysis of data showed the following (table 10):

Intra-group analysis:

Group I: Concerning peak, plateau, mean airway pressures, (St. comp) and (Dyn. comp) there was a significant difference when compared both Pre_{PCV} and $Post_{VCV}$ values.

Group II: Concerning peak, plateau, mean airway pressures, (St. comp) and (Dyn. comp) there was a significant difference when compared both Pre_{VCV} and $Post_{PCV}$ values.

Intra-abdominal pressure (IAP) (tables 11) and abdominal perfusion pressure (APP) (tables 12)values were recorded as basal, means of intra-operative values through (PCV) and (VCV) ventilation according to the studied group and 6 hours post-operative. Analysis of data showed the following:

a) Inter-group analysis: showed insignificant differences regarding comparison between the basal values.

b) Intra-group analysis:

I. Group I:

• Concerning basal IAP; there was a significant difference when compared with both $Post_{VCV}$ and 6hours post-operative values and insignificant difference when compared with Pre_{PCV} values. There was also a significant difference of IAP values between both Pre_{PCV} and $Post_{VCV}$.

• Concerning APP; there was insignificant difference when compared basal, Pre_{PCV} and $Post_{VCV}$.

II. Group II:

• As regards basal IAP; there was a significant difference when compared with both $Post_{PCV}$ and 6hours post-operative values and insignificant difference when compared with Pre_{VCV} values. There was also a significant difference of IAP values between both Pre_{VCV} and $Post_{PCV}$.

As regards APP; there was insignificant difference when compared basal, Pre_{VCV} and $Post_{PCV}$ values.

Table (1): Patients' characteristics. Data are displayed as mean ± SD.

	Group I	*Group II*
Age	34.80 ± 7.5	34.72 ± 4.9
Weight	102.72 ±7.0	104.80 ± 8.7
Height	1.6256 ± .05	1.6248 ± .05
BMI	38.924 ± 2.8	39.688 ± 2.4
IBW	54.756 ± 4.5	55.04 ± 6.2

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
BMI: body mass index
IBW: ideal body weight

Table (2): Heart rate. Data are displayed as mean ± SD.

	Group I		*Group II*	
Basal		86.6 ± 6.3		84.8 ± 9.1
Pre-plic	Pre_{PCV}	90.7 ±10.7	Pre_{VCV}	87.1 ± 7.7
Post-plic	$Post_{VCV}$	87.1 ± 6.8	$Post_{PCV}$	84.8 ± 9.1
Post-op		86.2 ± 4.5		85.5 ± 5.4

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
Basal: basal values before induction of anesthesia.
Pre-plic: pre-pliclication of the rectus muscle
Post-plic: post-pliclication of the rectus muscle
Post-op: values obtained 6 hours post-operative
$Pre_{PCV:}$ pressure controlled ventilation during pre-pliclication period
$Post_{VCV:}$ volume controlled ventilation during post-pliclication period
$Pre_{VCV:}$ volume controlled ventilation during pre-pliclication period
$Post_{PCV:}$ pressure controlled ventilation during post-pliclication period

Table (3): Mean arterial blood pressure. Data are displayed as mean ± SD.

	Group I		*Group II*	
Basal		94.8 ± 8.7		93.4 ± 10.6
Pre-plic	Pre_{PCV}	92 ± 7.3	Pre_{VCV}	92.4 ± 5.4
Post-plic	$Post_{VCV}$	93.4 ± 5	$Post_{PCV}$	91.8 ± 5.2
Post-op		94.9 ± 6.7		91.1 ± 6.6

Table (4): Ph values obtained from arterial blood gases. Data are displayed as mean ± SD.

	Group I		*Group II*	
Basal		7.3 8± .04		7.39 ± .02
Pre-plic	Pre_{PCV}	7.38 ± .03	$Post_{VCV}$	7.38 ± .04
Post-plic	Pre_{VCV}	7.37 ± .04	$Post_{PCV}$	7.38 ± .03
Post-op		7.39 ± .02		7.38 ± .04

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
Basal: basal values before induction of anesthesia.
Pre-plic: pre-pliclication of the rectus muscle
Post-plic: post-pliclication of the rectus muscle
Post-op: values obtained 6 hours post-operative
$Pre_{PCV:}$ pressure controlled ventilation during pre-pliclication period
$Post_{VCV:}$ volume controlled ventilation during post-pliclication period
$Pre_{VCV:}$ volume controlled ventilation during pre-pliclication period
$Post_{PCV:}$ pressure controlled ventilation during post-pliclication period

Table (5): $PaCO_2$ values obtained from arterial blood gases. Data are displayed as mean ± SD

	Group I		*Group II*	
Basal		39.38±5.3		39.1±2.6
Pre-plic	Pre_{PCV}	37.9±2.8	Pre_{VCV}	38.7±2.5
	CO_2-*g:*	4.76±1.1	CO_2-*g:*	4.56±1.0
Post-plic	$Post_{VCV}$	39.5±4.7	$Post_{PCV}$	39.8±5.4
	CO_2-*g:*	5.16±.9	CO_2-*g:*	4.96±1.0

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
Basal: basal values before induction of anesthesia.
Pre-plic: pre-pliclication of the rectus muscle
Post-plic: post-pliclication of the rectus muscle
Post-op: values obtained 6 hours post-operative
$Pre_{PCV:}$ pressure controlled ventilation during pre-pliclication period
$Post_{VCV:}$ volume controlled ventilation during post-pliclication period
$Pre_{VCV:}$ volume controlled ventilation during pre-pliclication period
$Post_{PCV:}$ pressure controlled ventilation during post-pliclication period
CO_2-*g:* Arterial-end tidal CO2 gradient

Table (6): HCO3 values obtained from arterial blood gases. Data are displayed as mean ± SD.

	Group I		*Group II*	
Basal		24.36±1.8		25.2±1.7
Pre-plic	Pre_{PCV}	24.5±2.5	Pre_{VCV}	24.9±2.6
Post-plic	$Post_{VCV}$	24.3±2.2	$Post_{PCV}$	25.3± 2.7
Post-op		25.3±2.6		25.8±2.9

Table (7): SPO_2. Data are displayed as mean ± SD.

	Group I	*Group II*
Basal	97.4 ± 1.3	97 ±1
Pre-plic	Pre_{PCV} 98.8 ± .2 *	Pre_{VCV} 98.9 ± .4 ¶
Post-plic	$Post_{VCV}$ 98.9 ± .2 *	$Post_{PCV}$ 98.8 ± .3 ¶
Post-op	97.4 ± 1	97.1 ± .8

Group I: Pre*PCV*-Post*VCV* group
Group II: Pre*VCV*-Post*PCV* group
Basal: basal values before induction of anesthesia.
Pre-plic: pre-pliclication of the rectus muscle
Post-plic: post-pliclication of the rectus muscle
Post-op: values obtained 6 hours post-operative
Pre*PCV:* pressure controlled ventilation during pre-pliclication period
Post*VCV:* volume controlled ventilation during post-pliclication period
Pre*VCV:* volume controlled ventilation during pre-pliclication period
Post*PCV:* pressure controlled ventilation during post-pliclication period
* Significant when compared with basal value within group I (*p*- value < 0.05)
¶ Significant when compared with basal value within group II (*p*- value < 0.05)

Table (8): PaO_2 values obtained from arterial blood gases. Data are displayed as mean ± SD.

	Group I	*Group II*
Basal	87.5 ± 6	90.2 ± 3
Pre-plic	Pre_{PCV} 273.2 ± 38 *	Pre_{VCV} 242.5 ± 27 ¶
Post-plic	$Post_{VCV}$ 274.7 ± 33 *	$Post_{PCV}$ 242.4 ± 29 ¶
Post-op	89.2 ± 4	90.9 ± 3

Table (9): Alveolar-arterial oxygen gradient (A-a O_2 g). Data are displayed as mean ± SD.

	Group I	*Group II*
Basal	11.2 ± 4.9	10.3 ±3.5
Pre-plic	Pre_{PCV} 35.8 ± 39.5 *	Pre_{VCV} 62.8 ± 28.1 ¶
Post-plic	$Post_{VCV}$ 64.4 ± 31.6 *#	$Post_{PCV}$ 33.7 ± 32.6 ¶ $
Post-op	11.9 ±5.4	12.1 ± 3.6

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
Basal: basal values before induction of anesthesia.
Pre-plic: pre-pliclication of the rectus muscle
Post-plic: post-pliclication of the rectus muscle
Post-op: values obtained 6 hours post-operative
$Pre_{PCV:}$ pressure controlled ventilation during pre-pliclication period
$Post_{VCV:}$ volume controlled ventilation during post-pliclication period
$Pre_{VCV:}$ volume controlled ventilation during pre-pliclication period
$Post_{PCV:}$ pressure controlled ventilation during post-pliclication period
* Significant when compared with basal value within group I (*p*- value < 0.05)
# Significant when compared with Pre_{PCV} value within group I (*p*- value < 0.05)
¶ Significant when compared with basal value within group II (*p*- value < 0.05)
$ Significant when compared with Pre_{VCV} value within group II (*p*- value < 0.05)

Table (10): Respiratory mechanics. Data are displayed as mean ± SD.

	Group I		*Group II*	
	Pre_{PCV}	**$Post_{VCV}$**	**Pre_{VCV}**	**$Post_{PCV}$**
Peak	19.1 ± 2.5	22.4 ± 2.3$^{\#}$	21.6± 2.4	19.4 ± 1.6$^{\$}$
Plateau	15.9± 2.4	19 ± 2.2$^{\#}$	18.2 ± 2.5	16.2 ± 1.9$^{\$}$
Mean	9.9 ±.57	9.2± .64$^{\#}$	8.8 ± .92	9.5 ± .92$^{\$}$
St. comp	52.2±11.5	41 ± 7.2$^{\#}$	43.2 ± 9.7	53.6 ± 9.9$^{\$}$
Dyn. comp	39.9± 6.7	32.8 ± 5.2$^{\#}$	34.6 ± 5.8	40.2 ± 5.7$^{\$}$

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
Peak: peak airway pressure
Plateau: plateau airway pressure
Mean: mean airway pressure
St. comp: static lung compliance
Dyn. comp: dynamic lung compliance
$Pre_{PCV:}$ pressure controlled ventilation during pre-pliclication period
$Post_{VCV:}$ volume controlled ventilation during post-pliclication period

Table (11): Intra-abdominal pressure (IAP). Data are displayed as mean ± SD.

	Group I	*Group II*
Basal	6.9 ± 1.4	6.4 ± 1.9
Pre-plic	Pre_{PCV} 7 ± 1.2	Pre_{VCV} 6.4 ± 1.9
Post-plic	$Post_{VCV}$ 7.6 ± 1.3$^{* \#}$	$Post_{PCV}$ 7.1 ± 1.7$^{\P \$}$
Post-op	8.9 ± .9*	7.2 ± 1.3¶

Table (12): Abdominal perfusion pressure (APP). Data are displayed as mean ± SD.

	Group I	*Group II*
Basal	88.1 ± 8.6	87.1 ± 10.2
Pre-plic	Pre_{PCV} 84.9 ± 7.1	Pre_{VCV} 86.1 ± 5.6
Post-plic	$Post_{VCV}$ 85.9 ± 5.3	$Post_{PCV}$ 84.8 ± 5.2
Post-op	86.1 ± 6.8	84.1 ± 6.5

Group I: Pre_{PCV}-$Post_{VCV}$ group
Group II: Pre_{VCV}-$Post_{PCV}$ group
Basal: basal values before induction of anesthesia.
Pre-plic: pre-pliclication of the rectus muscle
Post-plic: post-pliclication of the rectus muscle
Post-op: values obtained 6 hours post-operative
$Pre_{PCV:}$ pressure controlled ventilation during pre-pliclication period
$Post_{VCV:}$ volume controlled ventilation during post-pliclication period
$Pre_{VCV:}$ volume controlled ventilation during pre-pliclication period
$Post_{PCV:}$ pressure controlled ventilation during post-pliclication period
* Significant when compared with basal value within group I (p- value < 0.05)
# Significant when compared with Pre_{PCV} value within group I (p- value < 0.05)
¶ Significant when compared with basal value within group II (p- value < 0.05)
$ Significant when compared with Pre_{VCV} value within group II (p- value < 0.05)

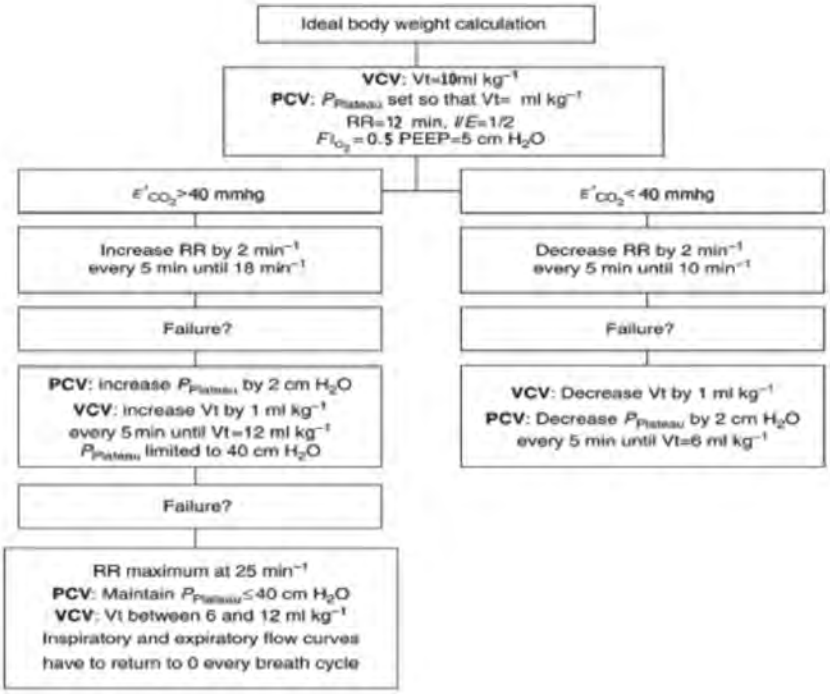

Fig. 1: Algorithm for intraperative mechanical ventilation.

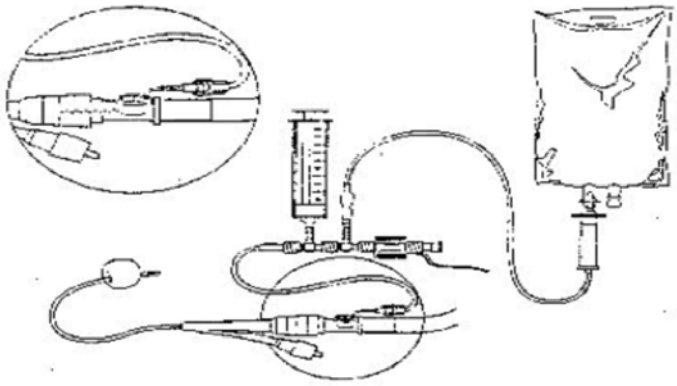

Fig. 2: Intravesical mesurement for IAP.

Discussion

Abdominal contouring procedures have been performed since the end of the nineteenth century. In 1910 Kelly first described the technique for large wedge excision of the abdominal panniculus. The modern abdominoplasty technique, including a low transverse incision with wide undermining in addition to rectus muscle plication, developed during the 60s. Over time, many variations in the technique have been described. They all have three fundamental objectives in common: resection of excess skin and fat, correction of the musculo-aponeurotic flaccidity (diasthesis recti) and improvement of appearance[10].

The tension caused by these mechanical changes, leads to increased intra-abdominal pressure (IAP), which may impair ventilatory function. In addition, other factors secondary to the procedure may alter respiratory function such as administration of anesthetic drugs and the anesthesia itself[11].

The number of obese patients undergoing surgery, either bariatric or non-bariatric (e.g abdominoplasty) is steadily increasing. These patients have a priori healthy lungs. However, the pathophysiological changes induced by obesity make these patients prone to perioperative complications, such as hypoxemia, hypercapnia and atelectasis. Immediately after the induction of general anesthesia, atelectasis develops, leading to a reduction in both ventilation-perfusion ratio and pulmonary compliance. It has been demonstrate that in anesthetized patients, PaO2 inversely related to BMI[12].

The selection of the optimal ventilation mode or the optimal control variable of ventilation for the obese patient is of interest to most of the anesthesiologists. According to Campbell and colleagues, VCV and PCV are not different ventilatory modes, but are different control variables within a mode. During VCV, airway pressure increases in response to reduced compliance, increased resistance, or active exhalation and may increase the risk of ventilator-induced lung injury. During PCV, the inspiratory flow and flow waveform are determined by the ventilator as it attempts to main-

tain an inspiratory pressure profile. The clinician should titrate the inspiratory pressure to the measured tidal volume[13].

This study was designed to evaluate the effect of pressure versus volume controlled ventilation on hemodynamics, gasometry, lung mechanics and intra-abdominal pressure in obese patients undergoing abdominoplasty.

As regards hemodynamics (HR and MBP), our results revealed that no significant difference. This result is in agreement with De Baerdemaeker et al., (2008)[13] who compared VCV and PCV during laparoscopic gastric banding in morbidly obese patients. The same result was also reported by Cadi et al., (2008)[9] who studied the effect of VCV and PCV during laparoscopic obesity surgery.

The effects of VCV and PCV on gasometry have been studied and showed controversial results. In our current study, as regards PH, $PaCO_2$, arterial-end tidal CO_2 gradient and HCO_3, our results showed insignificant differences between the two modes and many studies had similar conclusions to ours as De Baerdemaeker et al., (2008)[13], Esteban et al., (2000)[14] and Tugrul et al., (1997)[15]. In contrast to our results, Cadi et al., (2008)[9] reported differences PH, $PaCO_2$ and arterial-end tidal CO_2 gradient with tendency of PCV to be better.

As regards oxygenation parameters; SPO_2 and PaO_2; there were significant differences when compared basal values with intra-operative values during both VCV and PCV. This can be explained by the fact that mechanical ventilation improves oxygenation in obese patients[16]. Regarding the comparison between VCV and PCV on oxygenation; our results showed insignificant differences. These results were in agreement with many studies. De Baerdemaeker et al., (2008)[13] in his previously mentioned study, concluded that arterial oxygenation remained unchanged. Karcz et al., (2012)[17] concluded in his systemic review "State-of-the-art mechanical ventilation" that both modes are likely equivalent in supporting gas exchange.

Similarly, another systemic re-

view and meta-analysis published by Aldenkortt et al., (2012)[12] confirmed our results and it was performed for randomized trials testing ventilation strategies in obese patients undergoing surgery. This review included all the studies that were published between 1978 and 2011, and came from 10 different countries. He concluded that intraoperative oxygenation was similar with PCV and VCV.

In contrast to our results, Cadi et al., (2008)[9] reported that PCV compared with VCV during anesthesia for laparoscopic bariatric surgery improves gas exchange. The same result was reported also by Tugrul et al., (1997)[15] who compared PCV and VCV during one lung anesthesia.

In our results; A-a O2 gradient showed significant difference as it was higher during VCV compared with PCV. Cadi et al., (2008)[9] reported the same result and explained that by difference in the flow pattern being constant flow in VCV and decelerating in PCV which leads to better gas distribution.

As regards lung mechanics, peak, plateau, mean airway pressures, static and dynamic lung compliance, there was a significant difference when compared both PCV and VCV with tendency to be much better during PCV. These results are in agreement with Davis et al., (1996)[18] who compared PCV and VCV with different flow wave form. De Beer and Gould, (2013)[19] published review article about principles of artificial ventilation and mentioned the same results. In contrast, De Baerdemaeker et al., (2008)[14], concluded in his study that both VCV and PCV appear to be equally suited in morbidly obese patients. Also Karcz et al., (2012)[17] demonstrated that both modes are likely equivalent in pulmonary mechanics.

The possible explanation for our results regarding lung mechanics is different inspiratory flow wave form. PCV is characterized by decelerating pattern, while VCV is characterized by constant pattern. The ability of the decelerating flow waveform to distend the lung at the selected peak pressure throughout the inspiratory time

was described by Davis et al., (1996)[18]. This may facilitate alveolar recruitment, enhance diffusion, allow alveolar units with slow time constants to fill while preventing overdistension of normal alveoli and augment collateral ventilation.

Body contouring surgery, specifically abdominoplasty, has become increasingly popular; alteration of intra-abdominal pressure (IAP) during this procedure had been investigated by many authors. Talisman et al., (2002)[10], Neto et al., (2006)[22] and Huang et al., (2007)[20] showed increased IAP at the end of abdominoplasty. On the other hand, Al-Basti et al., (2004)[21] measured the IAP in obese multiparous patients submitted to abdominoplasty and reported that there was no significant increase in IAP.

In our current study; we reported significant increase in IAP after placation of rectus muscle regardless the type of ventilatory mode. This can be explained by the operative technique that has at least two maneuvers that result in IAP elevation: plication of the rectus muscle and flap resection[22].

We believe that it is the first study that compares PCV versus VCV effect on IAP. Facing the fact that the chest and abdomen are two spaces separated by the diaphragm and the events occur in one space do affect the other (Malbrain, 2006) and although we reported that PCV has better effect than VCV on lung mechanics; in our study, we found insignificant difference between PCV and VCV on IAP. This could be hypothesized by the dynamics of the abdominal wall that allow great volume changes without a proportional rise in IAP values (Neto et al., 2006).

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EFFECT OF PRESSURE VERSUS VOLUME CONTROLLED VENTILATION ON RESPIRATORY MECHANICS, HEMODYNAMICS AND INTRA-ABDOMINAL PRESSURE DURING ABDOMINOPLASTY

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COMPARISON OF THE EFFECTS OF LORNOXICAM TO PARACETAMOL WHEN ADDED TO LIDOCAINE FOR INTRAVENOUS REGIONAL ANESTHESIA IN PATIENTS UNDERGOING HAND AND FOREARM SURGERY

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Abstract

Background: *Lornoxicam is a new NSAID of the Oxicam class with analgesic anti-inflammatory and antipyretic properties. Lornoxicam is available in oral and parental form and is recommended for short term use in the postoperative period as it has a short term plasma elimination half-life of 3-5h.Paracetamol (acetaminophen) is an analgesic which relieves pain and reduces fever. Several studies have demonstrated peripheral antinoceptive properties of paracetamol in different pain models. The study was planned to evaluate the effect of intravenous paracetamol and lornoxicam when added to lidocaine in intravenous regional anesthesia for elective hand surgery regarding sensory and motor block, tourniquet pain and postoperative analgesia.*

Subjects and Methods: *This prospective double-blinded randomized study was conducted in patients undergoing hand or forearm surgery who were randomly assigned into 3 groups. The syringes in all groups contained 3 mg/kg of lidocaine. In control group (C) patients received 0.5% Lidocaine diluted with 0.9% normal saline to a total volume of 40 ml (n=20) in Lornoxicam group (group L) received 0.5% lidocaine diluted with normal saline plus Lornoxicam 8 mg to a total volume of 40 ml (n = 20) and Paracetamol group (group P) received 0.5% Lidocaine diluted with 250 mg of paracetamol (Perfalgan 10 mg/ml/Bristol-Myers-Squibb) diluted with saline to a total volume of 40 mL.*

Results: *The primary outcome of our study the addition of Lornoxicam 8 mg or acetaminophen 250 mg to lidocaine for IVRA decreased*

tourniquet pain, improved the speed of onset of sensory and motor block, prolonged the sensory and motor recovery times, decreased both intraoprative and postoperative analgesic requirements and improved the quality of anesthesia without causing any side effects.

Keywords: *Lornoxicam, Paracetamol, Regional anesthesia, Lidocaine.*

Introduction

Intravenous regional anesthesia (IVRA) was first used by August Bier in 1908[1]. This technique is easy to administer, reliable and cost-effective for short surgical procedures of the extremities performed on an ambulatory basis[2].

Delayed onset of action, poor muscle relaxation and Lack of postoperative analgesia are the major limitations of this technique[3]. Various additives have been combined with local anesthetics (LAs) to improve the quality of block, including opioids, clonidine, neostigmine and muscle relaxants. Also various nonsteroidal anti-inflammatory drugs (NSAIDs) have been demonstrated to enhance analgesia such as ketorlac[4] tenoxicam[5] and aspirin [6] when added to local anesthetics in IRVA.

Lornoxicam is a new NSAID of the Oxicam class with analgesic anti-inflammatory and antipyretic properties. Lornoxicam is available in oral and parental form and is recommended for short term use in the postoperative period as it has a short term plasma elimination half-life of 3-5h[6,7] Lornoxicam is also as effective as morphine but better tolerated when administered intravenously by patient-controlled analgesia for decreasing postoperative pain after discectomy[8]. Infiltration of the wound with combination of lornoxicam and local anesthetic improves the postoperative pain and decreases the need for opioids suggesting a local effect[9].

Paracetamol (acetaminophen) is an analgesic which relieves pain and reduces fever. Several studies have demonstrated peripheral antinoceptive[10,11] properties of paracetamol in different pain models perflgan (10 mg/ml, Bristol-Myers-Squibb, Anagni, Italy) is a solution of acetaminophen administered intravenously in order to relieve pain or reduce fever following surgery and was introduced into clinical practice in 2002.

We planned this study to evaluate the effect of intravenous paracetamol and lornoxicam when added to lidocaine in intravenous regional anesthesia for elective hand surgery regarding sensory and motor block, tourniquet pain and postoperative analgesia.

Subjects and Methods

This study was conducted at Kasr El-Aini hospital, Cairo University after approval of local Ethical committee & obtaining informed consent from all patients. Sixty adult patients of ASA physical status I and II, aged 20-60 years, who were scheduled for surgery of the hand or the forearm, were included in this study. Patients with Raynaud's disease, history of drug allergy, Sickle cell anemia, Liver diseases and kidney diseases were excluded from the study.

This prospective double-blinded randomized study was conducted in patients undergoing hand or forearm surgery who were randomly assigned into 3 groups. The syringes in all groups contained 3 mg/kg of lidocaine (2% Lidocaine, Rotexmedica, Trittan, Germany). In control group (C) patients received 0.5% Lidocaine diluted with 0.9% normal saline to a total volume of 40 ml (n=20) in Lornoxicam group (group L) received 0.5% lidocaine diluted with normal saline plus Lornoxicam 8 mg (AUG Pharma under license of Nycomed, Austria) to a total volume of 40 ml (n = 20) and Paracetamol group (group P) received 0.5% Lidocaine diluted with 250 mg of paracetamol (Perfalgan 10 mg/ml/Bristol-Myers-Squibb) diluted with saline to a total volume of 40 mL.

Forty-five minutes before the surgical procedures, patients were premedicated with IM 0.07 mg/Kg of midazolam. After the patient had been taken to the operating room, mean arterial blood pressure (MAP), peripheral oxygen saturation (SPO2) and heart rate (HR) were monitored. Two cannulas were placed, one 22-gauge intravenous (IV) cannula in the dorsum of the hand of the surgical extremity and a second 20-gauge IV cannula in the non-operative arm for crystalloid infusion.

The operative arm was elevated for 2 min then exsanguinated with an esmarch bandage. A pneumatic double tourniquet was placed a round upper arm, and the proxi-

mal cuff was inflated to 250 mmHg. Circulatory isolation of the arm was verified by inspection, absence of radial pulse, and loss of pulse oximetry tracing in the epsilateral index finger. After the bandage was removed, 40 ml of the respective solutions were injected over 20 seconds by an anesthesiologist.

The Sensory block was assessed by pinprick performed with a 22-gauge needle every 30 seconds until the dermatomal sensory block of medial and antebrachial cutaneous, ulnar, median and radial nerves achieved. Motor function was assessed by asking the patients to flex and extend his/her wrist and fingers and complete motor block was noted when no voluntary movement was possible. Sensory block onset time was noted as the time elapsed from drug injection to complete sensory block achieved in all dermatomes. Motor block onset time was the time elapsed from injection of study drug to complete motor block.

After complete sensory and motor blocks were achieved, the distal tourniquet was inflated to 250 mmHg, and the proximal tourniquet was releases and the surgery was started.

MAP, HR and SPO_2 level were recorded before application of tourniquet and after the application of tourniquet every five minute and were measured after release of the tourniquet, and postoperatively at 30 min, 1hr, 2hr, 3hr, 4hr, 6hr and 24 hours.

Pain due to the tourniquet was assessed with visual analogue scale (VAS) scores (0= no pain and 10= worst pain imaginable). Levels of sedation were assesses with the Ramsey sedation scale as follows: 1) patient is anxious and agitated or restless or both, 2) patients is cooperative, oriented and tranquil, 3) patient responds to command only, 4) patient exhibits brisk response to light glabellar tap or loud auditory stimulus (5) patient exhibits a sluggish response to light glabellar tap or loud auditory stimulus, and 6) patients exhibits no response.

Both VAS and sedation levels were recorded before and after the application of tourniquet and during the operation (10,15,20, 30,40,50 and 60 min). When pain due to tourniquet was >3 on the VAS, patients were given fentanyl in 10 μg increments up to 1μg/kg (Fentaryl-hmllin 50 mcg/ml, lan-

gesfeid, Germany) and total administered dose requirement time were noted. Oxygen was administered with face mask if SPO2 was lower than 91%. At the end of the operation patients were asked to qualify the operative conditions such as tourniquet pain or incisional pain and the quality of the anesthesia was graded by the anesthesiologist who was blinded to the study drug as follows: (4) excellent = no complaint from the patient; (3) good = minor complaint with no need for supplemental analgesics; (2) moderate = complaint that required a supplemental analgesic ……; (1) poor = unsuccessful, patient was given general anesthesia.

At the end of the operation the surgeons, who was blinded to the patient group, were asked to quality and score the operative conditions such as excessive bleeding and disturbing movement of the arm as follows; 4 = excellent; 3 = good; 2 = acceptable and 1 = poor.

The tourniquet was not deflated before 30 min and was not inflated more than 1.5 hours. At the end of the operation, the tourniquet deflation was performed by cyclic deflation technique (the tourniquet was deflated three times in a cyclic manner with 10 seconds period of deflation). Sensory recovery time was noted (time elapsed after tourniquet deflation up to recovery of pain in all innervated areas determined by pinprick test). Motor block recovery time was noted (the time elapsed after tourniquet deflation up to movement of fingers).

Also, first analgesic requirement time was noted (the time elapsed after tourniquet release to the first patient request of analgesic). In post-anesthetic care unit, patients received tramadol 50 mg when VAS >3 with maximum dose of 600 mg/day and total amount of tramadol administered to each group were recorded.

During the first 2 hours in the post-anesthetic care unit and later in the surgical ward, patients were questioned for circumoral numbness and tingling, nausea and vomiting, skin rash, tinnitus, gastric discomfort and other side effects were noted if encountered and MAP, HR and VAS scores were assed every 2 hours postoperatively during the first 24 hours.

Statistical Methods:

Data was analyzed using IBM SPSS Advanced Statistics version 20.0 (SPSS Inc., Chicago, IL). Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between 3 groups was done using ANOVA or Kruskal-Wallis test. Comparison of repeated measures was done using ANOVA for repeated measures. A p-value <0.05 was considered significant.

Results

The study included 3 groups, Groups C (control), L (Lornoxicam group) and P (paracetamol group). Table 1 shows baseline and operative characteristics of the three studied groups. The three groups were comparable in age, sex and body weight. There was no significant difference in the operative and tourniquet time between the three groups.

Table 2 shows a comparison between the three groups regarding characteristics of anesthesia and analgesia. The three groups were significantly different from each other regarding onset time of sensory and motor block. Control group had the longest onset time of sensory and motor block. Also, recovery time of sensory and motor block were significantly shorter in control group compared to the lornoxicam and paracetamol groups. However, recovery time was comparable between the latter two groups. Time to fentanyl request was significantly shorter and fentanyl dose was significantly larger in control group compared to the other two groups, although both time to fentanyl request and dose were comparable between lornoxicam and paracetamol groups. Similarly, a significantly higher number of control group patients needed fentanyl ($p=0.007$) or tramadol ($p<0.001$) for analgesia. Tramadol consumption was significantly higher in control group compared to lornoxicam and paracetamol groups, the latter two groups showed comparable tramadol consumption.

VAS score was comparable in the three groups at the beginning of surgery. Starting from 10 minutes up to 50 minutes intraoperatively, VAS score of lornoxicam and paracetamol groups was significantly lower than control group, with no significant difference be-

tween lornoxicam and paracetamol groups (figure 1). VAS score was significantly lower in lornoxicam and paracetamol groups compared to control group throughout the postoperative period up to 24 hours. There was no significant difference between lornoxicam and paracetamol groups (figure 2).

There were no side effects noticed during the study related to systemic absorption of local anesthesia such as circumoral numbness and tingling, convulsion, respiratory depression and hemodynamic instability and also there were no side effects noticed related to tourniquet such as nerve palsy.

Table (1): Baseline and operative characteristics of the three studied groups.

	Group C (n = 20)	**Group L (n = 20)**	**Group P (n = 20)**	**p value**
Age, years (mean±SD)	30.7±6.8	31.0±6.9	30.2±6.9	0.896
Sex, male/female	8/12	10/10	8/12	0.847
Weight, kg (mean±SD)	74.9±8.7	72.9±10.7	72.2±8.2	0.542
Operative time, minutes (mean±SD)	57.1±9.4	57.0±9.4	61.7±6.5	0.118
Tourniquet time, minutes (mean±SD)	68.2±9.1	68.2±9.2	71.9±6.3	0.355

Table (2): Anesthesia and analgesia characteristics of the three studied groups

	Group C (n = 20)	Group L (n = 20)	Group P (n = 20)	p value
Sensory block				
Onset (minutes)	7.1±0.5[a]	3.0±0.2[b]	5.3±0.6[c]	< 0.001
Recovery (minutes)	4.6±0.5[a]	6.4±0.3[b]	6.7±0.4[b]	< 0.001
Motor block				
Onset (minutes)	9.9±0.7[a]	7.3±0.3[b]	8.2±0.5[c]	< 0.001
Recovery (minutes)	5.9±0.6[a]	7.8±0.6[b]	8.0±0.4[b]	< 0.001
Fentayl				
Use (yes/no)	13/7	4/16	5/15	0.007
Time (minutes)	19.7±1.2[a]	31.3±2.8[b]	29.2±1.3[b]	< 0.001
Dose	69.6±5.2[a]	47.5±6.5[b]	43.0±4.5[b]	< 0.001
Tramadole				
Use (yes/no)	14/6	3/17	4/16	< 0.001
Consumption	36.1±5.9[a]	16.7±2.9[b]	17.5±2.9[b]	0.001
Quality of Anesthesia Score				
Anesthetist	3.4±0.5	3.8±0.4	3.8±0.4	0.016
Surgeon	2.4±0.5	2.7±0.6	2.7±0.5	0.070

Data as mean±SD
Groups was different superscript letters (a,b) are significantly different (A p-value < 0.05)

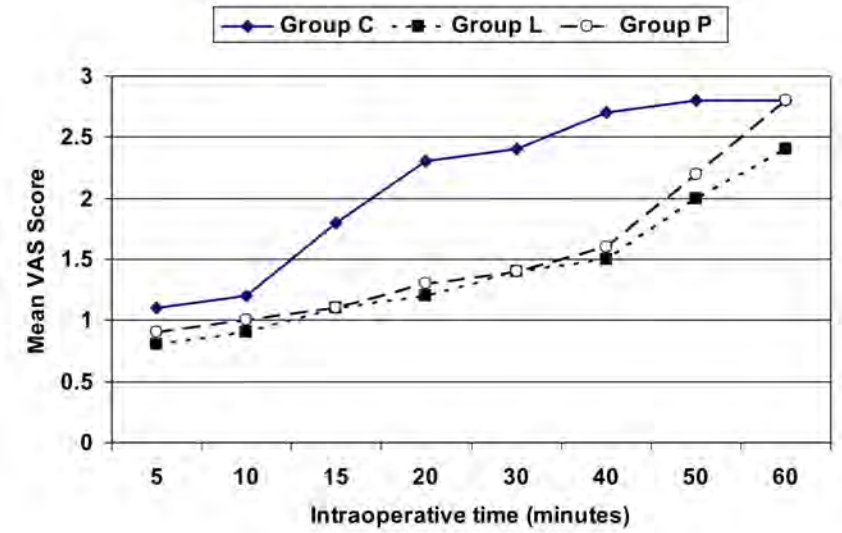

Fig. 1: Intraoperative VAS score in the three studied groups.

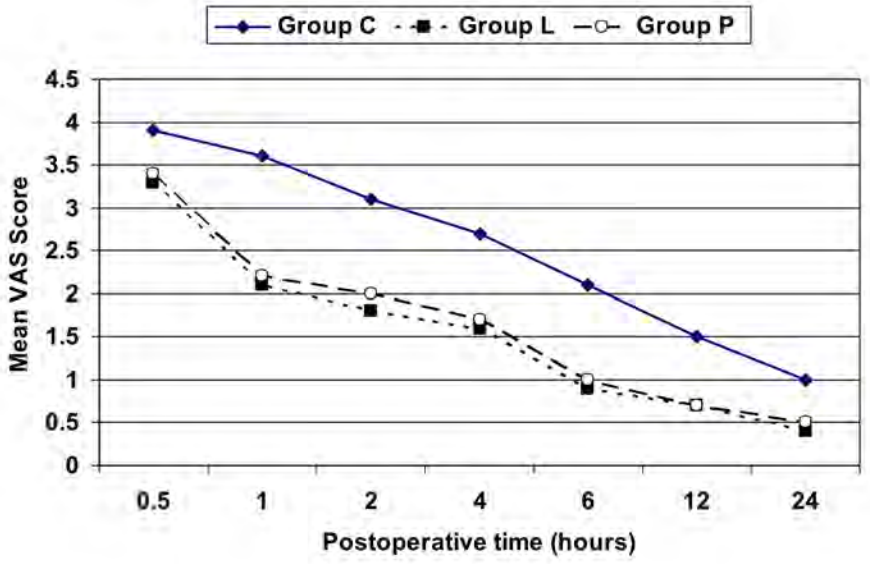

Fig. 1: Postoperative VAS score in the three studied groups.

Discussion

The primary outcome of our study the addition of Lornoxicam 8 mg or acetaminophen 250 mg to lidocaine for IVRA decreased tourniquet pain, improved the speed of onset of sensory and motor block, prolonged the sensory and motor recovery times, decreased both intraoprative and postoperative analgesic requirements and improved the quality of anesthesia without causing any side effects.

The addition of Lornoxicam shortened the sensory block onset time more than did addition of acetaminophen. Various NSAIDs such as lornoxicam[12,13], acetaminophen and ketorlac[4,15] have been used to improve the quality of analgesia in IVRA. It's known that increasing pH of local anesthetics improves the nerve penetration and onset of blockade [2]. Alkalization with bicarbonate has been found to be an effective adjunct for IVRA[16].

It was found pH of lidocaine-normal saline solution was 6.7, pH of lidocaine- normal saline-lornoxicam mixture was 7.6 and that of lidocaine-normal saline-acetaminophen mixture was 5.88. So, alkalization of LA with lornoxicam enhanced the sensory and motor block onset times by increasing the percentage of the free base. This might explain the rapid onset time of sensory block in group (L) compared with group (P) and group (C).

NSAIDs are largely used together with local anesthetics in IVRA to improve the quality of anesthesia. A systematic review by Choyce and Perg(2) suggested that NSAIDs offer a good adjuncts to IVRA. The major analgesic effect of NSAIDs is attributed to isoenzyme CoX-2 inhibition[17]. Reuben and Dupart[18] have found in another study that NSAIDs decrease the afferent nociceptive signals and synthesis of inflammatory mediators arising at the site of surgery.

Reuben and Duprat[18] had found that patients had less pain with decreased analgesic requirements in first postoperative hour when ketlorac was added to LA in their study. This was attributed to residual ketorlac in the operative arm and to lesser extent redistribution of ketoralc to systemic circulation after deflation of the tourniquet[15]. These findings are

related to our study as there was prolonged postoperative analgesia and decreased analgesic requirements with adding paracetamol or lornoxicam to local anesthestics in IVRA.

Paracetamol is generally considered as a week inhibitor of prostaglandins synthesis. Several studies have demonstrated several mechanisms for anti-nociceptive effect of paracetamol, including N-methyl-D-aspartate[19], and the effect on cannabinoid receptors [11,20].

A recent study done by Can bay et al[21] have demonstrated that paracetamol has peripheral anti-nociceptive effect. They have found also that pre-treatment with 50mg of IV paracetamol decreased propofol-induced injection pain. In another study acetaminophen may reduce pain through interference with delivery of peripheral B-endorphins[22].

Buritova and Besson[23] have demonstrated that Lornoxicam shows anti-nociceptive effect at a peripheral site and this action may be medicated via No-CGMP pathway and the opening of K+ channels.

It has been found that ischemia and oxidation stress may lead to tourniquet pain[24] Coderre and Colleagues[25] have demonstrated that antioxidant therapy my decrease ischemic tourniquet pain due to oxidative damage. Lornoxicam exhibits antioxidant effects in rats[26], so when lornoxicam was added to LA in IVRA might attenuate the tourniquet pain by antioxidant mechanism.

A Limitation of this study design was attributed to the fixed dose of intravenous acetaminophen 250 mg. the effective dose has yet to be determined. As when doses over 250 mg of paracetamol were mixed, the total volume of IVRA was too large and the control of the content of LA would be very difficult.

In conclusion, this study indicated (has found) that addition of lornoxicam to lidocaine in IVRA significantly shortened the onset of sensory block than did addition of acetaminophen. The addition of lornoxicam or acetaminophen to LA or IVRA have decreased the motor block onset time and pro-

longed both sensory and motor block recovery times. Both lornoxicam and acetaminophen have prolonged tourniquet pain onset time and improved postoperative analgesia.

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COMPARISON OF THE EFFECTS OF LORNOXICAM TO PARACETAMOL WHEN ADDED TO LIDOCAINE FOR INTRAVENOUS REGIONAL ANESTHESIA IN PATIENTS UNDERGOING HAND AND FOREARM SURGERY

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SOME PLATELET PROPERTIES OF RESTING APHERESIS PLATELET CONCENTRATE FOR THE LAST 6 HOURS OF A 48-HOUR STORAGE PERIOD

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Abstract

Background: *Apheresis platelet (PLT) units are not routinely agitated during transit. Aim of the study was to evaluate effects of resting (6 h of interruption of agitation) vs continue agitation of platelet concentrate (PC) stored for 48 h in the blood bank.* ***Materials and Methods:*** *apheresis PLT units were collected with Trima cell separator (n=20, terumo BCT), continuously agitated, starting routinely within 1 hour of collection, extending for 42 h at room temperature (20-24 °C). An identical apheresis PLT unit was stored with continuous agitation (designated as the control group, CA6h), and the other was held without continuous agitation for 6 h (WCA6h); by stopping the agitator. We studied some in vitro platelet quality variables as PLT count, PLT unit volume; swirling, PH, lactate dehydrogenase (LDH) concentration, CD62P and CD42b.* ***Results:*** *were compared with those of PC continuously agitated. Results: The mean platelet yield of individual apheresis-PC unit for (CA6h) and (wCA6h) were 3.25±0.20x 10^{11}, 3.25±0.19x 10^{11} respectively with no statistically different (P=0.92). LDH, and CD62P did not differ significantly (P=0.54), (P=0.07), between CA6h (51.11±6.68), (17.33±3.46) and WCA6h (52.39±6.42), (19.54±4.12) respectively. WCA6h showed highly significant lower CD42b expression (149.11±38.99) vs CA6h (223.41±42.88) (P=0.001) Likewise, the mean pH values were significantly different: WCA6h (6.88±0.15) and CA6h (6.99±0.12) (P=0.025).* ***Conclusions:*** *PC stored under agitation for 42 h at 22-24 °C and rested for 6 h had preserved PLT count and platelet yield, LDH and CD62p as PC kept under continuous agitation for the whole 48 h storage period.*

Keywords: *apharesis platelet, resting, interrupted agitation, platelet concentrate.*

Introduction

Platelet Concentrate (PC) from single donor is prepared by platelet apheresis, processed over about 1 hour yielded one therapeutic dose of platelets, called PC [1]. PCs are collected using different synthetic materials, centrifugation/leucocyte-removal processes, and stored in different types of bags. Upon exposure to artificial surfaces and high centrifugation forces, blood cells can undergo various levels of cellular activation/fragmentation and release reactions which may not only influence the extent of the platelet storage lesion but may also contribute to poor clinical effectiveness of the PC and transfusion reactions[2].

Apheresis platelets (PLTs) differ in many ways from whole blood–derived PLT concentrates: they are a) collected with a different anticoagulant and/or preservative, b) stored in different containers, c) may contain different PLT subpopulations, and d) can be stored for up to 7 days[3].

It is assumed that continuous, gentle agitation of the PC maintains the pH levels at or above 6.2 throughout the period that the platelets are stored at room temperature (22-24˚C) in special, gas-permeable plastic bags to ensure their in vitro quality and in vivo effectiveness. This procedure facilitates the transfer of oxygen to the PLTs and the removal of carbon dioxide from the storage bag[4]. The most noted effect from interruption of agitation for lengthy periods (24 hr) is an increase in the rate of pH decline during storage. This reflects an increase in production of lactic acid due to enhanced glycolysis and reduced oxidative metabolism[3].

As continuous agitation is not practical, because substantive periods of discontinued agitation occur between remote collection and manufacturing sites and during shipping to hospitals, there has been interest in better defining the effects of interrupted agitation[3]. This creates problems. Is an interruption of agitation for 1 minute, 1 hour, 1 day, or even 2 or 3 days consistently harmful? Should brief periods of interruption of agitation be cause for discarding the PCs. AABB standards permit up to 24

hours of interrupted agitation[5].

PCs are stored for up to 5 days under agitation conditions at room temperature until transfusion, where complex structural and functional changes of platelets occur, summarized as platelet storage lesion (PSL) Preparation mode and storage conditions play an important role in the development of PSLs[1]. Increased release of platelet α-granules and cytosolic proteins, increased procoagulant activity, and altered glycoprotein (GP) expression, all of which are characteristic of platelet activation[6]. Both activation state and the reactivity of the platelets can be determined using flow cytometry. Activation markers of interest that can be studied in such a way include P-selectin (CD62P), as well as GPIb expression (CD42)[7].

There are various receptors on the glycoprotein (GP) layer of the platelet, include the selectins (such as GMP 140, or P-selectin), the integrins (such as GP I, GP II, GP III, GP IV, GP V), and other receptors, which assist in platelet adhesion, aggregation and coagulation. Glycoprotein GP Ib is important in the attachment of platelets to von Willebrand factor (vWF) and the vascular subendothelium. Von Willebrand Factor binds to GP Ib in the presence of ristocetin [8]. CD62 (P-selectin, GMP-140, PADGEM) is (CD62p), a constituent of α-granules in resting platelets, can be detected on the activated platelet surface after α-granule secretion[9].

Aim of The Study

Determining the influence of resting (6 h of interruption of agitation) vs. continuing agitation of apheresis PC, and comparing some properties of apheresis PLT units that were stored with continuous agitation (CA6h) as a control and units stored with 6 h of resting "without continuous agitation" (WCA6h) as a test units after 42 h of storage from the time they were collected for whole 48 h storage time under Blood Bank-compatible conditions.

Materials and Methods

PLT collection and preparation of matched, identical components:

Twenty PCs were prepared in the Zagazig University Blood

Transfusion Center. Platelet collection and storage from healthy male donors (age 20-40 years) meeting the directive for platelet cytapheresis (platelet count >200 103/µl; body weight >50 kg) and who gave written and informed consent. PCs were prepared according to the manufacturer's instructions by the Trima Accel (Software Version 5.1, terumo BCT) cell separator, using the blood anticoagulant (acid citrate dextrose (ACD)) ratio 11:1. PLTs suspended in 100% plasma with a plasma target volume of 200- 300 ml and a platelet target yield of $3x10^{11}$ to $3.5x10^{11}$ The target level of PLTs was 6.0 to $7x10^{11}$ per unit. Two identical components in PLT containers (Trima) were prepared. These machines perform leukoreduction during the donation process via a leukoreduction system chamber (LRS chamber) [10]."Preparations with total PLT contents of less than $6.0x10^{11}$ were not utilized in this study".

Day of collection:

For the primary series of experiments, the collection was divided equally by weight between the two integrally attached containers within 30 to 40 minutes of the apheresis procedure.

Storage with or without CA:

PLT component of each matched pair was placed on a flatbed agitator (Helmer, 65-70 cycles/min) at 20 to 24°C within 30 minutes of the completion of collection and maintained under agitation for 42 h. The 20 PCs were then divided into two groups of twenty PC each. The first group PC units, designated as the control units, continued under continuous agitation for the subsequent 6 h (CA6h), while the second group, designated as the test units was removed from the agitator and placed for 6 hours stored stationary (resting) under the same environmental conditions in the incubator without agitation (WCA6h). After a total of 48 h of storage the CA6h and WCA6h PCs were taken out of the incubator and tested immediately.

In vitro PLT testing:

Before obtaining all samples, the components were thoroughly mixed manually for a few seconds. Samples aseptically taken from both components (CA and WCA).

The qualitative and quantitative tests performed were platelet count, volume, and swirling score, pH, LDH concentration and platelet activity tests (CD62p% and CD42b expression).

Platelet count, PLT volume, PLT yield:

PLT counts were obtained using hematology analyzer Sysmex KX-21N cell counter (sysmex corporation, Kobe, Japan) with EDTA taken samples. The PLT volume (ml) was calculated by subtracting empty bag weight from component weights of full bag "weighing the contents of the storage bag, in grams, on a scale and the result, in grams, was divided by 1.03 (1.03 g/ml is the density of the storage medium composed of 100% plasma)[11]. To compare the retention of PLT concentrations in matched apheresis PLT units, PLT content "Yield" throughout storage was calculated by multiplying the PLT count by the PLT volume measured[12].

PLT yield ($x10^{11}$) = Count (10^3/ ul) x 10^3 ul/ml X Volume (ml) according to instruction of the manufacture.

Swirling score:

"Swirling" is a visual effect caused by defraction when platelets are manually re-suspended and held up to a strong light. The presence of swirling indicates the suspension contains high-quality, discoid platelets[13].

Qualitative measure of discoid PLTs morphology scored as:

Score 0: Homogeny turbid and is not changed with pressure.

Score 1: Homogeny swirling only in some part of the bag and is not clear.

Score 2: Clear homogenic swirling in all part of the bag.

Score 3: Very clear homogeny swirling in all part of the bag.[12]

pH:

Viability-related variables[14]. The pH was determined of the PCs at room temperature (20-24°C) with an Accumet basic (AB15) pH meter (Fisher Scientific,USA).

Lactate dehydrogenase release (LDH):

Enzyme marker of cell viability [15,16], a marker for disintegration of platelets[9]. LDH release was measured by COBAS INTEGRA

400 plus (Germany) of all PCs units after 48 h of storage (including the continuous agitation or 6 h of resting).

Platelet activity tests:

Monoclonal antibody labeling:

PC samples were stained for 20 min at room temperature in the dark by incubating with 10 µl of fluorochrome-labelled monoclonal antibodies per $1x10^6$ platelets. fluorescein isothiocyanate (FITC)-conjugated CD42b (GPIbα) antibodies (clone HIP1), phycoerythrin (PE)-conjugated CD62p (P-selectin/ GMP-140/platelet activation dependent granule-external membrane protein; (Clone AK-4), from (BD Pharmingen™San Jose, California, USA) were used. After incubation with fluorochrome-conjugated antibodies, the samples were washed twice by adding 2·0 ml filtered PBS-ethylenediaminetetraacetic acid (PBS-EDTA) with 0·1% Na-azid, and were centrifuged at 1200RPM for 10 min at 18 °C.

Flowcytometry analysis:

Flowcytometric analyses were performed using a flowcytometer equipped with its accompanying software (FACSCalibur and Cell Quest, respectively, Becton Dickinson, USA). The flowcytometer settings were optimized for the acquisition of platelets by logarithmic signal amplification in all four detectors (forward and side scatter channels and fluorescence channels FL1 and FL2), and at least 10,000 PLT events were collected. For analysis, a gate was set around intact platelet population as defined by forward and side scatter characteristics. The percentage of positive platelets of total platelet expressing activation markers (CD62p) and surface membrane glycoproteins (CD42b) above that of background (negative control) as well as the mean flourescence intensity (MFI) was recorded. Daily controls of optics and fluorescence intensity was performed using standardized beads (CaliBrite; BD Bioscience, San Jose, CA, USA).

Statistical Analysis:

Analysis done using SPSS version 17 (SPSS Inc., Chicago, IL, USA), data were expressed as means and SDs for WCA and CA components. Statistical comparisons were assessed with the two-

tailed paired t test. A significant p value was taken to be less than 0.05. Percentage differences were determined between control and test results relative to the mean control value, multiplied by 100 percent(3). The cutoff value for a new diagnostic test (used for determining cut off value for LDH measurements, CD62P % CD24b expression) "calculated by using statistical technique" Mean ± 2SD" of test values, an interval obtained of a test value coming outside this interval will be less than 5%[17].

Results

The data are summarized and given as means ± standard deviations of the values determined for the CA6h and WCA6h PC. Shown in Table 1.

PLT count, PLT yield per unit:

The platelet counts in the CA6h and WCA6 PC were in the normal range and statistically not different (P<0.0.9). Platelet yield of individual apheresis-PC unit was calculated and analyzed, (mean ± SD) for control (CA6h) and test (wCA6h) were 3.25±0.20x 10^{11}, 3.25±0.19x 10^{11} per unit respectively, statistically not different (P<0.092), so, resting at the end of storage did not affect the platelet count, or platelet yield in the PC units stored for a maximum of 48 h.

Swirling score:

Swirling with score 3 was observed in 25%, and 15% of CA6h, WCA6h PC units, respectively, while score 2 swirling was noticed in 75% and 85% of CA6h, WCA6h PC units respectively, with statistically no significant difference (P=0.071). No unit had scored 1 swirling. Thus 6 h of resting seemed not affect the swirling properties of the PC.

PH:

The pH of all studied apheresis-PC did not drop below 6.8 in the control PC or below 6.7 in PC that were rested for 6 h throughout the 48 h of storage, with statistically significant difference (P=0.025).

Lactate dehydrogenase release:

LDH release in the two groups of PC (CA6h and WCA6h) was not significantly different (P=0.54), with reference range calculated statistically (Mean ± 2SD) (37.8-64.5) U/bag.

Platelet activity tests:

The mean value of P-selectin expression (CD62p) was not significantly different in the WCA6h PC than in the CA6h PC (P=0.07). CD42b expression for WCA6h and CA6h showed a highly significant decrease in the rested units (P<0.001). Reference range calculated statistically for (CD62p) (10.4-24.3%) and for CD42b expression (137.7-309.2) (Table 1).

Mean percentage difference in mean levels of some PLTs parameters subjected to a 6hour interruption of agitation compared to continuously agitated units with paired t test were presented in (Table 2), which showed a highly significant mean percentage difference inCD42b expression, significant with LDH release, non significant with CD62p %.

For each variable studied, percentage difference were determined between control and test results of mean PLT variables values relative to the mean control value, multiplied by 100 percent to quantitate differences between variables, shown in (Figure 1).

Table (1): Means ± standard deviations (mean±SD) and range of some platelet parameters of the values determined for the agitated CA6h (n=20) and not agitated WCA6h (n=20) PC for the last 6 hours of a 48-hour storage period.

Assay	48hours		t	p	Reference
	Control(CA6h)	Test(WCA6h)			
Platelet count ($x10^3/\mu l$)	1415.35±74.99 (1252-1550)	1412.55±74.06 (1245-1540)	0.11	0.9 NS	--
Volume/ml	229.9±9.96 (215-250)	229.9±9.96 (215-250)	0.0	1.0 NS	200- 300ml
Platelet yield ($x10^{11}$/ unit)	3.25±0.20 (2.99-3.84)	3.25±0.19 (2.99- 3.82)	0.09	0.92 NS	$>3\times10^{11}$ in ≥90% of units*
Swirling	2.25±0.44 (2.0- 3.0)	2.15±0.37 (2.0-3.0)	0.36	0.71 NS	≥ 2
PH	6.99±0.13 (6.8-7.2)	6.88±0.15 (6.7-7.2)	2.32	0.025 S	> 6 - 2 in ≥90% of units*
LDH U/bag	51.11±6.68 (41.25-61.20)	52.39±6.42 (52.39-62.91)	0.61	0.54 NS	(37.8 - 64.5) U/bag
CD62P(%of positive)	17.33±3.46 (9.53-23.52)	19.54±4.12 (10.53-27.68)	1.84	0.07 NS	(10.4 - 24.3) %
Cd42b (MFC)	223.41±42.88 (159.69-299.00)	149.11±38.99 (93.78-212.56)	5.7	0.001 < HS	(137.7 - 309.2)

P values <0.05 were considered statistically significant.

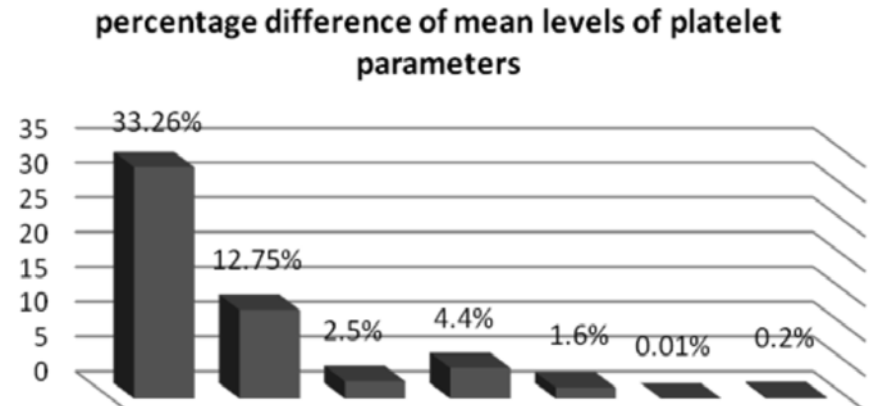
*Standards for platelet yield and pH values of platelet concentrate as required by the AABB.

NS: non significant, S: significant, HS: highly significant, U/bag: unit per bag, MFC: mean fluorescence channel.

Table (2): Mean percentage difference and range of difference of some PLTs parameters (control-test) subjected to a 6hour interruption of agitation with paired t test.

Assay	48hours		Mean percentage difference±SD, Range of difference	Paired T	p
	Control(CA6h) mean±SD	Test(WCA6h) mean±SD			
LDH U/bag	51.11±6.68	52.39±6.42	2.735± 5.73 (-2.28-21.57)	2.136	0.045 S
CD62P %	17.33±3.46	19.54±4.12	12.64±4.15 (3.9-17.9)	13.6	0.01 NS
CD42b MFC	223.41±42.88	149.11±38.99	33.06±6.78 (20.76-41.27)	21.79	<0.001 HS

P values <0.05 were considered statistically significant
S: Significant, NS: Non significant, HS: Highly significant.



Data presented in table 1 were utilized to calculate percentage difference.

Fig. 1: Percentage difference of mean levels of PLTs parameters subjected to a 6hour interruption of agitation relative to the mean control value, multiplied by 100 percent.

Discussion

Agitation permits the maintenance of pH at or above 6.2 during storage, as key to maintain in vivo viability[3]. Although CA is well documented as the optimal means for maintaining PLT properties during storage, there is the need to routinely ship PLTs to hospitals from collection and/or manufacturing sites without instrument-based agitation[14]. There are no standards for rest or hold periods for PLTs prepared by apheresis[18]. There are limited data describing the maintenance of in vitro properties of apheresis PLTs after an interruption of agita-

tion during actual shipment of PLTs[3]. The primary goal to assess the influence of interrupting CA, which is the accepted standard for maximally maintaining PLT properties during storage[11].

In this work we studied to what extent 6 h of resting after 42 h of storage from the time they were collected, affect platelet quantity (count , platelet content "yield" per bag), quality (swirling, pH), viability (LDH release) and activity (CD62P%, CD42b expression).

In the current study regarding PLT count/units, ninety five percent (1/20) units of apheresis-PC met the desired quality control criteria according to AABB standards required for Apheresis platelets $>3x10^{11}$ in ≥90% of units[12]. PLT yield per unit in the CA6h and WCA6 PC were in the normal range. So, Platelet count, PLT yield were maintained in both PC (CA6h, WCA6) units stored for 48 h.

Swirling was present within score 2, 3 in all units studied CA6h and WCA6h PC. No unit had scored 1 swirling. Thus 6 h of resting seemed not affect the swirling properties of the PC. Which was similar with finding of Naghadeh H et al[4] who observed no differences in the swirling score between CA6h and WCA6h PC prepared from platelet-rich plasma. Results corresponded also with the findings of Singh R et al [12], Bertolini F et al[19], and also with Hunter S et al[20] that showed that the maintenance of grade 2 to 3 swirling essentially guarantees the quality of PC.

According to AABB standards required for Apheresis PLTs pH at end of allowable storage >6 • 2 in ≥90% of units[13]. We found that pH of our all studied apheresis-PC did not drop below 6.8 in the control PC or below 6.7 in PC that were rested for 6 h throughout 48 h of storage. So, although our pH value decreased but was maintained within acceptable range, and mean pH percentage difference were decreased relative to continuously agitated products by 1.6%. In previous study by Vassallo R et al[5] with whole blood-derived PLTs have demonstrated that a contiguous 24-hour interrupted agitation results in maintenance of PC pH value of 6.5 or

greater .Another study by Moroff G et al[11] showed that Trima apheresis PLT components that were stored with (CA) and (WCA) during two separate periods, immediately after collection and between Day 2 and Day 3 of storage, mean pH levels on Day 5of storage were decreased in (WCA) units with relative to (CA) products by 0.25. We could explain the differences between these results and ours to the difference in preparation method of the platelets, the period of stopping agitation and time at which the study done during the storage period.

LDH release measurements in the two groups of our PC (CA6h and WCA6h) indicating that stopping agitation did not have major effects on platelet viability, which was agreed with finding of Naghadeh H et al[4] observed that 6 h of resting did not have a deleterious effect on pH, LDH release and ristocetin-induced platelet aggregation (GPIb-related).

So, the mean difference in levels of variable parameters in our study regarding the platelet quantity, quality and viability appear to be small possibly without major effect on PLTs viability.

Increased P-selectin (CD62P) expression during storage has been reported by several authors, whereas GPIb (CD42b) has been shown to decrease during storage[21].

Triulzi D el al[22], by using the appearance of P selectin (CD62) on the surface of platelets as an index of activation, have suggested that more than one third of the platelets in the concentrate express CD62 within 4 h of storage. Lozano M et al[23] also found that 20 to 30% of platelets in platelet concentrates expressed CD62 and reported that CD62 expression correlated poorly with percent recovery after transfusion.

The percent increase of platelet activation between CA and WCA units after 48h of storage for CD62P% was 12.75% in our study which was close to Moroff G et al [11] who found differences in CD62-positive PLTs being less than 20% for CA and WCA units after 5 days of storage (Utilized two periods without agitation, immediately after collection for 7 to 8

hours and a subsequent period for 24 hours between Day 2 and Day 3 of storage). The percentage difference for CD42b expression was found 33.26 for our study. A study by Sandgren P et al[9] found that storage of PCs reduce expression of GP1bα on the surface of platelets; this reduction is assumed to decrease the adhesive capacity. On the other hand, Wang C et al[7] reported that activated platelets exhibit down regulation of GPIb (CD42; the thrombin binding site), also suggested that a 65% reduction in this expression of GPIb, has no effect on platelet adhesion during flow conditions also, Tynngård N[21] reported a decline in the surface expression of GPIb with platelet storage up to ten days, together with impaired ability of the platelets to be become activated by thrombin as storage time increased. The membrane content of GPIb with storage has been reported as a 35% loss.

So, in this study the baseline platelet activity changes observed was corresponding to the findings of others, these small differences appear to be only quantitative and may reflect differences in PLT product, heterogeneity of PC prepared using the standard protocol, manipulation during collection or processing of the platelets and the different periods of storage without agitation.

In conclusion, From a clinical point of view, the differences between CA and WCA of PLTs units, for various in vitro variables measured after 48h of storage were limited, although some differences were significant, these minimally influenced differences of in vitro properties relative to those of matched continuously agitated products would appear not to reflect any major changes that might influence in vivo viability.

Of course more research is needed to understand how agitation can best be modified prior to transfusion, and how exactly this interruption of continuous agitation for 6 h, after 42 h of continuous agitation, affects PC quality, quantity and viability needs more investigation.

Conflict of Interest:

None of the authors has any conflict of interest.

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SOME PLATELET PROPERTIES OF RESTING APHERESIS PLATELET CONCENTRATE FOR THE LAST 6 HOURS OF A 48-HOUR STORAGE PERIOD

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OUTCOME OF CORONARY ARTERY BYPASS IN PATIENTS WITH CHILD-PUGH CLASS A LIVER CIRRHOSIS

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Abstract

Objectives: *Coronary artery bypass grafting in cirrhotic patients carries high risk of morbidity and mortality. Most of these complications are related to hepato-renal failure, bleeding and postoperative wound infection rather than cardiac problems.*

Methods: *Prospective study to evaluate elective CABG early postoperative outcome of elective CABG in patients with Class A Child Pugh liver cirrhosis.*

Results: *From October 2007 to April 2011total 59 patients with Class A liver failure underwent elective CABG.37male, 22female. 42hypertensives and 28diabetics. Mean1st 24hours chest tube drainage was 853.80±56.10ml, minimal 130ml maximum 3500ml. 12 patients (20.3%) were re-explored for bleeding. Mean ventilation time was 10.48±6.65 hours. Mean ICU stay was 59.52±13.91hours. Two patients (3.38%) died one patient due to hepato-renal failure (re explored 3 times) while the 2nd patient died after delayed recovery due to cerebral hemorrhage. 20 patients (33.89%) had wound infection, two need debridement and rewiring. Mean hospital stay was 9.18±2.29days. Total morbidity was 49%. Total mortality was 3.38%.*

Conclusion: *Elective CABG can be tolerated satisfactorily in class A Child Pugh cirrhotic patients with high incidence of the postoperative complications specially bleeding and wound infection.*

Introduction

Open heart surgery in cirrhotic patients had relatively high risk of morbidity and mortality when compared to non cirrhotic patients mainly Postoperative liver decomposition, bleeding and high incidence of postoperative wound infection[1-5].

Child Pugh classification[6] (Table 1) consists of certain clinical,

laboratory and radiological parameters to classify liver cirrhotic patients into A, B and C classes. Class A has the best condition while class C has the worst condition.

Model for End-Stage Liver Disease (MELD) score[7] are calculated in cirrhotic patients to help in prediction of the excepted morbidity and mortality. MELD score ranging from 6 to 40, patients with score of 6 are the best ill patients while patients of 40 are the sickest one.

The expected morbidity and mortality is much higher in patients with advanced Child Pugh Classification and high MELD score but there is no accurate predicator of outcome of open heart surgery in liver cirrhosis patients. Some studies used Child Pugh Classification others used Model for End-Stage Liver Disease (MELD) score to evaluate the outcome of CABG in liver cirrhosis patients but most of these studies of had limited number of patients[4,5].

The number of cirrhotic patients undergoing CABG is increasing in Egypt as it has one of the highest percentages of hepatitis C patients in the world ranging from 10% to 13% of the population[8], most of these patients are class A Child Pugh classification so we had this prospective study to evaluate the outcome of CABG in A class Child Pugh patients. All preoperative, operative and postoperative details are collected and analyzed.

Patients and Methods

From April 2008 to April 2011 a prospective study to evaluate the outcome of elective CABG in class A cirrhotic patients. All patients had full clinical evaluation and full laboratory evaluation including complete blood picture, complete liver function and complete renal functions test. Enzyme-linked immunosorbent assay (ELISA) test was used in diagnose hepatitis markers. Abdominal ultra sound is done for all patients to evaluate the liver condition and diagnosis portal Hyperion. No liver biopsy was taken. Child Pugh classification and MELD score are calculated for all patients.

Inclusion criteria:

1- Elective CABG.

2- Class A Child Pugh.

3-Good left ventricular function with ejection fraction above 35%.

Exclusion criteria are:

1- Class B and C Child Pugh classification.

2- Emergency or urgent CABG.

3- Redo CABG.

4- Open heart surgeries rather than CABG like valve surgery or combined surgery like CABG+ valve surgery.

5- Patients with poor left ventricular function with ejection fraction below 35%.

6- Renal failure patients on regular dialysis.

All preoperative, operative and postoperative data including 3 months follow up after discharge home are collected and analyzed.

All patients continue on same drugs they usually use until the morning of the surgery except anti-platelets, which are stopped for 7 days before surgery.

After the patients are anesthetizes, midline sternotomy is done. Pedicled left internal mammary artery (LIMA) is harvested in all patients.

All operations were performed utilizing conventional cardiopulmonary bypass (CPB) giving cold antigrade crystalloid cardioplegic solution repeated every 30 minutes. CPB was conducted using a membrane oxygenator and mild hypothermia (35C).

Packed red blood cells were administered when haematocrit was less than 25%. Fresh frozen plasma and platelets were administered when platelet count was less than 40000/ml or as a part of control postoperative bleeding.

Postoperative complications were classified as follow:

- Pulmonary: pneumonia, ventilator dependence greater than 48 hours, excessive pleural effusion requiring an additional drainage.
- Infectious (superficial and deep sternal wound infection).
- Bleeding complications (re-exploration because of excessive mediastinal bleeding or cardiac tamponade requiring drainage).
- Renal complications (increase in serum creatinine greater than 1.5mg/dl, oliguria (<0.5 ml/kg/min) for more than 6 hours postoperatively or any other indication

for dialysis).

- Other postoperative complications related to liver diseases, such as encephalopathy, hyperbilirubinemia and gastrointestinal bleeding as a result of varices were also recorded.

Mortality is defined as death during a hospitalization for surgery, regardless of length of stay, or within 30 days from hospital discharge.

Values of continuous variables were expressed as mean and standard deviation performed with computerized statistical packages (SPSS 18.0 software, SPSS, Chicago, IL, USA).

Results

Total 59 patients were eligible for the study. Main cause of liver cirrhosis was hepatitis C (42 patients) and Hepatitis B (17patients) with no Alcoholic cirrhosis.47 male and 12 female .28 patients were diabetics while 42 were hypertensives. Mean MELD score was15.2±3.38 (minimal 8, maximum 23). Table 2 summarizes the preoperative patient's profile.

Mean number of grafts was 2.72±0.57. Mean cardiopulmonary bypass time was 62.27±6.40 minutes (minimum45, maximum78). Mean Cross-clamp time it was 41.53±5.85 (minimum 28 maximum 55min). No patients need intra aortic balloon pump. Table 3 summarizes the operative data.

The postoperative mean chest tube drainage was 853.80±56.10ml; minimal 130ml maximum 3500 ml. Mean packed RBCS transfusion was 2.18±1.68 units. Mean fresh frozen plasma (FFP) Transfusion was 3.64±2.48 units. Mean platelets transfusion was 3.99±1.30 units. No postoperative myocardial infarction diagnosed by ECG and repeated cardiac enzyme. 12 patients were re-explored for bleeding (20.3%), 8 of them had cardiac tamponade. Mean ventilation time was 10.48±6.6. Mean ICU stay was 59.52±13.91 hours. 20 patients (33.89%) had wound infection, 15 of them had leg wound infection, 2 patients with mediastinitis needed debridement and rewiring. one patient had left sided clotted haemothorax diagnosed by CT chest drained though left mini thoracotomy. 21 patients (35.59%) had left pleural effusion, all treated medi-

cally except 6 patents needed repeated pleural taping. Four patients readmitted due to mediastinitis, 2 of them had rewiring. Mean Hospital stay was 9.18±2.29 days. Total morbidity was 49%. Total mortality was 3.38%. (2 patients), 1st was re-explored 3 times for bleeding and died due to hepato-renal failure, 2nd patient had delayed recovery with right sided hemiplegia .CT brain showed cerebral hemorrhage. Table 4 summerizes the postoperative outcome.

Table (1): Child-Pugh Classification of Severity of Liver Disease (6).

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirrubin, mg/dL	</= 2	2-3	>3
Albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time Seconds over control INR	 1-3 <1.8	 4-6 1.8-2.3	 >6 >2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

INR: International Normalised Ratio. A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (de-compensated disease).

Table (2): Preoperative Demographic and Laboratory Results.

Variable	Mean & Percent
AGE	53.22±8.23
MALE	37(62.71%)
FEMALE	22(37.28%)
HYPERTENSIVE	12(16%)
EF	52.76±6.79
DIABETICS	28(47.45%)
MELD score	15.2±3.38(8-23)
CREATININE	1.03±0.18
UREA	18.13±6.80
BILIRUBIN	0.90±0.28
AST	34.02±15.87
ALT	29.76±13.83
ALBUMIN	3.96±0.36
HB	12.78±1.40
PLATLETS	168.40±55.65

EF: ejection fraction; HB: hemoglobin; **ALT:** Alanine Aminotransferase; **AST:** Aspartate Aminotransferase.

Table (3): Operative Data of the Patients.

Variant	Mean & ST
GRAFTS	2.72±0.57(Min2, Max4 grafts)
CPB(min)	53.28±20.31min
CXT(min)	38.14±16.08min

CPB: cardio-pulmonary bypass, **CXT:** cross clamp time, **Min:** minute. **Min:** minimum, **Max:** maximum.

Table (4): Postoperative Outcome and Laboratory Results.

Variable	Mean & SD
VENTILATION TIME	10.48±6.65
ICU TIME	59.52±13.91
HOSPITAL STAY	9.18±2.29
BLOOD LOSS	853.80±56.10
RF-EXPLORATION	12(20.3%)
TAMPONADE	8(13.55%)
PRBCS TRANSFUSION (unit)	2.18±1.68
FFP(unit)	3.64±2.48
PLALETS TRANSFUSION(unit)	3.99±1.30
DEATH	2(3.38%)
ALT	64.56±45.01
AST	42.38±16.18
TB	0.98±0.52
UREA	18.90±6.93
CREATININE	1.05±0.43

SD: standard deviation; **TB:** total bilirubin; **ALT:** Alanine Aminotransferase; **AST:** Aspartate Aminotransferase; **ICU:** intensive care Unit; **FFP:** fresh frozen Plasma; **PRBCS:** packed red blood cells.

Discussion

Open heart surgery in cirrhotic patients carries high risk of postoperative morbidity and mortality[1-5]. There is Limited experience in open heart surgery in class A cirrhotic patients and most of these studies are of limited number[3,10-12], emergency cases are included[13] or wide variety of surgical procedure are included[6]. The total morbidity of open heart surgery in class A cirrhotic patients ranging from 39% to 60% [13-17]. Most of these complications are not related to cardiac complications and mostly related to hepato-renal failure, bleeding and high incidence of postoperative infection[1-5,8,9]. There is no accurate predicator of outcome of CABG surgery in

those patients as some studies used Child Pugh classification while others used MELD score as prognostic values for open heart surgery in cirrhotic patients [18,19].

Bleeding is major clinical problem of CABG in patients with liver disease as there are major alterations in the haemostatic pathways in most patients with liver disease including altered platelet and endothelial function, altered clotting factors and conditions such as hyperfibrinolysis, dysfibrinogenemia and renal failure which may be superimposed on these underlying abnormalities[20]. Also Cardiopulmonary Bypass machine aggravates the coagulopathy inducing platelet dysfunction, fibrinolysis, and hypocalcemia[21].

The postoperative high incidence of delayed wound healing and high infection rate is expected in this group of patients due to hypoalbumenia and relatively high incidence of blood products transfusion[20].

In our study the total morbidly percent was 49% most of these complications are related mainly to bleeding, infection, liver decompensation and renal impairment rather than low cardiac output. In our study elective CABG in class A cirrhosis while other studies with relatively high morbidity emergency cases are included and other cardiac operations rather than CABG[3,12-15].

The mortality of open heart surgery in liver cirrhotic class A patients differs from study to other ranging from 5.2%[2] to 15% [13]. Mostly related to sepsis and hepato-renal failure. In our study as emergency cases and other procedure rather than elective CABG are exclude, the postoperative mortality in was 3.38% mainly due to hepto-renal failure and cerebral hemorrhage. Table (4) summarizes the postoperative outcome and laboratory results.

Conclusion

Elective Coronary artery bypass surgery in patients with class A Child Pugh cirrhosis can be tolerated satisfactorily with higher incidence of the postoperative complications specially bleeding and infection problems.

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OUTCOME OF CORONARY ARTERY BYPASS IN PATIENTS WITH CHILD-PUGH CLASS A LIVER CIRRHOSIS

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NUTRITIONAL MANAGEMENT OF PREDIALYSIS RENAL FAILURE PATIENTS USING GUM ARABIC

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Abstract

Background: *Adequate nutritional management is a mainstay in treatment of predialysis renal failure. The current protocols have many drawbacks. GA proved effective for those patients in previous studies.*

Aim of work: The present study aimed to investigate the effect of GA on biochemical and clinical parameters in predialysis patients and their need to dialysis.

Materials and Methods: *The study comprised two groups: Group I (Treatment group): included 22 patients and received Arabic gum in addition to the conventional therapeutic intervention. Group II (Control group): included 19 patients who received conventional therapeutic intervention. In the treatment group, patients received a daily dose of 100 gm of Arabic gum dissolved in water or natural juice equally divided into morning and evening dose of 50 gm. All patients were subjected to careful history taking, thorough clinical examination and laboratory investigations including renal function, serum electrolytes and blood picture. All participants were followed monthly for 4 months.*

Results: *In the study follow up, treatment with gum Arabic resulted in progressive significant improvement in renal function (creatinine clearance, serum creatinine and serum urea). There were also significant decline in serum Ca, P, Na and K levels in the treatment group. In addition, treatment group had better blood pressure control and none of patients needed dialysis in the study period in comparison with 7 patients in the control group. Uremic fetor disappeared in 18 patients of*

the treatment group while no improvement occurred in the control group.

Conclusions: *Nutritional supplementation with Gum Arabic in predialysis CRF patients results in general improvement of laboratory and clinical parameters with subsequent delay in disease progression and need of dialysis.*

Keywords: *Chronic renal failure, gum Arabic, renal nutrition.*

Introduction

Diet potentially plays a major role in the progression and complications of predialysis CKD. Moderate protein consumption along with a diet low in sodium might slow kidney disease progression. Increasing vegetable protein intake might decrease serum phosphorus, uremic toxins, and kidney damage.[1]

A properly implemented dietary treatment for patients with chronic renal failure (CRF) can correct several metabolic and endocrine disturbances and delay initiation of dialysis, but concerns exist about the risk of malnutrition and protein depletion.[2] Also, the practical implementation of protein restriction through dietary intervention has been hindered on multiple levels, including patient nonadherence and and lack of health care resources.[3] Furthermore, larger scale studies demonstrated lack of effectiveness of protein restriction strategy.[4]

Consequently, more kidney protective strategies are needed to reduce the burden of complete kidney failure from chronic kidney disease (CKD).[4]

Gum arabic (GA) is a branched-chain, complex polysaccharide, either neutral or slightly acidic. Pharmacologically, GA has been claimed to act as an anti-oxidant, and to protect against experimental hepatic-, renal- and cardiac toxicities in rats. It has also been claimed to alleviate the adverse effects of chronic renal failure in humans.[5] However, clinical data on its efficacy in human studies are scarce.

Aim of Study

The present study aim to inves-

tigate the efficacy of gum Arabic in predialysis chronic renal failure patients.

Materials and Methods

The present study is an interventional longitudinal case control study. It was conducted at Mansoura Nephrology Center, Mansoura, Egypt after obtaining the required permissions. Informed consent was obtained from all participants.

Patients were selected to participate in the study had chronic renal failure in the predialysis stages with no known allergy or contraindication to Arabic gum or any of its ingredients.

The study comprised two groups: Group I (Treatment group): included 22 patients and received Arabic gum in addition to the conventional therapeutic intervention. Group II (Control group): included 19 patients who received conventional therapeutic intervention.

In the treatment group, patients received a daily dose of 50 gm of Arabic gum dissolved in water or natural juice equally divided into morning and evening dose of 25 gm. The used sample was subjected toxicological, biochemical and microbiological assessment in the National Nutrition Institute Laboratories and food science and Technology department, faculty of Agriculture, Tanta university. Both groups received low protein diet.

All patients were subjected to careful history taking, thorough clinical examination with particular notion to uremic fetor which was diagnosed if the patient had a urine-odor breath. Laboratory investigations included creatinine clearance, blood urea, serum creatinine, serum phosphorous, calcium, potassium and sodium, fasting blood sugar, blood bicarbonate, hemoglobin, hematocrit value and platelets count. All participants were followed monthly for 4 months.

Data obtained from the present study were computed using SPSS versions 17 under the platform of Microsoft Windows XP, Professional Edition. Continuous data were expressed in the form of mean ±

SD while categorical data were expressed in the form of count and percent. Comparison of continuous data were performed utilizing student t test, while categorical data were done using Chi-square test. P value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of the studied patients are shown in table-1. No significant differences were detected between groups regarding the laboratory data, causes of renal failure, presence of hypertension or gender distribution.

In the study follow up, treatment with gum Arabic resulted in progressive significant (P<0.002) increases of creatinine clearance in comparison with the control group (Table-2; Fig.1). Also serum creatinine decreased significantly in the treatment group than in control group (Table-3; Fig. 2). Mean blood urea level decreased significantly (P<0.001) at the end of four months than in the control group (Table-4; Fig.3).

Concerning electrolyte excretion, serum sodium decreased significantly (P<0.001) in treatment group than in the control group (Table-5; Fig.4). Also, serum potassium decreased significantly (P<0.001) in the treatment group than in control group (Table-5; Fig.4). In addition, Mean serum Calcium decreased significantly (P<0.001) in the study group than in control group (Table-6; Fig.5). Further, mean serum phosphorus decreased significantly (P<0.001) in the study group than in control group (Table-7; Fig.6).

It was also found that mean fasting blood sugar decreased significantly (P<0.001) in the study group than in control group (Table-8; Fig.7). Both hemoglobin and hematocrit increased significantly (P<0.001) in the study group than in control group (Tables 9 & 10; Fig. 8 & 9). Mean platelet count increased significantly in the third (P=0.023) and the fourth month (P=0.045) in the study group than in control group (Table-11; Fig. 10). However, no significant differences existed between serum bicarbonate levels in the studied groups.

Throughout the study period,

there was a progressive improvement of blood pressure in the treatment group in comparison (Fig. 11). Seven patients in the control group started hemodialysis, yet only one patient started dialysis in the treatment group because of symptomatic uremia.

Laboratory data at the end of the 4 months follow up period are shown in table-12. All data (except bicarbonate) showed significant change.

Table (1): Patient characteristics.

Characteristics	Mean ± SD		P
	Treatment (n=22)	Control (n=19)	
Age	41.68 ± 10.44	45.63 ± 9.6	NS
Height	171.55 ± 8.57	165.26 ± 6.2	NS
Blood Urea	123.31 ± 34.79	105.95 ± 13.37	NS
Creatinine clearance	15.82 ± 9.50	16.05 ± 8.01	NS
Serum Creatinine	4.80 ± 1.82	4.13 ± 0.98	NS
Serum Phosphorus	4.41 ± 0.22	3.72 ± 0.66	NS
Serum Calcium	10.31 ± 0.38	9.16 ± 1.29	NS
Potassium	4.40 ± 0.77	4.05 ± 0.57	NS
Serum Sodium	139.23 ± 2.37	135.61 ± 1.63	NS
Fasting Blood Sugar	120.50 ± 22.53	96.42 ± 8.41	NS
Blood Bicarbonate	26.21 ± 4.38	21.76 ± 4.54	NS
Hemoglobin	11.32 ± 1.73	10.94 ± 1.77	NS
Hematocrit	34.62 ± 6.03	34.93 ± 6.40	NS
Platelet count * 10^3	218.01 ± 83.00	237.5 ± 26.00	NS
○ Cause of renal failure			
• Hypertensive nephrosclerosis	16	19	NS
• Obstructive uropathy	1	0	NS
• FMF	1	0	NS
• Urate nephropathy	1	0	NS
• Uncertain	3	0	NS
○ Hypertension	15	7	0.045
○ Gender,			NS
Male	13	10	
Female	9	9	

Table-2, Fig.1: Mean Cr. clearance among four months.

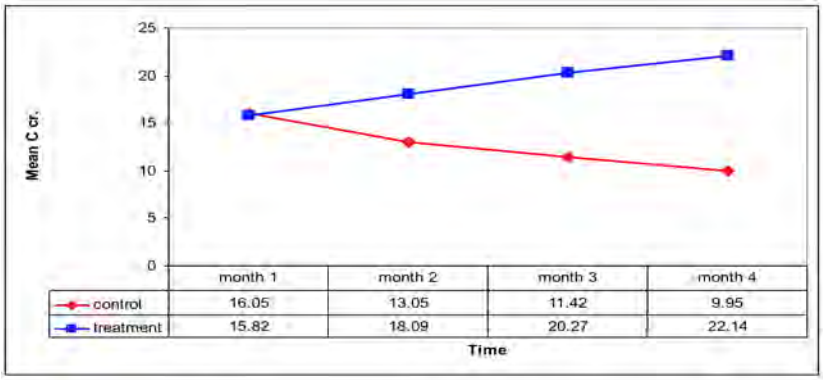

Table-3; Fig.2: Serum creatinine in the studied groups.

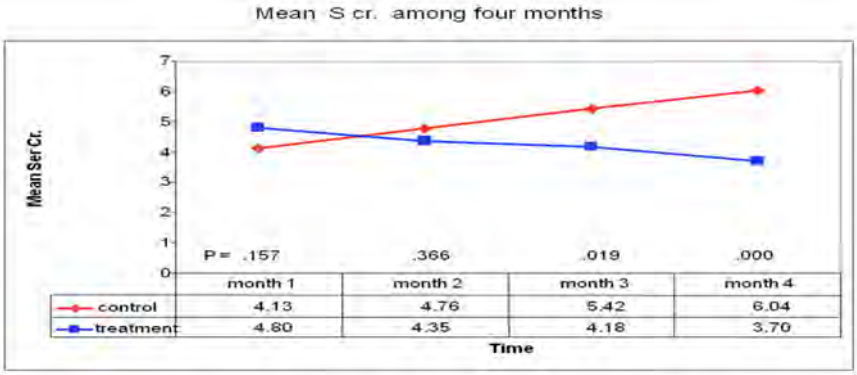

Table-4; Fig.3: Urea in the studied groups.

Table-5; Fig.4: Serum sodium in the studied groups.

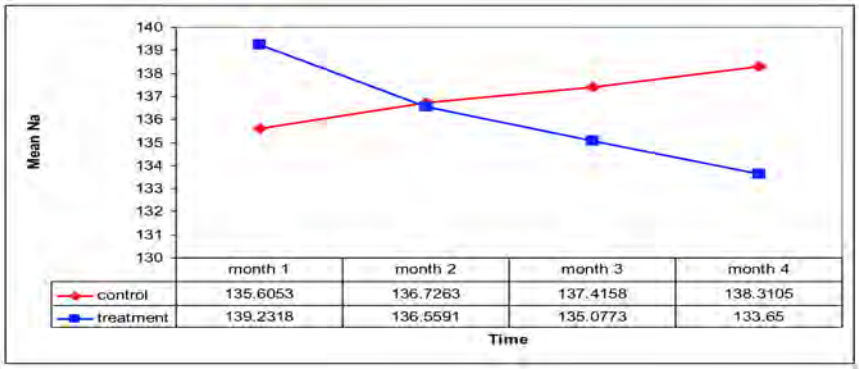

Table-6; Fig.5: Serum potassium in the studied groups.

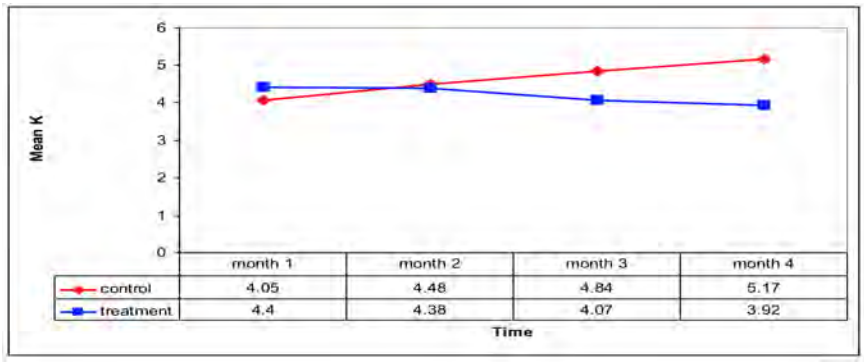

Table-7; Fig.6: Serum calcium in the studied groups.

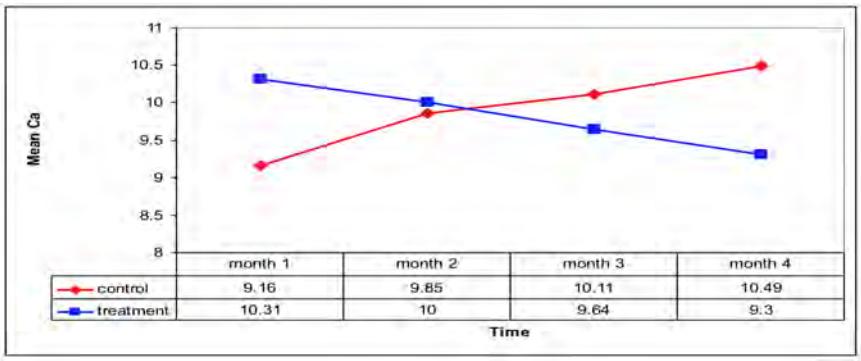

Table-8; Fig.7: Serum Phosphorus in the studied groups.

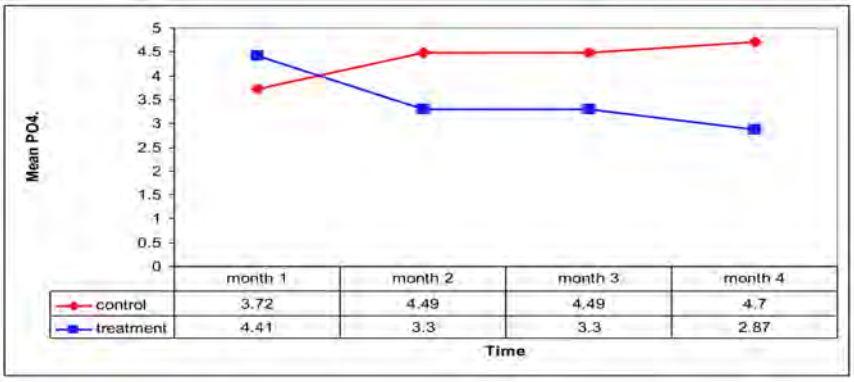

Table-9; Fig.8: Fasting blood sugar in the studied groups.

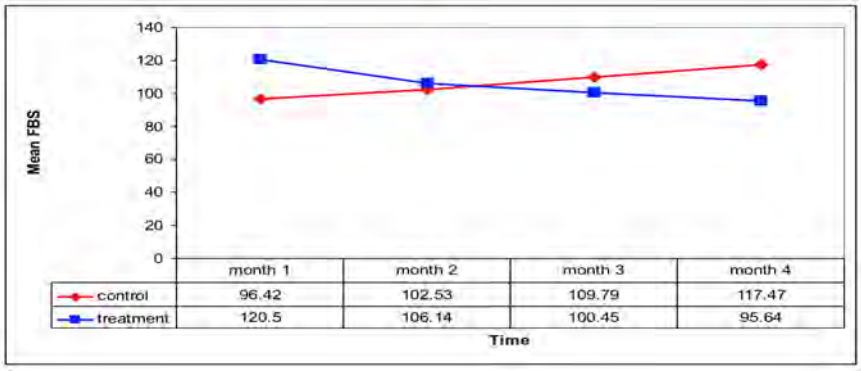

Table-10; Fig.9: Hb in the studied groups.

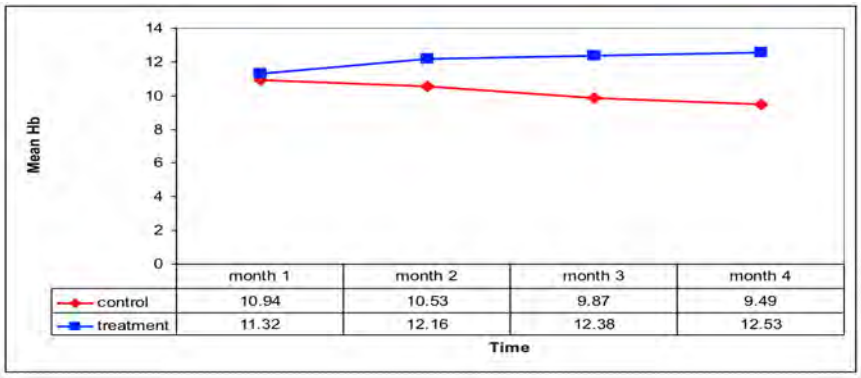

Table-11; Fig.10: Hematocrit value in the studied groups.

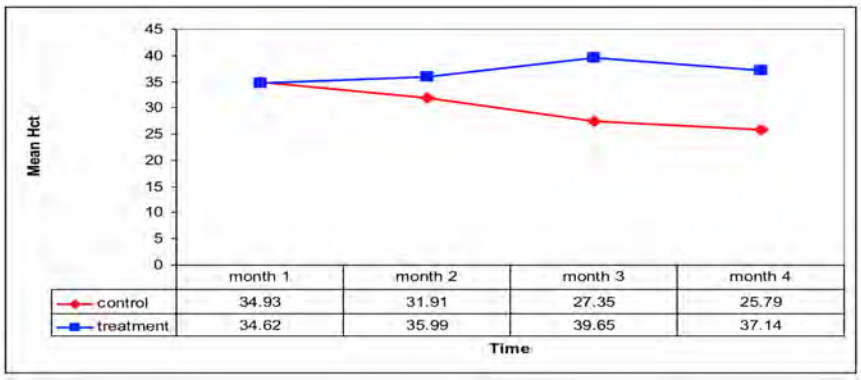

Table-12; Fig.11: Platelets count in the studied groups.

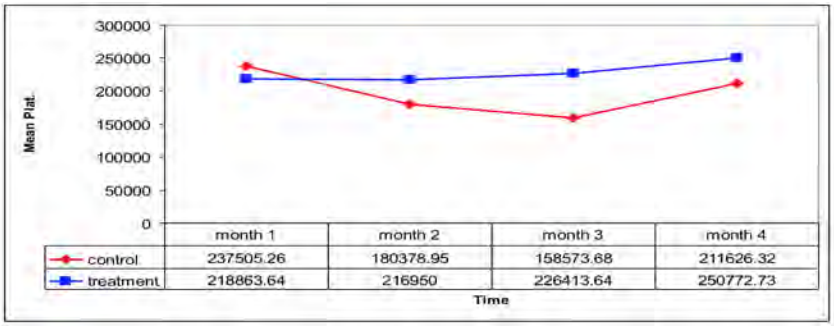

Fig. 11: Blood pressure in the studied groups.

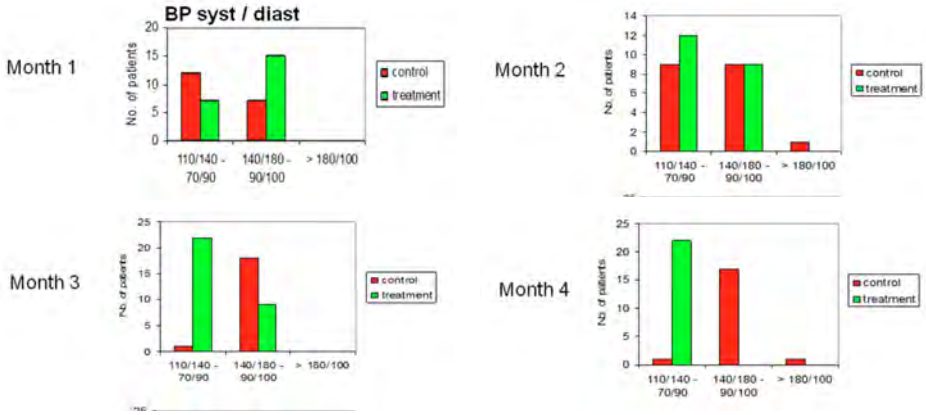

Table (12): Laboratory data after month 4.

	Mean ±SD		P
	treatment	control	
Urea	71.27 ± 23.16	171.42 ± 13.82	< 0.001
Ccr	22.14 ± 15.56	9.95 ± 3.34	0.002
S.Cr	3.70 ± 1.73	6.04 ± 1.18	< 0.001
PO4	2.87 ± 0.60	4.70 ± 0.39	< 0.001
CA	9.30 ± 0.51	10.49 ± 0.65	< 0.001
K	3.92 ± 0.60	5.17 ± 0.53	< 0.001
Na	133.65 ± 2.60	138.31 ± 1.49	< 0.001
FBS	95.64 ± 7.50	117.47 ± 8.14	< 0.001
HCO3	21.76 ± 1.95	23.27 ± 4.49	0.16
Hb	12.53 ± 1.65	9.49 ± 1.48	< 0.001
Hct	37.14 ± 5.31	25.79 ± 3.49	< 0.001
Plat * 10^3	250.00 ± 65	211.00 ± 54.6	0.045

Discussion

Dietary management is an essential element in the management of predialysis CRF and it can protect against disease progress and need of dialysis.(6)

This study was designed to investigate the effect of gum arabic treatment on the metabolic profile of chronic renal failure patients and also as a complementary conservative measure aimed at improving the quality of life.

Two groups of patients in different stages of chronic renal failure were selected. None of the patients was on dialysis. Both groups received low protein diet. The study group received 50 gm/day gum arabic divided into morning and evening dose of 25 gm. They were followed up monthly for four months. Both blood urea and creatinine significantly decreased, urea from 171 to 71 (P<0.001) and creatinine from 6.04 to 3.7 (P<0.001). Creatinine clearance also significantly increased from 9.95 to 22.14 ml/min (P<0.002). This finding can be explained by the reports that gum arabic increased fecal nitrogen excretion and decreased both urea production and urea nitrogen recycling.(7)

A study in animal models of experimental CRF showed that consumption of diets containing fermentable carbohydrates resulted in a greater fecal nitrogen excretion, coupled with a reduction in serum urea concentration.(8)

In Bliss et al.(9) reported that

patients with CRF on a low-protein diet (LPD) with 50 g gum arabic per day had a greater fecal nitrogen excretion and lower serum urea than did patients on only LPD.

Also, in the present study, serum sodium and serum potassium decreased significantly (P<0.001) than in the control group which is consistent with the finding of Ali et al.[5]

Mean serum calcium decreased significantly (P<0.001) in the study group than in control group in spite of calcium content of gum arabic which may be insufficient to treat hypocalcemia in chronic renal failure. GA has a high cation-binding capacity, particularly for calcium (Ca2+). Degradation of GA in the cecum releases the sequestered bile acids and the acidic pH generated during the fermentation process renders them insoluble. The bound calcium is also released and forms insoluble complexes with bile acids, thereby promoting their excretion.[10]

In addition, mean serum phosphorus decreased significantly (P<0.001) in the study group than in control group. The mean fasting blood sugar decreased significantly (P<0.001) in the study group than in control group (figure 8). Mixtures of different types of gum (not including GA) have been shown to inhibit glucose movement in vitro, and lower postprandial blood glucose and plasma insulin in human subjects when incorporated in a drink containing 50 g glucose.[11,12]

Infusion of meals containing starch showed that a decrease in the digestion rate of starch in the upper small intestine accounted for part of the effect of viscosity on glycemic response, whereas the main effect of gum was apparently to slow gastric emptying.[13]

Both hemoglobin and hematocrit increased significantly (P<0.001) in the study group than in control group. This may be explained by the increased gastrointestinal excretion of toxic nitrogenous compounds retained in chronic renal failure that suppress erythropoiesis by the bone marrow. Blood pressure control improved markedly by the end of four months as

shown also by decreasing antihypertensive medications. Patients in the study group reported improved well-being. Neither became acidotic or uremic, and neither required dialysis during the study period. They also reported disappearance of uremic fetor and better sleep.

In spite of these encouraging results, an important limitation of the present study is the short follow up period. In relation to the chronic and long-standing nature of the disease, a longer follow up period is warranted in future studies.

Conclusions

Nutritional supplementation with Gum Arabic in predialysis CRF patients results in general improvement of laboratory and clinical parameters with subsequent delay in disease progression and need of dialysis.

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NUTRITIONAL MANAGEMENT OF PREDIALYSIS RENAL FAILURE PATIENTS USING GUM ARABIC

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DIFFERENTIAL EXPRESSION OF P16 ^{INK4a}PROTEIN AND S100A4 PROTEIN IN GASTRIC CARCINOMA

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Abstract

Purpose: *Gastric cancer (GC) is an extremely common disease worldwide. The p16 ^{INK4a} protein, is a tumor suppressor protein that inhibits CDK4 and CDK6, which phosphorylate the RB protein. S100A4 is known to be involved in cancer cell motility by virtue of its ability to activate non-muscle myosin. The present study aims at investigating role of p16 ^{INK4a}, and S100A4 in progression of gastric carcinoma by analyzing p16 ^{INK4a} and S100A4 protein expression in gastric carcinomas and correlating their expression with clinicopathological findings.*

Patients and Methods: *Forty cases including 30 non-consecutive retrospective selected gastric carcinomas (4 GI, 16 GII, 10 GIII), and 10 cases of normal gastric tissue at resection margins of peptic ulcers, were taken as control. Cases were collected in the period 2009-2012, selected from files of pathology department, Faculty of Medicine- Benha University and Egyptian National Cancer Institute (NCI). Follow up of the selected cases was recorded for 18 months. Correlations between S100A4 and p16 ^{INK4a} immunoreactivity and clinicopathological characteristics were evaluated.*

Results: *showed significant inverse correlation between p16 ^{INK4a} expression and grade of carcinoma ($P<0.05$) as well as high significant correlation with type of gastric carcinoma ($P<0.01$). S100A4 was positively correlated with tumor grade, lymph node metastasis, distant metastasis and tumor-node-metastasis (TNM) staging ($P<0.05$). S100A4 expression was also significantly correlated with poor patient survival. The worst survival was correlated to cases with low p16^{INK4a}/high S100A4 expression ($P<0.01$).*

Conclusion: *The immunohistochemical expression of both p16^{INK4a}*

and S100A4 in gastric carcinoma is associated significantly with tumor grade. Expression of S100A4 is significantly associated with lymph node and distant metastases, and poor prognosis. Estimation of both markers can be used in planning the therapy and patient's follow up.

Key words: *p16INK4a, S100A4, tumor suppressor gene (TSG), gastric carcinoma (GC), immunohistochemistry (IHC).*

Introduction

Gastric cancer is the fourth most common cancer in the world with relative frequency of 7.8% of all cancers. It is the second leading cause of cancer-related mortality worldwide, accounting for 11.3% of cancer deaths (de Martel C et al., 2012). In Egypt, cancer stomach is in the eleventh rank constituting 2.1% of all cancers with male to female ratio 1.55 and median age of 53 year (El-Bolkainy et al., 2013). However, the worldwide incidence of gastric cancer has declined rapidly over the recent few decades. Part of the decline may be due to the recognition of certain risk factors such as H. pylori and other dietary and environmental risks (Hannah, et al; 2012).

The current staging classifications of gastric carcinomas do not produce accurate predictions of patient outcomes. Molecular biomarkers may account for this diversity and several prognostic factors have been identified (Fareed, et al; 2009). However, none of these methods have been proven to be robust enough to be incorporated into routine practice.

A critical point in the cell cycle is the G1/S transition checkpoint which is frequently altered in tumors. This is controlled by cyclins and cyclin-dependent kinases (CDK), which complex to induce the progression of cells into the S-phase by phosphorylating retinoblastoma protein. INK4 comprising $p16^{INK4a}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$ are family of CDK inhibitors and binds specifically to CDK4 and CDK6, thereby preventing kinase activities (Hanan, et al; 2009).

P16, a 156-amino acid protein, is encoded by the INK4A (CDKN2A, MTS1) gene located on chromosome 9p21. It exerts its function by competing with cyclin

D in binding to CDK4 preventing the activation of this kinase and inhibiting its productive interaction. Thus, p16 protein can specifically associate with CDK4 and disrupt the formation of active kinase complexes preventing transition of cells from G1 to S phase (Osanai, et al; 2011). Therefore, inactivation of $p16^{INK4a}$ leads to activation of cyclin/CDK complexes, resulting in cell cycle progression. Alterations of $p16^{INK4a}$ gene are known to occur in many primary tumors (Hannah, et al; 2012).

S100A4 protein is the well-studied member of S100 family. It is localized in the nucleus, cytoplasm, and extracellular space and possesses a wide range of biological functions. It appears to take part in the homeostasis of growth with apparent involvement in growth factor signal transduction and apoptotic cell death (House et al., 2011). Overexpression of the S100 calcium-binding protein A4(S100A4) is involved in epithelial-to-mesenchymal transition, oncogenic transformation, angiogenesis, cytoskeletal integrity and cancer metastasis. (Cabezo´n et al., 2007 & Stein, et al, 2011 and Zhao, et al, 2013). S100A4 is also named as metastasis-associated Mts1 and fibroblast-specific protein 1 (FSP1) because of its close association with cancer metastasis and constitutive expression in fibroblasts (Malashkevich et al., 2008). Therefore, S100A4 protein expression is associated with patient outcome in a number of tumor types (House et al., 2011). The present study aims at investigating role of p16 INK4a, and S100A4 in progression of gastric carcinoma by analyzing p16 INK4a and S100A4 protein expression in gastric carcinomas and correlating their expression with clinicopathological findings.

Materials and Methods

Clinical investigations and tissue samples: The present study was based on 40 cases including ten cases of normal gastric tissue at resection margins of peptic ulcer (non-neoplastic lesion) were taken as control and 30 non-consecutive retrospective selected gastric carcinomas. Carcinoma cases included 18 cases of adenocarcinoma, 6 cases of mucinous carcinoma and 6 cases of signet

ring carcinoma. Cases were collected in the period 2009-2012. They were selected from files of pathology department, Faculty of Medicine- Benha University and Egyptian National Cancer Institute (NCI). Cases were selected according the availability of clinical and follow-up data for at least 18 months. Only patients with primary gastric cancer who had not undergone previous irradiation or chemotherapeutic treatment were included in the study.

Staging was carried out according to the TNM Classification System (Rosai, 2011).

Formalin-fixed, paraffin-embedded gastric tissues were used. Three sections of 4 micron thickness were obtained from each case. One section was H & E stained for diagnosis and grading. Two experienced pathologists blindly and independently confirmed the histological diagnosis of each gastric lesion and agreed on the grading. Other sections were mounted on positively- charged slides, immunohistochemically stained with antibodies against S100A4 and p16 INK4a using the Ultra Vision Detection System (Anti-polyvalent, HRP/DAB, ready-to-use, Lab Vision corporation).

Immunohistochemical staining: tissue sections mounted on positively- charged slides, were heated at 60℃ for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining. After antigen retrieval with microwave treatment in 10mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6.0, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 minutes. Sections were washed 3 times with cold 0.01 M phosphate buffered saline (PBS). After blocking with 10% normal rabbit serum, sections were incubated with polyclonal antibody against $p16^{INK4a}$ (clone 16P07 Neomarkers; dilution 1:100) and prediluted ready to use S100A4 (Neomarker, LAB VISION, USA). Both were incubated overnight at 4° C. The prepared DAB-substrate-chromogen solution was applied and incubated for 5-15 minutes until color intensity has been reached. Lastly, sections were counterstained with Mayer's Hematoxylin. Samples of endometrial

adenocarcinomas known to over-express p16 ^{INK4a} were used as positive controls. A tissue section of breast cancer was used as positive control for S100A4. Negative controls were performed by omitting the primary antibody step.

Interpretation and evaluation of $p16^{INK4a}$ immunohistochemical staining: The stains were interpreted as positive if there was distinct diffuse cytoplasmic or nuclear reactivity in the neoplastic cells, greater than any background staining in the matched non-neoplastic gastric tissue, in more than 20% of the cells within the different cases (Chen, et al, 2013).

Interpretation and evaluation of S100A4 immunohistochemical staining: S100A4 was stained yellow-brown in the cytoplasm and nucleus. The degree of immunostaining based on the staining intensity and percentage of positive cells. The intensity grading scale was according to the following criteria: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining yellow-brown) and 3 (strong staining, brown). Moderate and strong staining indicated tumors with high S100A4 expression, while no and weak staining indicated low S100A4 expression (Li, et al, 2013).

Statistical analysis: Statistical analysis was performed using the SPSS (version 16.0 for windows) software package according to Sperman's correlation coefficient. Correlation between several variables was computed using Fisher's exact test. P value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.

Results

A- Immunohistochemical results of $p16^{INK4a}$ staining":

All 10 cases (100%) of normal gastric tissue showed positive cytoplasmic expression of $p16^{INK4a}$, while among 30 cases of gastric carcinoma, 13 cases (43.3%) were positive and 17 cases (56.7%) were negative. This difference between expression of $p16^{INK4a}$ in normal and malignant neoplastic gastric tissue was statistically highly significant (P<0.01) (Fig 1).

As regarding the type of cancer,

11 cases (61.1%) of adenocarcinoma were positive for $p16^{INK4a}$, while all cases (100%) of signet ring carcinoma showed negative expression. Out of 6 mucinous carcinomas, only 2 cases (33.3%) were positive (P<0.01).

In relation to the tumor grade, all cases (100%) of gastric carcinoma grade 1 showed positive $p16^{INK4a}$ expression. Among cases of grade II, 7 out of 16 cases (43.75%) were positive, and 9 cases (56.25%) were negative. Among the 10 cases of gastric carcinoma GIII, only 2 cases (20%) were positive for $p16^{INK4a}$ expression and 8 cases (80%) were negative and this relationship is statistically highly significant (P<0.05) (Fig 2).

All stage I cases (100%) showed positive $p16^{INK4a}$ expression, while only one case (12.5%) of stage II was positive. Six cases (75%) of stage III and 2 cases (20%) of stage IV were positive for $p16^{INK4a}$ expression, this relationship is statistically insignificant. (P>0.05).

- Concerning to state of lymph node metastasis, 4 out of the 10 lymph node negative cases (40%) showed positive $p16^{INK4a}$ expression, while positivity was seen in 9 of the 20 lymph node positive cases (45%). This relationship was statistically insignificant (P>0.05).

Insignificant correlation was also detected between $p16^{INK4a}$ expression and distant metastasis (P>0.05). Eleven (55%) out of 20 metastasis free cases were positive for $p16^{INK4a}$ expression and 2 (20%) out of 10 cases with distant metastasis were positive.

Correlation with patient survival was also statistically insignificant (P>0.05). Half of the cases of living patients (50%) showed positive $p16^{INK4a}$ expression and 5 dead cases were positive (35.7%). (Table 1).

(B) Immunohistochemical results of S100A4 staining:

All of the 10 cases of normal gastric tissue (100%) showed low cytoplasmic expression of S100A4, while 23 cases (76.7%) of gastric carcinoma showed high expression. This difference was highly statistically significant (P<0.01) (Fig 1).

As regarding the type of carcinoma, 12 cases (66.7%) of adenocarcinoma high S100A4 expression, while all cases (100%) of signet ring carcinoma showed low expression. Among 6 cases of mucinous carcinoma only 1 case (16.7%) was reported with low expression of S100A4 ($P<0.05$).

In relation to the tumor grade, the expression of S100A4 was increased together with loss of differentiation. Only one case (25%) of grade 1 showed high expression while 12 cases of G2 (75%) and all cases of G3 (100%) showed high expression, and this relationship is statistically highly significant ($P<0.05$) (Fig 3).

As regarding the stage of gastric carcinoma, one case (25%) of stage I showed high expression. In stage II and III, high S100A4 expression was shown in 6 cases (75%). All cases (100%) of stage IV cases showed high S100A4 expression. This relationship is statistically highly significant. ($P<0.05$).

- Concerning the state of lymph node, S100A4 expression is markedly among cases with positive L.N. metastases. All of these cases, (100%) showed high expression. In the 10 lymph node negative cases (70%) showed low S100A4 expression. This relationship was statistically highly significant ($P<0.01$).

- All cases (100%) of those with distant metastasis showed high expression. In absence of distant metastasis, 13 cases (65%) showed high expression. A statistically significant correlation was found between S100A4 expression and occurrence of distant metastasis ($P<0.05$).

Regarding patient survival, the expression of S100A4 was related to poor prognosis. All of dead cases (100%) showed high expression, while 9 cases (56.25%) of living patients showed high expression. This was a statistically highly significant relationship ($P<0.01$). (Table 2).

(C) Correlation between $p16^{INK4a}$ & S100A4 expression in gastric carcinoma:

Carcinoma cases were classified into 4 groups according to the

expression of both $p16^{INK4a}$ & S100A4. The worst prognosis was correlated to the group of Low $p16^{INK4a}$/high S100A4. This correlation was highly statistically significant ($p<0.01$). The group of high $p16^{INK4a}$/low S100A4 showed good prognosis and this correlation was also statistically significant ($p<0.05$) (Table 3).

Table (1): Correlation between $p16^{INK4a}$ expression and clinicopathological data in examined cases.

Clinicopathological variable	*Total*	*$p16^{INK4a}$ expression*		*P value*
		Negative	*Positive*	
Type of tissue examined				
Control	**10**	**0**	**10 (100%)**	**P<0.01**
Gastric carcinoma	**30**	**17 (56.7%)**	**13 (43.3%)**	
Type of carcinoma				
-Adenocarcinoma	**18**	**7 (38.9%)**	**11 (61.1%)**	**P<0.01**
- Mucinous carcinoma	**6**	**4 (66.7%)**	**2 (33.3%)**	
-Signet ring carcinoma	**6**	**6 (100%)**	**0**	
Tumor Grade:				
G I	**4**	**0**	**4 (100%)**	**P<0.05**
G II	**16**	**9 (56.25%)**	**7(43.75%)**	
G III	**10**	**8 (80%)**	**2 (20%)**	
TNM stage:				
Stage 1	**4**	**0**	**4 (100%)**	
Stage 2	**8**	**7 (87.5%)**	**1 (12.5%)**	**P>0.05**
Stage 3	**8**	**2 (25%)**	**6 (75%)**	
Stage 4	**10**	**8 (80%)**	**2 (20%)**	
L.N. metastasis:				
Negative	**10**	**6 (60%)**	**4 (40%)**	**P>0.05**
Positive	**20**	**11 (55%)**	**9 (45%)**	
Distant metastasis:				
Negative	**20**	**9 (45%)**	**11(55%)**	**P>0.05**
Positive	**10**	**8 (80%)**	**2 (20%)**	
Patient's survival:				
Alive	**16**	**8 (50%)**	**8 (50%)**	**P>0.05**
Dead	**14**	**9 (64.3%)**	**5 (35.7%)**	
Total	**40**	**17**	**23**	

NB: There is a highly significant correlation between type of carcinoma and $p16^{INK4a}$ expression ($P<0.01$), also a significant inverse correlation between $p16^{INK4a}$ expression and tumor grade ($P<0.05$).

Table (2): Correlation between S100A4 expression and clinicopathological data in examined patients.

Clinicopathological variable	*Total*	*S100A4 expression*		*P value*
		Low	*High*	
Type of tissue examined				
Control	**10**	**10 (100%)**	**0**	**P<0.01**
Gastric carcinoma	**30**	**7 (23.3%)**	**23 (76.7%)**	
Type of carcinoma				
-Adenocarcinoma	**18**	**6 (33.3%)**	**12 (66.7%)**	**P<0.05**
- Mucinous carcinoma	**6**	**1(16.7%)**	**5 (83.3%)**	
-Signet ring carcinoma	**6**	**0**	**6 (100%)**	
Tumor Grade:				
G I	**4**	**3 (75%)**	**1 (25%)**	**P<0.01**
G II	**16**	**4 (25%)**	**12 (75%)**	
G III	**10**	**0**	**10 (100%)**	
TNM stage:				
Stage 1	**4**	**3 (75%)**	**1 (25%)**	
Stage 2	**8**	**2 (25%)**	**6 (75%)**	**P<0.01**
Stage 3	**8**	**2 (25%)**	**6 (75%)**	
Stage 4	**10**	**0**	**10 (100%)**	
L.N. metastasis:				
Negative	**10**	**7 (70%)**	**3(30%)**	**P<0.01**
Positive	**20**	**0**	**20 (100%)**	
Distant metastasis:				
Negative	**20**	**7 (35%)**	**13 (65%)**	**P<0.05**
Positive	**10**	**0**	**10 (100%)**	
Patient's survival:				
Alive	**16**	**7 (43.75%)**	**9 (56.25%)**	**P<0.01**
Dead	**14**	**0**	**14 (100%)**	
Total	**40**	**17**	**23**	

NB: A highly significant correlation between S100A4 expression and grade of the carcinoma, TNM stage, L.N. metastasis, as well as patient survival (P<0.01). Also there is a significant correlation between S100A4 expression and each of carcinoma type and distant metastasis (P<0.05).

Table (3): The correlation between patient survival and the expression of $p16^{INK4a}$ & S100A4.

Patient Categories	*Total*	*Survival*	
		Alive	*Dead*
Low $p16^{INK4a}$/low S100A4	**3**	**3**	**0**
Low $p16^{INK4a}$/high S100A4	**14**	**4**	**10**
High $p16^{INK4a}$/low S100A4	**4**	**4**	**0**
Highp16^{INK4a}/high S100A4	**9**	**5**	**4**
Total	**30**	**16**	**14**

NB: A highly statistically significant correlation was found between survival and low $p16^{INK4a}$/high S100A4 (p<0.01) and between survival and high $p16^{INK4a}$/low S100A4 (p<0.05).

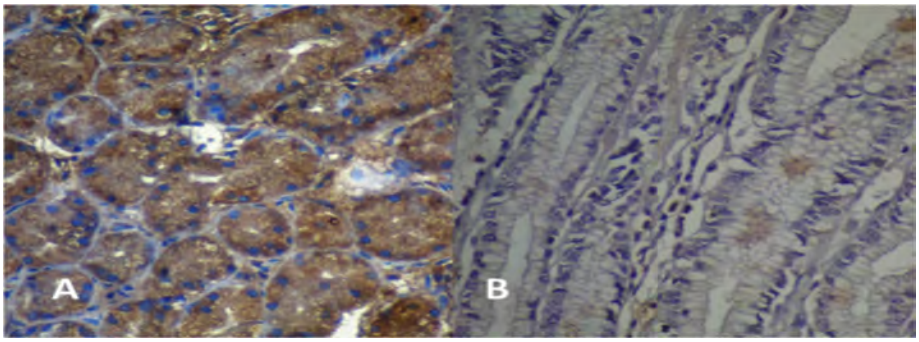

Fig. 1: normal gastric mucosa: **A)** positive $p16^{INK4a}$ expression all-over the stained cells, **B)** negative S100A4 expression all-over the stained cells (IHC X 400).

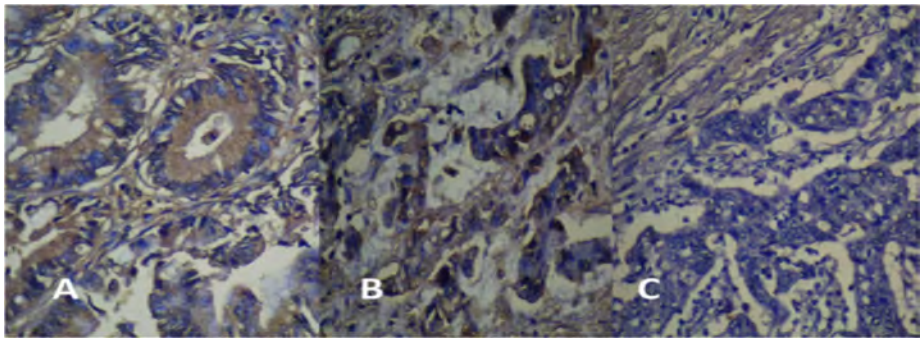

Fig. 1: $p16^{INK4a}$ expression in gastric carcinoma, **A)** grade I gastric carcinoma, positive cytoplasmic expression in all malignant glands in the field. **B)** GII gastric carcinoma, positive expression in <20% of malignant cells. **C)** GIII gastric carcinoma, completely negative expression of $p16^{INK4a}$ (IHC x 400).

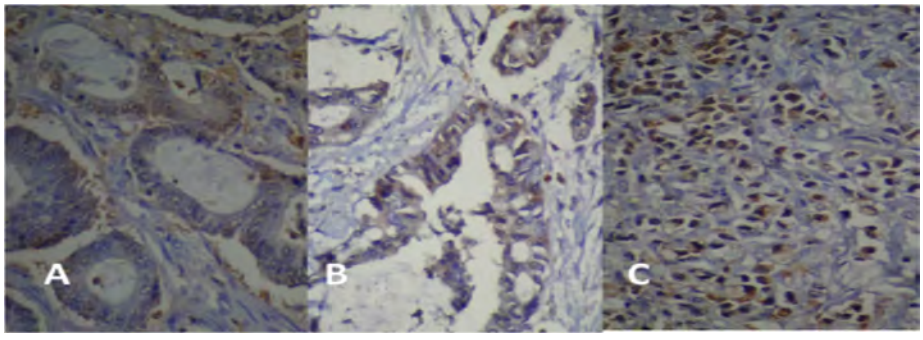

Fig. 1: S100A4 expression in gastric carcinoma: **A)** grade I gastric carcinoma, negative expression in all malignant glands in the field. **B)** GII gastric carcinoma, positive cytoplasmic expression in <20% of malignant cells. **C)** GIII signet ring carcinoma, highly positive cytoplasmic expression of S100A4 (IHC X 400).

Discussion

Recent research has revealed a rapid increase in the number of alterations underlying oncogenesis and the proteins which regulate the cell cycle. The protein p16 is a cell cycle regulator acting as a cyclin-dependent kinase inhibitor (CDKI). Because of its antiproliferative effect, p16 has been suggested to be a tumor suppressor gene. Deletions, mutations and functional inactivation of p16 occur with a frequency second only to p53 in most human malignancies (Argyris, et al; 2013).

Here it was reported that $p16^{INK4a}$ expression in non-neoplastic and neoplastic gastric tissues is completely different. All cases (100%) of normal gastric mucosa showed positive expression of p16INK4a in comparison to (43.3%) of carcinoma cases which were positive to $p16^{INK4a}$, and this relation was statistically highly significant ($P<0.01$).

These results were in agreement with Osanai, et al; (2011) who found that the immunohistochemical expression of p16 was observed in only 32.4% of the carcinoma cases. Xiu-Sheng, et al; (2001) found that the positive rate of P16 protein expression in gastric carcinoma was significantly lower than that in normal gastric mucosa and dysplastic gastric mucosa ($P<0.05$).

On the contrary, Tsujie, et al; (2000) who reported that less than 10% of non-tumor gastric mucosal cells were $p16^{INK4a}$ positive, whereas the expression of $p16^{INK4a}$ in gastric cancer cells varied widely from 0 to 100% (mean, 24.5%). Also Rocco, et al; (2002) found that in non-cancerous gastric tissues the immunostaining of p16 was weak and limited to antral glands. Lesnikova, et al; (2009) found that $p16^{INK4a}$ expression was not seen in normal cervix tissue. Similarly Zhao, et al; (2003) reported that the frequency of loss of P16 protein expression in the gastric cancer tissue, adjacent non-tumor tissue, and distal normal tissue was 77.5%, 55.0%, and 17.5%, respectively ($P<0.005$). This difference in the results may be attributed to difference in the type or grade of carcinoma cases in each study.

In the current study, a significant inverse correlation ($p<0.05$) between p16INK4a expression and grade of gastric carcinoma cases was found, all cases of grade I, (43.75%) of grade II, and (20%) of grade III gastric carcinoma cases showed positive $p16^{INK4a}$ expression. These results were parallel to results reported by Tsujie, et al; (2000) who reported a clinicopathologic survey indicated that a low or no expression of $p16^{INK4a}$ was associated with poorly differentiated carcinoma ($p=0.0133$). Rocco, et al; (2002) found that the intensity of immunostaining was inversely related to the grade of differentiation of these tumors. The loss of expression of $p16^{INK4a}$ in high grades of carcinomas means loss of its antiproliferative activity. This may be an important factor in uncontrolled gastric cell proliferation and progression of the tumor towards high grades.

On the contrary to the current results Osanai, et al; (2011) reported that There was no statistically significant relationship between the immunohistochemical expression of p16 and the degree of histological differentiation of tumor, when analyzed the relation to immunoreactivity score ($p=0.81$). Also, Xiu-Sheng, et al; (2001) reported that the positive rate of P16 protein expression in mucoid carcinoma 10.00% was significantly lower than that in poorly differentiated carcinoma 51.22%, undifferentiated carcinoma 57.69% and signet ring cell carcinoma 62.50% ($P<0.05$). The key differences between this study and similar previously reported IHC studies are firstly, the number of cases analyzed; secondly the absence of p16 in the lowest grade of differentiation may reflect clonal expansion of the cells with a more aggressive phenotype.

As regarding type of carcinoma, positive $p16^{INK4a}$ expression was reported in 61.1% of adenocarcinomas, 33.3% of mucoid carcinomas, while all cases of signet ring carcinoma were negative. This was a highly statistically significant correlation ($p<0.01$). These results could be explained by the behavior of different types of gastric carcinomas; 66.7% of mucinous carcinomas which are considered a less differentiated carcinomas and

signet ring carcinomas which are always considered a high grade cancer, and this explains their negative expression for p16 which is found to be negative in high grade tumors.

Rocco, et al, (2002) reported that the overexpression of p16 seems to be a common event in the development of both intestinal and diffuse type of gastric cancer and it is likely that it may be driven by features of the neoplastic state. Also, Xiu-Sheng He, et al, (2001) reported that the positive rate of P16 protein expression in mucoid carcinoma 10.00% was significantly lower than that in poorly differentiated carcinoma 51.22%, undifferentiated carcinoma 57.69% and signet ring cell carcinoma 62.50% (P<0.05).

Regarding lymph node metastasis, positive $p16^{INK4a}$ expression was reported in 40% of cases with absent lymph node metastases and in 45% of cases with positive lymph node metastases. This relationship was statistically insignificant (P>0.05).

Concerning distant metastases in this study, 80% of gastric carcinomas with distant metastases were negative to $p16^{INK4a}$ expression and 55% of cases without distant metastases were positive to p16INK4a. This relationship was also statistically insignificant (P>0.05).

As regard the stage of gastric carcinoma, all stage I cases (100%) showed positive $p16^{INK4a}$ expression, while (12.5%) of stage II cases were positive, (75%) of stage III cases and (20%) of stage IV cases were positive for $p16^{INK4a}$ expression, this relationship is statistically insignificant (P>0.05).

These results were supported by results reported by Osanai, et al; (2011) who reported that Statistical analysis showed no significant relationship between staging and $p16^{INK4a}$ protein expression (p=0.485).

In this study, the correlation between $p16^{INK4a}$ protein expression and patient's survival was statistically insignificant (p>0.05). This agrees with Tsujie, et al, (2000), who reported that the level of p16 expression did not correlate with patients' prognosis. Chen, et

al, (2013) also did not identify a correlation between p16 levels and patient survival.

Different studies showed that S100A4 plays a role in tumor growth, motility and invasion suggesting that it is directly linked to the progression of human carcinoma as in colorectal carcinoma (Boye et al., 2010) and in prostatic carcinoma (Yong-Wook et al., 2010). In this study, it was reported that all cases (100%) of non-neoplastic gastric tissue showed low S100A4 expression while (76.7%) of gastric carcinoma cases showed high expression of S100A4 and this difference was statistically significant (P<0.01).

This agrees with Yonemura, et al, (2000) who reported that S100A4 expression was detected in 51 (55%) of 92 primary gastric cancers and Li, et al, (2013) who reported that 53 (62.35%) of gastric carcinoma cases exhibited S100A4 overexpression, in which immunostaining was observed in the cytoplasm or the nucleus of the tumor cells.

In relation to the tumor grade, the expression of S100A4 was increased together with loss of differentiation. (25%) of grade 1 showed high expression, (75%) of G2 and all cases of G3 (100%) showed high expression, and this relationship is statistically highly significant (P<0.05). This agreed with Yonemura , et al, (2000) who reported a strong relationship between S100A4 expression and histological differentiation of gastric adenocarcinomas. In their meta-analysis on colorectal carcinoma, Liu, et al, (2013) detected a higher S100A4 expression with poor differentiation. This could be explained by the highly variable feature of S100A4 expression which might indicate the influence(s) of cell cycle regulators, and especially epigenetic factor(s) in the transcription of this gene.

Concerning the state of lymph node metastasis, we found that all cases with positive L.N. showed high S100A4 expression. In the 10 lymph node negative cases, 7 cases (70%) showed low expression. This relationship was statistically highly significant (P<0.01).

Also in this study we reported

strong S100A4 expression in 100% of cases with distant metastasis. In absence of distant metastasis, (65%) showed low S100A4 expression. A statistically significant correlation was found between S100A4 expression and occurrence of distant metastasis ($P<0.05$).

In this work, it was found that the S100A4 expression is increased in relation to the stage of gastric carcinoma. (75%) of stage I showed low expression while (100%) of stage IV cases showed high expression. This relationship is statistically highly significant. ($P<0.01$).

Comparing to other studies, Li, et al, (2013) reported that S100A4 overexpression was closely associated with the gastric LN metastasis ($P=0.000$) and distant metastasis ($P=0.024$). Zhao, et al, (2013) found that gastric S100A4 was positively correlated with lymph node metastasis and tumor-node-metastasis (TNM) staging ($P<0.05$). The same were the results of Wang, et al, (2010) who reported that gastric Expression of S100A4 in gastric cancer is associated significantly with lymph node and distant metastases

In the meta-analysis on colorectal cancer done by Liu, et al, (2013), the results suggested a significant association between high S100A4 expression and advanced TNM stage, as well as the presence of lymph node metastasis. Pooled data also suggested an evident trend towards higher S100A4 expression with poor differentiation and distant metastases.

As regarding endometrioid carcinoma, Xie, et al, (2007) reported that S100A4 expression was significantly higher in stage III and IV tumors compared with stage I.

These results could be explained by the function of S100A4. It stimulates cell motility, invasion, angiogenesis and participates in the regulation of cell death. Invasion and motility is probably promoted through induction of epithelial to mesenchymal transition EMT. Cell motility, invasion, and angiogenesis all contribute to stimulation of metastasis. S100A4 enhances the turnover of

myosin IIA filaments at the leading edge of migrating cells, resulting in increased motility, which could contribute to an increased metastatic capacity of cancer cells, Then S100A4-stimulated plasmin activation may also contribute to the observed activation of MMP-2 and MMP- 13 which help tumor cells to travel through the surrounding stroma (Boye and Mælandsmo, 2010).

In this study, it was found that the expression of S100A4 was related to poor prognosis. All of dead cases (100%) showed high expression, while (43.75%) of living patients showed low expression. This was a statistically highly significant relationship ($P<0.01$).

In their studies on gastric carcinoma, Zhao, et al, (2013) & Wang, et al, (2010) reported S100A4 as a marker for poor prognosis. Also Yonemura, et al, (2000) reported that patients with S100A4-positive tumors survived significantly poorer than did those with S100A4-negative tumors

On other studies Liu, et al, (2013) detected that more than twelve studies investigated the relation between S100A4 and patient survival and practically demonstrated a significant association between S100A4 overexpression and worse prognosis in CRC patients. Stein, et al (2011) study was carried upon both colorectal and gastric cancer and they reported that a high S100A4 expression correlates with aggressive tumor growth and poor prognosis in colorectal cancer. Overexpression of S100A4 is also related to aggressiveness and metastasis in gastric cancer. This correlation to poor prognosis could be explained by the strong correlation between high S100A4 levels and advanced tumor stage.

On the contrary, in their study, Pedersen, et al (2002) did not find any association between the expression levels of S100A4 and clinical outcome. But they thought that this conflict occurred because they investigated snap-frozen, acetone-fixed tumor biopsies, while others examined archival formalin-fixed, paraffin-embedded specimens. Such differences in preservation and fixation could possibly affect the results.

The classification of patients into 4 groups according to expression of both markers reveals that the worst prognosis was related to the group of low $p16^{INK4a}$/ high S100A4 expression and this was highly statistically significant ($p<0.01$). This could be explained by the relation between advanced tumor stage and high S100a4 levels plus the relation between the negative p16INK4a and loss of tumor differentiation.

In conclusion, this study suggests that $p16^{INK4a}$ is related to the differentiation of the gastric carcinoma while S100A4 upregulation is positively associated with the growth, invasion, metastasis and differentiation of gastric carcinomas. $p16^{INK4a}$ and S100A4 may be useful markers to predict progression of gastric carcinoma, while S100A4 may be a promising marker for detection of progression, aggressive behavior and prognosis of gastric carcinomas. Estimation of both markers could be valuable in planning the therapy and patient's follow up.

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CONTENTS