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A PROSPECTIVE RANDOMISED TRIAL COMPARING RHOMBOID EXCISION AND LIMBERG FLAP CLOSURE VERSUS KARYDAKIS FLAP RECONSTRUCTION FOR TREATMENT OF SACROCOCCYGEAL PILONIDAL DISEASE

Mohamed E. Abellatif MD, Nashaat Noaman MD, Ahmad Negm MD, Ashraf Abass MD, Amro El-Hadidy MSc, Gamal El-Morsy MD and Ramadan Ellithy MD

Department of General Surgery, Faculty of Medicine, Mansoura University, Egypt

Abstract

Background And Objective: *Pilonidal disease is common among adults and carries a great morbidity in terms of high recurrence rate and patient discomfort. A gold standard treatment has not yet been established.*

Settings: *This study took place in Mansura University Hospital, Faculty of Medicine*

Patients: *from February 2008 to March 2010, Patients were randomly assigned to undergo either Limberg rhomboid flap or Karydakis flap reconstruction. The follow-up period ranged from 8 months to two years, with the mean follow-up period about 18 months. Surgical findings, complications, recurrence rates and degree of patient satisfaction were compared.*

Main Outcome Measures: *The primary outcome measure in this study was the postoperative complications including early wound infection and wound failure*

Results: *There were120 patients (115 males and 5 females) with a median age of 22 years (range, 18-40 years). There were no significant differences between the two groups in terms of complication rate, length of hospital stay, or recurrence rate. Operative time was longer in the*

Limberg rhomboid flap group. Patients in the Karydakis group reported feeling completely healed more quickly postoperatively. There was significant difficulty with defecation in the Karydakis group in comparison to Limberg group. The two groups reported similar rates of satisfaction.

Conclusions: *The Limberg rhomboid flap technique and the Karydakis procedure appear to generate comparable outcomes.*

Keywords: *Pilonidal disease, surgical treatment, Limberg, Karydakis*

Introduction

Pilonidal disease is a common surgical entity which represents a wide range of patients attending the outpatient's surgical clinics. The incidence appears to be high especially among young men and some estimated it is about 8.8% in the army recruited youth[1,2]. Although the debate which still present about the etiological nature of the disease, many authors believed in the theory stated that it is an acquired disease and its occurrence can be largely affected by the habitual and job nature of the sufferers.

Many surgical and non surgical modalities were described for treatment of saccrococygeal pilonidal disease (SPD); these modalities represented the management dilemma which ranged from no treatment at all or conservative treatment (such as painstaking clearance and hair control by shaving or depilation with topical agents) to a more extensive surgery with flap transposition[3]. This surgical challenging appears due to the nature of postoperative complication facing surgeons namely, high rate of wound infection, impaired wound healing, and higher rates of recurrence [4]. Beside all these, management should take in consideration that surgery must be simple, inexpensive, associated with low hospitalization periods and rapid wound recovery especially among those patients with low income and depend only on daily salary[5].

Surgical modality in which wide excision with or without marsupialization which favored by many as it offers a low recurrence rate, on the other hand left behind a large midline raw area to be healed secondary in months add-

ing a more burden on the patients quality of life[6]. So, many surgical techniques exist hoping to deal with that problems to decrease the overall postoperative morbidity, including excision with primary midline closure, oblique excision with asymmetrical closure methods, such as Karydakis flap reconstruction, Limberg rhomboid flap, V-Y plasty and, Z- plasty[6,7,8]. The main objective behind most of flap techniques as a treatment of SPD is that they produce a lateralization and flattening of the natal cleft and by these techniques if successfully done will decrease the overall postoperative morbidity and recurrence rate [9,10,11].

In this prospective study, we compare the results of rhomboid excision and Limberg flap closure with Karydakis flap reconstruction in the management of the SPD.

Patients and Methods

Setting :

Consecutive patients who were treated for benign sacrococcygeal pilonidal disease at Mansoura University Hospital, Mansoura, Egypt, during the period from February 2008 to March 2010 were eligible for this study. Informed consent was obtained from all patients to be included in the study, after explanation of the nature of the disease and possible treatment. The study was approved by the local ethical committee.

Outcome measures :

The primary outcome measure in this study was the postoperative complications including early wound infection and wound failure.

Inclusion criteria :

All patients with benign non-complicated SPD.

Exclusion criteria :

Exlusion criteria included patients with complicated sacrococcygeal Pilonidal disease such as pilonidal abscess at the time of admission, for which unroofing and drainage procedures were required.

Interventions :

The patients were then randomized into two groups: Group 1 underwent excision and Karydakis

flap reconstruction; Group 2 underwent rhomboid excision and Limberg flap repair. The surgical treatment options, complication rates, hospitalization, and work-off periods were analyzed.

Follow up :

All patients were discharged on the second postoperative day in normal circumstances and were advised to keep the sacrococcygeal area clean and shaved. Oral antibiotic coverage was recommended after discharge. Skin sutures were removed on the fourteen postoperative days. Follow-up examinations were made at the end of the first, sixth, and twelfth months after surgery. Beyond 1.5 years, the patients were monitored by phone calls at every 6 months. The duration of hospital stay, early wound complications, time of return to active work, recurrent disease and the time of recurrence, and patient complaints including numbness and dissatisfaction were recorded.

Sample size :

To guarantee adequate statistical power, a sample size calculation was performed. Because the reported rate of postoperative complications associated with flap procedures varies between 1% and 15% (12) to accomplish a statistical power of 80% and by setting the alpha level at 5%, a sample size of 57 patients per arm was essential in double-sided testing. We determined a sample size of 65 patients per arm, allowing up to eight patients to drop out.

Randomization and allocation to intervention :

All patients were subjected to careful history taking, clinical examination, and laboratory tests. Randomization was achieved through a computer - generated schedule, and the results were sealed into envelopes. The envelopes were drawn and opened by a nurse not otherwise engaged in the study in the operating room (Fig. 1).

The statistical analysis of data done by using excels program and SPSS (SPSS, Inc, Chicago, IL). Program statistical package for social science version 10. The description of the data done in form of mean (+/-) SD for quantitative data.And Frequency & proportion

for Qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative date student t-test was used to compare between two groups. Paired sample t-test to compare one group at different time. Chi square test was used for qualitative data. Correlation coefficiency was done to detect association between variables. N.B: P is significant if < or = 0.05 at confidence interval 95%

Surgical Procedure

The evening before the day of surgery, the surgical site was prepared by shaving the hair widely around the natal cleft. The patients were operated on after general or spinal anaesthesia. A single dose of a third generation cephalosporin was given intravenously before starting surgery and the patient was placed in a prone position with the cheeks of the buttocks strapped apart. The surgical area was disinfected by application of 10% povidone-iodine. Between 0.5 and 1 mL of methylene blue was injected through the most prominent opening of the pilonidal sinus to help to clarify the extent of the diseased tissue.

Karydakis flap group :

Karydakis flap reconstruction was performed in conformity with the original procedure described by Karydakis(10). A vertical eccentric elliptical incision carried down to the post sacral fascia, complete removal of unhealthy tissue with the normal tissue around the cyst and sinus tracts, mobilization of the medial wound edge by undercutting the adipose tissue at a depth of 1 cm, the advancement of the flap across the midline to the post sacral fascia and suturing of its edge to the lateral one. A single multiple-hole, closed suction drain was inserted underneath the flap. Interrupted 2-0 absorbable sutures were used to fix the flap to the fascia, whereas skin approximation was performed using interrupted 2-0 polypropylene sutures (fig. 2). The flap must be adequately mobilized at least 1.5 cm from the midline and repaired without any for of tension.

Limberg flap technique :

Using sterile skin-marking pen, a rhombic shape with its long axis in the midline should enclose all the pathological area to be excised and the flap line is

mapped on the skin. The excision is extended down to the level of the postsacral and gluteal fascia with complete removal of all pathological area.A Limberg fasciocutaneous flap which is usually located on the adjacent right buttock, based inferiorly is used to cover the rhombic defect. After complete haemostasis by electrocautery and removal of adhesive tapes, full mobilization of the flap was done and repair completed without any kind of tension. A suction drain is placed beneath the flap through a separate stab incision. The wounds are sutured in three layers. The fascial and deep subcutaneous layers are approximated with interrupted 2-0 Vicryl sutures, the superfacial subcutaneous layer with inverting sutures of 2-0 Vicryl sutures, and the skin is closed with 2-0 Prolene sutures (Fig. 3).

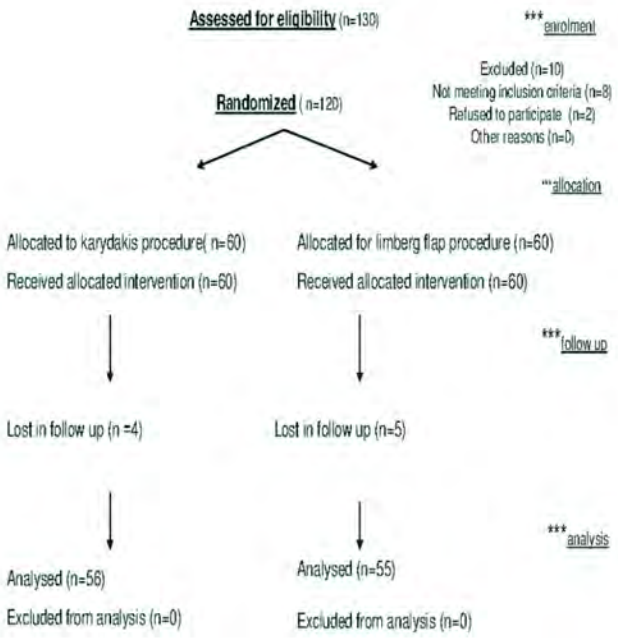

Fig. 1 : Flow diagram of the process through the phase of a randomized trial (9. e, enrollment, intervension allocation, flow up, and data analy sis).

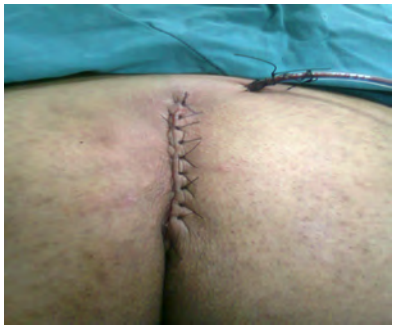

Fig. 2 : The resulting final wound is lateral to the midline.

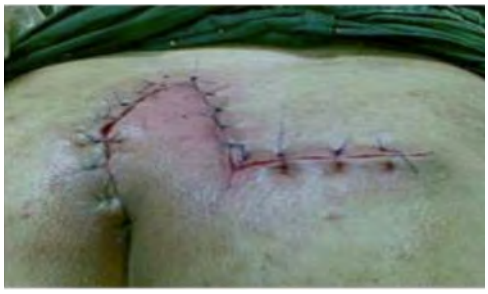

Fig. 3 : The final result of rhomboid excision and limberg flap transposition.

Generally the drains were removed in the outpatient's clinic when a daily drainage below 20 mL was obtained. In case of wound infection or haematoma, the wound was drained by the removal of a few sutures, covered with daily dressings, and oral antibiotic was given for another 5 days.

Results

The study flow chart is shown in figure 1. Of 130 consecutive patients seen during the recruiting period, 120 patients (115 males and 5 females) with a median age of 22 years (range, 18-40 years) were evaluated and entered the study. These patients were randomly diveided into two groups (60 patients in karydakis group and 60 patients in Limberg group) the two groups were matched for age, sex, pathological diagnosis.

Data collected during the in-hospital and early postoperative period are shown in Table 1. The male: female ratio for the Karydakis group was 57 / 3 and 58 / 2 for the Limberg group. No differences were detected between the 2 groups, in terms of patient age or gender, the type of aesthesia used, or the type of disease presentation.

There was a significant difference in the mean duration of the operation (52.7 ± 7.9 min) for the Limberg rhomboid flap method and (49.2 ± 5.5 min) for the Karydakis procedure, (P = 0.005).

There was no significant difference between the two groups with respect to the overall incidence of early complications (P = 0.44). It was noted that in both groups, most patients with early complications were diagnosed during outpatient visits after discharge. One patients (1.7%) in the Limberg rhomboid flap group experienced wound infection that required early suture removal (partial) and healing by secondary intention, but neither patient required complete reopening of the wound. For two Patients (3.3%) in the Karydakis group, early suture removal and secondary healing were required. In other patients who developed complications, prolonged antibiotic use, and/or simple drainage was sufficient to overcome the condition. No partial or total flap necrosis that necessitated further surgical intervention was detected. The comparative results of data collection during follow up are summarised in Table 2. No differences were detected between the 2 groups regrading time of return to work, for Karydakis group it was (20 ± 6.01) days and for Limberg group (22.38 ± 4.8) days (p = 0.030). Patients in the Karydakis group reported a significantly shorter time to feeling completely healed (30.2 ± 8.9) days relative to the Limberg rhomboid flap group (32.6 ± 6.7) days (p = 0.07). In the Limberg rhomboid flap group, the disease recurred in 2 patients (3.3%) within 6 to 9 months; in the Karydakis group, 1 patient (1.7%) was diagnosed with recurrent disease that developed after 4 months of surgery (p = 0.55). As presented in Table 2, there was not a significant difference between groups in terms of the degree of satisfaction expressed by patients (P = 0.35). Out of 60 patients prospectively investigated in the Karydakis group, 47 (78.3%) rated their degree of satisfaction as “excellent, ” 10 (16.7%) rated it as “good, ” and 3 (5.0%) rated it as “unsatisfied.” However, 66.7% of patients in the Limberg group rated their satisfaction level as “excellent, ” 26.7% as “good, ” and 6.7% as “unsatisfied.” Defecation was performed more easily after the Limberg rhomboid flap group when compared with the Karydakis flap group (p = 0.002) (Table 3).

Discussion

Pilonidal disease is a common health problem that carries high morbidity and patient discomfort. The natural history of the disease is so variable as small percentage of patients may have no complain at all and the problem focused only in that a small pits present in their backs whereas there are other large percentage of patients who show a more dramatic history with chronic intermittent inflammatory process with acute exacerbation and abscess formation. According to the theory postulated by Karydakis, SPD occurs only when 3 main factors coexist (1) invader loose hair, (2) force that facilitate the hair insertion into skin, (3) vulnerability of that underlying skin (these is related mostly to the depth of intergluteal sulcus). Fortunately, many authors believed that the main causes of SPD recurrence after complete excision are related also to the above mentioned factors[13].
A wide range of surgical and non surgical treatment methods have been described for SPD but no standard treatment has been approved by all surgeons until now and there is also no treatment present without risk of complication especially the disease recurrence. While most of the conservative treatment methods help in elimination of many SPD predisposing factors, still surgery with its all modalities forms aim to eliminate the main cause of SPD which is the presence of deep intergluteal sulcus.[3,14,15]. So, surgeons were so urge to discover new plastic reconstructive methods for that purpose such as Z-plasty, W-plasty and V-Y plasty. The main general advantages from post excision flap closure of the defect are the short hospital stay, the low recurrence rates which estimated to be about 5% among all flap techniques and high patients' satisfaction index.[11,16]. Karydakis was using an asymmetrical excision and primary closure aiming for lateralization of the incisional line away from the midline. This kind of repair produce a flattened gluteal furrow which prevents hair penetration into the natal cleft, sound and acceptable cosmetic results beside if any postoperative infection occurs the defect will not be as large as Limberg flap.[17,18]. The Limberg technique which was originally re-

served for complex or recurrent pilonidal disease, now it used widely as a plastic technique for primary SPD. It involves creation of a flap to achieve primary closure and obliteration of any deep natal cleft. So, it relocates hair follicles away from the midline and prevents any frictional forces to be involved in creation of a new disease. Limberg technique offers many advantages such as low recurrence and complication rate, but appears to have a lack in cosmetic appeal especially among female patients.[19, 20, 21].

Surgical drainage of the wound is still a matter of debate, in time where are many surgeons approved its usage to decrease the incidence of postoperative complications such as fluid collection and wound dehiscence, its effect on recurrence rate remain unclear [22,23]. Akinic et al, observed a very low recurrence rate (0.9%) with routine using of a suction drain and subcuticular skin closure. On the other hand Gurer et al[17], in his prospective randomized study stated that routine cavity drainage after karydakis procedure has no effect on the disease recurrence rate. In this study, we used drains in both groups and we suggest that is one of the reasons for the low rates of complications in our series including recurrence rate (2.5%).

Although, in our study, we discharged all patients in both group in the second postoperative day unless a complication such as infection had occurred. This suggests that the mean hospital stay duration after flap procedures could be shortened safely as long as patients can be regularly checked in the outpatient's clinics. We still believe that length of hospital stay is a good measure for any pilonidal surgery success and one of the main items to be in consideration when assessing the patient satisfaction. For Karydakis operation, the mean hospital stay appears to be short, for example Morden et al.[24] highlighted that the mean hospital stay duration after the Karydakis operation as 0.08 days. On the other hands, the mean hospital stay seems to be longer for patients treated with Limberg technique. In a study by Daphan et al[20], a mean hospital stay of 5.9 days was observed.

In this study, the rate of wound infection for Karydakis flap was 3.3% versus 1.7 for Lamberg flap. In a study examining the MLF procedure by Mentes et al,[23]. An infection rate of 0.8 % was noted. Hower, Karydakis[9], stated that in his original report that the rate of wound infection was 8.5% in a cohort of 5.876 patients. As regard postoperative collection and dehiscence, in our study the rate was 1.7% and 3.3% for karydakis flap verus 3.3% and 1.7% for Limberg flap recpectively.

No statistically difference between the two groups in term of recurrence rate (p = 0.55). During a mean follow up of 18 months (ranged from 8 months to two years), with 111 patients at 1 year after surgery at least, 2 (3.3%) recurrences developed in the karydakis group whereas only one (1.7%) recurrence developed in the Limberg group. The documented incidence of recurrence after the Karydakis procedure is between 0% and 4.4%[16,20,25], and there is a well-recognized incidence of recurrence of up to 5.3% after the classic Limberg flap [20, 25, 26].

In the present study, time feel ready to return work in karydakis group was 20.25±6.01 days and for Limberg procedure was 22.38±4.5 days with (p= .30).

Akıncı et al[3] reported that the mean time to return to normal activity following pilonidal surgery has been reported to range from 12.4 to 20 days after Karydakis flap reconstruction, while Daphan et al[20] stated that it was from 8 to 9.4 days after the MLF procedure.

The most important item to be mentioned here is that many patients may feel completely ready for return to full physical activity without appropriate concomitant complete healing. In our study, the time required for patients to feel completely healed was shorter among those who underwent the Karydakis procedure (p = 0.07). So, all patients should be strictly advised for regular visits at outpatients' clinics for early detection of any postoperative complication and healing process follow up.

In our study 78.3% of patients in the Karydakis group rated their

satisfaction level as"excellent," and 5% of patients reported feeling dissatisfied with the procedure. In the other hand, 40% of patients in Limberg group rated their feeling level as"excellent," and 6.44 % reported feeling of dissatisfaction with the procdure, mostly due to the low cosmotic ability of Limberg procedure. In a study by Mahdy[27], the superiority of the classic and MLF techniques were documented in terms of patient satisfaction and comfort.

There was significant difficulty with defecation in the Karydakis group in comparison to Limberg group in our study (p = 0.002). This observation is hardly to be explained but it may be related to geometrical reasons associated with flap design and flap tension [15].

In conclusion, the results of the currently reported study suggest that for patients with SPD, Limberg flap technique and Karydakis flap reconstruction have no significant superiority over each other, in terms of either in hospital stay, rate of complications and return to full physical activity. In this study, early postoperative difficulty of defecation was low in karydakis group and so the Limberg procedure seems to be superior to karydakis technique as regard to this point. Although we recommend excision and flap technique for treatment of non infected SPD, certain parameters must be evaluated when we chose certain flap including long term results especially recurrence rate and cosmetic outcome.

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Mohamed E. Abellatif MD, Nashaat Noaman MD, Ahmad Negm MD, Ashraf Abass MD, Amro El-Hadidin MD, Gamal El-Morsy MD and Ramadan Ellithy MD

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SURGICAL PLANNING OF FUNGAL SINUSITIS WITH INTRACRANIAL EXTENSION IN IMMUNE-COMPETENT PATIENTS ACCORDING TO RELATION TO IMPORTANT NEURO VASCULAR STRUCTURES

Montasser A. Foda MD, Kamal Balkheur FRCS(C), Yasser Haroon MD*, Mamdouh M. Fadel MD, Ahmad Haroon MD and Hassan Othman MS*****

*Deparments of Neurosurgery, *E.N.T., **Radiology, and ***Pathology, Al-Noor Specialist Hospital, Holy Makkah, KSA.*

Abstract

Background: *Fungal sinusitis is a common disease in healthy young adults in our locality. It may destroy the skull base and invade the intra-cranial and intra-orbital compartments causing neurological and ocular manifestations.*

Objectives: *The aim of this study is to evaluate the results of combined neurosurgical and ENT surgical managements for patients with fungal sinusitis with intracranial extension.*

Methods: *We managed 10 cases with fungal sinusitis with intracranial extension. All patients were immune-competent. The average age of patients in this study was 31.5 year (range 17 - 46 years). There were 6 males and 4 females. All patients presented with headache, 5 with nasal obstruction and polyps, 5 with unilateral Proptosis, one case with trigeminal neuralgia and squint, one case with epilepsy and 2 with deterioration of level consciousness. CT was done in all cases, MRI in 8 patients, CT angiography and conventional cerebral angiography in one patient. Surgical intervention was decided according to the relation of*

the fungal granuloma to important intracranial structures. Endonasal approach alone was done when the granuloma not related to the optic nerve, internal carotid artery "ICA" or cavernous sinus (n=3). Combined subfrontal and endonasal approaches were used when granuloma closely related to one or more of the previous structures (n=5). Transcranial approach alone was done in 2 patients with isolated sphenoid fungal sinusitis, that was associated with a mycotic ICA aneurysm in one patient and with a temporal lobe abscess in the other. In addition antifungal treatment was used for 8-12 weeks. patients were followed up clinically and radiologically for 3 months period.

Results: *Showed one patient died 2 months post-operatively from fungal meningitis. No morbidity related to the operative procedures were recorded in this stud. Proptosis was completely corrected in the 5 cases. Follow up CT showed eradication of the fungal granuloma in all surviving patients. Histologically fungal infection included Aspergillosis and mucormycosis.*

Conclusion: *Team ENT and neurosurgical work and early diagnosis are mandatory in the management of fungal sinusitis with intracranial extension in immune-competent patients. Surgical planning to the relation of fungal granuloma to important neurovascular structures is the corner stone for safe removal of granuloma.*

Key words: *Invasive fungal sinusitis - intracranial fungal granuloma - mycotic fungal cerebral aneurysm - fungal brain abscess.*

Introduction

Traditionally, fungal infections of the paranasal sinuses have been considered uncommon and were thought to occur only in immune compromised individuals. However, the occurrence of fungal sinusitis has increased recently in the immune competent population [(1)]. It is now believed that the fungi are important etiologic agents of sinusitis [(2)].

The intracranial extension generally presents with neurological symptoms, and the patient often

consults a neurosurgeon or an ophthalmic surgeon. So, it is very important for these surgeons to recognize fungal sinusitis as the cause. Although, it seems that this entity is more common in the immune compromised, especially poorly controlled diabetic patient. It is also prevalent in the immune competent population[3].

Paranasal sinus mycoses were first reported by Mackenzie[4]. Since then, several varieties have been identified, including allergic, indolent and invasive forms[5]. Not uncommonly, the fungal granuloma will erode the skull base and extend into the intracranial and intraorbital compartments. However, bone destruction is not necessary for the development of intracranial or intraocular complications, because the fungus can extent along vascular channels. Ocular sequelae can include diplopia as a result of involvement of the extra-ocular muscles, as well as proptosis, chemosis and loss of vision. Neurologic sequelae can include meningitis, brain abscess and cavernous sinus thrombosis[6].

The current management strategy calls for a multimodality approach, including aggressive surgical debridement, the use of antifungal and/or anti-allergic agents[7,8]. It is not yet understood which combination of these therapies constitutes optimal management. Furthermore, in case of intra cranial extension, it is not clear which surgical (endonasal, transcranial or combined) approach has to be used [6,7,8,9]. So the aim of this study is to evaluate the results of combined neurosurgical and ENT surgical approaches for immune-competent patients having fungal sinusitis with intracranial extension, according to the relation of the granuloma to eloquent neurovascular structures.

Materials and Methods

This study included 10 immune-competent patients with fungal sinusitis that invaded the skull base and had an intracranial extension.

Complete history taking and full ENT, ophthalmic and neurological examinations were done for all patients with special concern

about:

- History of absence of diabetes or immunodeficiency state. All patients were cancer free and non had received immunosuppressive therapy.
- Symptoms of headache, nasal obstruction or discharge, anosmia, vomiting, nausea, epistoxis, pre - orbital pain, facial pain, facial swelling, altered sensorium, seizures, weakness of limbs, diplopia, visual disturbance and fever.
- Presence of blackish or brownish discharge, pale or dark mucosa in the nose, ophthalmoplegia, chemosis, proptosis, raised intracranial pressure, visual blurring or hemiparesis.

Radiological evaluation was done pre and postoperatively:

- **Preoperative:**

C-T was done for all patients to assess soft tissue extension and bony erosion. In fungal sinusitis, CT will show central sinus high attenuation [10,11]. MRI, was performed in 8 patients for better visualization of the intracranial extension and to identify the relation of the fungal granuloma to important neurovascular structures namely, the optic nerves, ICA and cavernous sinuses. A decrease in signal intensity on T1 and a marked decrease in signal intensity (signal viod) on T2 weighted images were reported to be characteristic of fungal disease [11,12].

In addition, CT angiography and conventional cerebral angiography were done for one case with sub arachnoid hemorrhage (SAH).

- **Post operative :**

C-T was done 1 month and 3 months post operatively.

Laboratory and histopathological examinations :

- **Preoperative :**

Complete laboratory investigations were done for all patients for selection of immune-competent patients and preparation for surgery.

Fungal culture and biopsy:

There are special stains required for detecting the fungus. these include methamine silver for mucor, rhizopus and absidia. The aspergillus has uniform septate

hyphae branching at 45 degree. The mucor shows non septate, non uniform branching at 90 degrees. Copious mucin, abundant eosinophils and charcot - lyden crystals are characteristic of allergic fungal sinusitis (AFS) and one has to stain deeply to look for fungal hyphae [3]. The culture used is sabauraud media. The biopsy from the middle turbinate and polyps were examined histopothologically.

- **Post operative :**

All surgical specimens were examined histopothologically.

Operative techniques:

a. Endonasal approach alone was used when the granuloma not related to the optic nerve , ICA or cavernous sinus.

b. Combined subfrontal and endonasal approach were used when the granuloma closely related to optic nerve, ICA or cavernous sinus .

In the first part of operation, craniotomy was done to remove the intracranial and intraorbital components of the granuloma extradurally. Then, the granuloma within the frontal, posterior ethmoidal and sphenoid sinuses was removed under vision to decompress the optic nerves, ICA and cavernous sinus. Periosteal graft was used to secure the defect in the skull base. After closure of the cranial wound, endonasal removal of the granuloma within the anterior ethmoid and maxillary sinuses was done endoscopically by ENT team.

c. Transcranial approach alone was done for patients with isolated sphenoid fungal sinusitis.

Postoperative antifungal treatment was used for 8-12 weeks. Fluconazole was used in 8 patients and amphotricin B was used in 2 patients.

Results

Clinical characteristics of the patients are listed in table 1.

Age ranged from 17 to 46 years with average of 31.5 year.

There were 6 males and 4 females patients .

The most common presentation of the disease was headache in all patients, nasal abstraction and polyps in 5 cases, unilateral proptosis in 5 cases, trigeminal neuralgia and squint in one case, epilepsy in one case and deterioration of level of consciousness in 2 cases.

Radiological findings(table 2):

As diagnosed by CT, 8 patients had fungal pansinusitis and two had isolated sphenoid fungal sinusitis.

In the group of patients with fungal pansinusitis (n=8), the fungal granuloma caused destruction of the anterior skull base (posterior wall of the frontal sinus, cribriform plate of ethmoid and/or planum sphenoidale). Five of them also had intra-orbital extension. Subfrontal fungal granuloma was observed in all 8 patients and, in addition, suprasellar granuloma was seen in 2 patients.

On the other hand, patients with isolated sphenoid fungal sinusitis (n=2) had destruction of the body of the sphenoid sinus. Both patients had left parasellar fungal granuloma and one of them, in addition, had a suprasellar granuloma.

One patient had SAH in the suprasellar cistern. In this patient, CT angiography and 4-vessels angiography confirmed the presence of a 10 mm left ICA bifurcation aneurysm.

As shown in MR imaging, the fungal granuloma was intimately related to the optic nerve in 6 patients, ICA in 5 patients and cavernous sinus in 2 patients. One patient had left temporal brain abscess.

According to surgical approaches :

a. Endonasal approach alone was used in 3 cases .
b. Combined subfrontal and endonasal approaches were used in 5 cases.
c. Transcranial approach alone was done in 2 cases .

Regarding to laboratory and histopothological results:

There were 8 cases with aspirgellosis and 2 cases with mucormycosis.

There were 5 patients with allergic fungal sinusitis and 5 with chronic invasive fungal sinusitis.

Case presentation:

Case 1: Twenty Seven years old male patient presented with chronic headache for two years. CT and MR showed fungal pansinusitis with small intracranial component due to destruction of the cribriform plate. However, the fungal granuloma was not intimately related to the optic nerve, ICA or the cavernous sinus (figure 1). The fungal granuloma was removed via endonasal approach. CT showed no evidence of recurrence of the fungal infection after three months.

Case 2: Forty-two years old male patient presented with left proptosis and chronic headache for one year. CT and MR scan showed fungal pansinusitis with small intracranial component due to destruction of the cribriform plate and planum sphenoidale. The fungal granuloma was intimately related to the optic nerves and left ICA (figure 2). Combined transcranial and endonasal approach was used to eradicate the fungal granuloma. Through a bifrontal craniotomy, the fungal granuloma at the base of the skull and within the sphenoid, frontal and posterior ethmoid sinuses was removed extradurally under direct vision. After closure of the cranial wound, endonasal approach was used to remove the granuloma within the anterior ethmoid and maxillary sinuses microscopically. CT showed no evidence of recurrence of the fungal infection after three months.

Case 3: A female patient, 46 years old, was admitted through ER with fever, deterioration of the level of consciousness, and neck stiffness. She was referred to our hospital as a case of pituitary apoplexy. CT showed sphenoid fungal sinusitis, destruction of the body of the sphenoid, supra- and left para-sellar fungal granuloma, together with SAH in the suprasellar cistern. Routine preoperative CT angiography showed 10 mm left ICA bifurcation aneurysm that was closely related to the parasellar lesion. Four-vessels angiography confirmed the diagnosis of the aneurysm (Figure 3). Patient was operated through a left fronto-

temporal craniotomy. First, clipping of the aneurysm was done intradurally. The wall of the terminal part of left ICA was thicker than normal and whitish in colour. Also, the dura covering the sellar region was thick and inflamed. No intradural fungal granuloma was found in the para- or suprasellar regions. After closure of the dura, the fungal granuloma (fungal balls) in the supra- and left parasellar region and within the sphenoid sinus was removed extradurally. The planum sphenoidale and left wall of the body of the sphenoid bone was already destructed by the granuloma. Histopathological examination showed mucormycosis. Follow-up CT showed removal of the fungal granuloma. The patient showed good post-operative recovery and was stable for 3 weeks. However, she died 2 month post-operatively from uncontrolled fungal meningitis.

Case 4 : 41 years old male patient presented with intractable left trigeminal pain and diplopia due to 6^{th} nerve palsy for several months. CT and MR showed isolated sphenoid fungal sinusitis, destruction of the body of the sphenoid, left parasellar fungal granuloma and small left temporal cystic lesion most probably a chronic abscess (Figure 4). Patient was operated through left temporal craniotomy. Intraoperatively, he had a purulent infection involving the sphenoid sinus, body and left greater wing of sphenoid. Adjacent dura and brain was infiltrated by the purulent infection. The fungal granuloma was removed and the fungal brain abscess was also drained. Histopathology showed mucormycosis. Patient did well postoperatively and was maintained on antifungal treatment for 3 months. Follow-up CT brain showed removal of the fungal granuloma. After 3 months, the trigeminal pain greatly improved and epilepsy is controlled by carbimazepine.

Outcome :

No morbidity or mortality related to the operative procedures was observed in our patients. However, case number (9) died two months post-operatively from fungal meningitis although she was recieving amphotricin. Proptosis was com-

pletely corrected in the five patients. Headache also improved post-operatively in all surviving patients.

Follow-up CT showed eradication of the fungal granuloma in all survivors with no evidence of recurrence during the follow-up period.

Table 1 : Clinical characteristics and surgical treatment in study group.

Case No	Age/ Ys	Sex	Main presentation	Location	Type of surgery
1	27	M	Headache, nasal obstruction, polyps	PNS , IC	Endonasal approach
2	22	M	Headache, nasal obstruction , polyps	PNS , IC	Endonasal approach
3	35	F	Headache , unilateral proptosis	PNS , IC, IO	Combined subfrontal and endonasal approach
4	42	M	Headache, nasal obstruction , polyps , unilateral proptosis	PNS , IC, IO	Combined subfrontal and endonasal approach
5	25	M	Headache , unilateral proptosis	PNS , IC, IO	Combined subfrontal and endonasal approach
6	17	M	Headache, nasal obstruction , polyps	PNS , IC	endonasal approach
7	26	F	Headache , unilateral proptosis, deterioration of level of consciousness	PNS , IC, IO	Combined subfrontal and endonasal approach
8	34	F	Headache, nasal obstruction , polyps , unilateral proptosis	PNS , IC, IO	Combined subfrontal and endonasal approach
9	46	F	Headache, deterioration of level of consciousness, epilepsy	PNS , IC	Trans cranial approach
10	41	M	Headache , trigeminal neuralgia , squint	PNS , IC	Transcranial approach

PNS = Paranasal sinuses IC = Intracranial IO = Intraorbital

Table 2: Radiological findings in study group (n=10):

Investigation	Findings	Number
CT scan	Pan-sinusitis	8
	Isolated sphenoid sinusitis	2
	Destruction of anterior skull base	8
	Intra-orbital granuloma	5
	Destruction of the body of sphenoid	2
	Subfrontal granuloma	8
	Suprasellar granuloma	3
	Parasellar granuloma	2
MR	Granuloma not intimately related to neurovascular structures	3
	Granuloma intimately related to optic nerve	6
	Granuloma intimately related to ICA	5
	Granuloma intimately related to cavernous sinus	2
	Brain (left temporal) abscess	1
CT & 4-vessels angiography	Left ICA bifurcation aneurysm	1

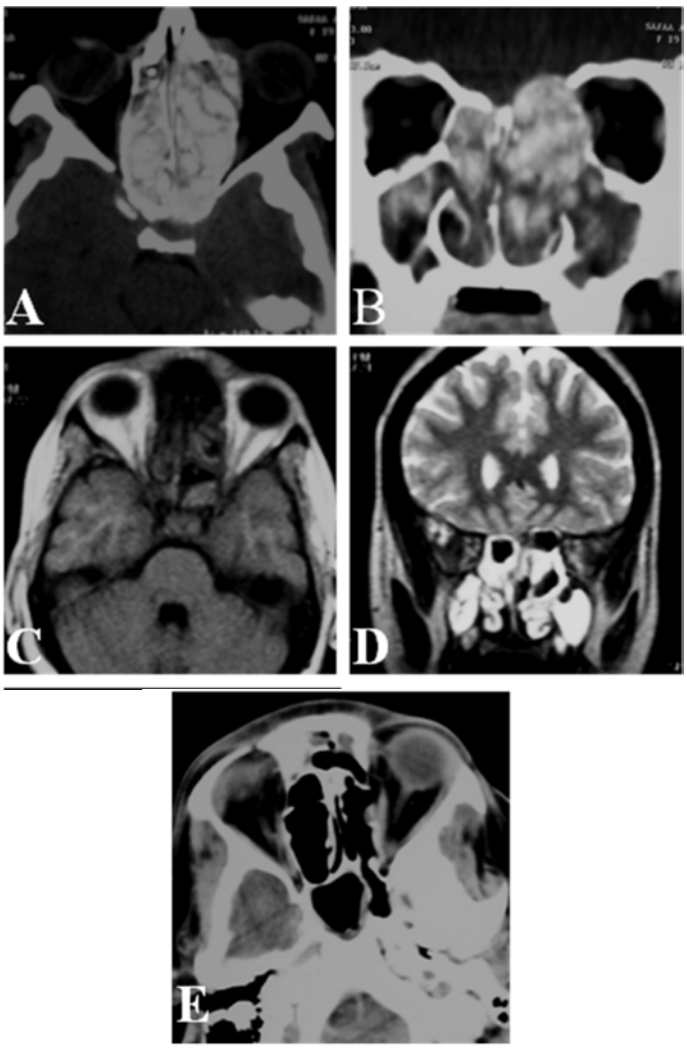

Figure 1: (A) Axial and (B) coronal CT images showed hyperdense lesion involving all paranasal sinuses suggesting fungal pansinusitis with intracranial extension through the cribriform plate of ethmoid. (C) The hypo-intense signal on T1-weighted axial MR image and the signal void on the T2 weighted coronal image (D) confirmed the diagnosis of fungal sinusitis. MR also showed that the fungal granuloma was not intimately related to eloquent brain structures. Endonasal removal of the granuloma was done in this patient. (E) three months follow-up CT brain showed no evidence of recurrence of the granuloma.

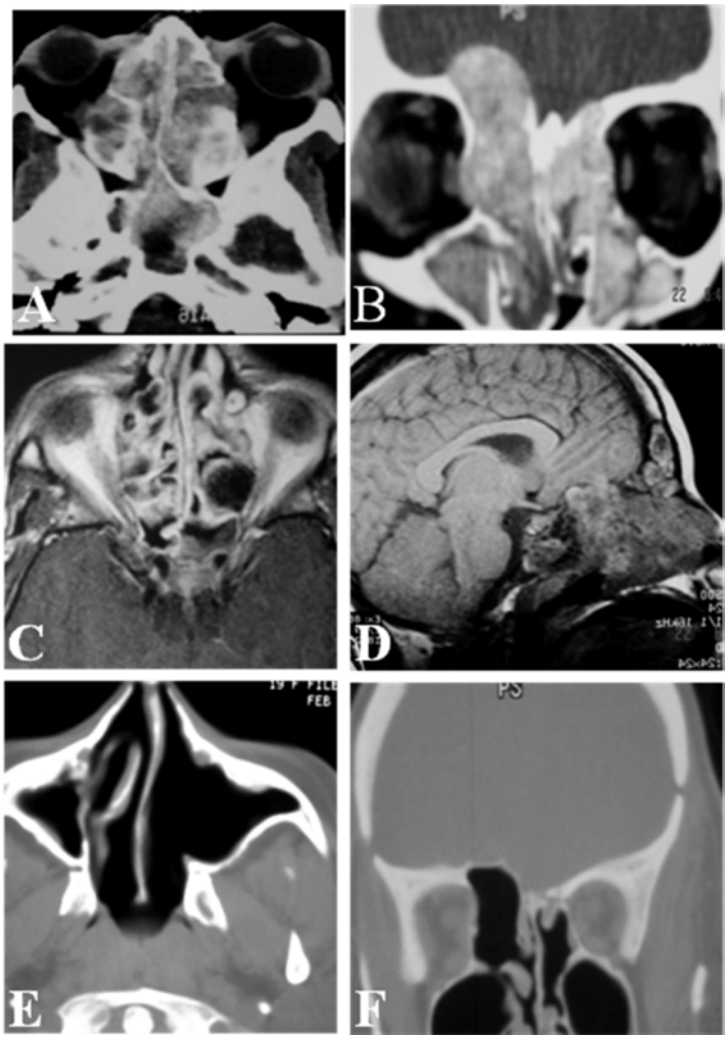

Figure 2 : (A) Axial and (B) coronal CT images in this patient showed fungal pansinusitis with intracranial and intraorbital extension. (C) Axial MR image showed lateral displacement of both optic nerves by the granuloma. (D) The sagittal MR image showed subfrontal extension of the granuloma through the cribriform plate and planum spheniodale. Combined transcranial and endonasal approach was used to remove the granuloma and to decompress the optic nerves. Three months follow-up CT brain in (E) axial and (F) coronal section showed no evidence of recurrence of the granuloma.

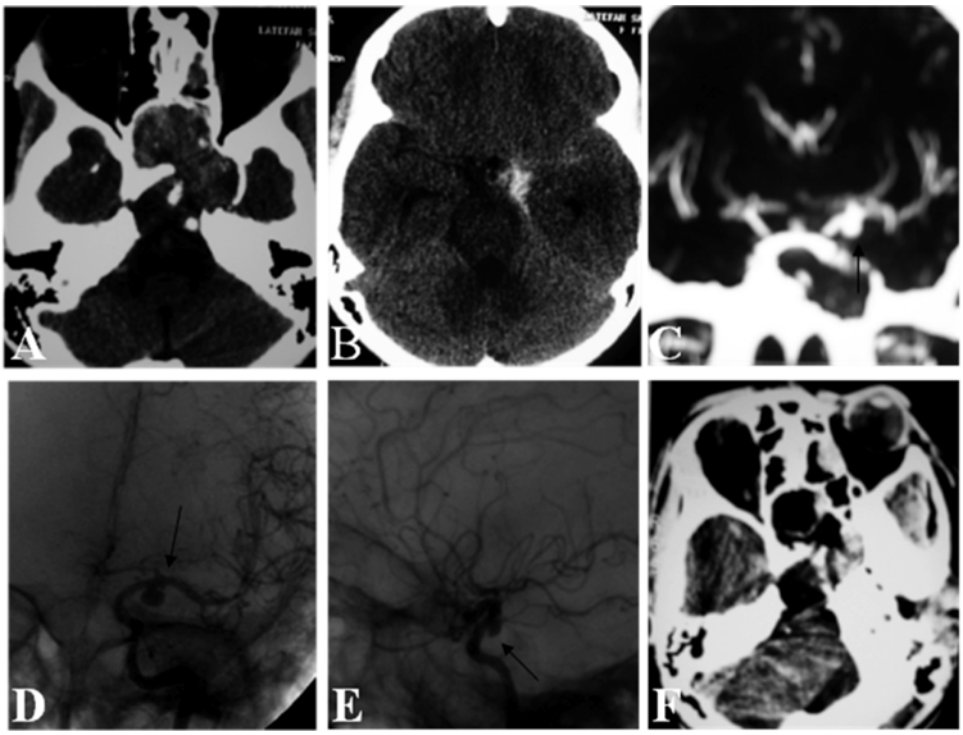

Figure 3 : The axial CT image in (A) showed isolated sphenoid fungal sinusitis with left parasellar extension, (B) showed SAH in the left side of suprasellar cistern. (C) Reformated CTA image in the coronal plan showed left ICA bifurcation aneurysm. Four vessels angiography, A-P (D) and lateral (E) views confirmed the diagnosis of Left ICA bifurcation aneurysm. This patient was operated through right pterional approach for clipping of the aneurysm and for removal of the granuloma. (F) one month post-operative CT follow-up showed complete removal of the granuloma.

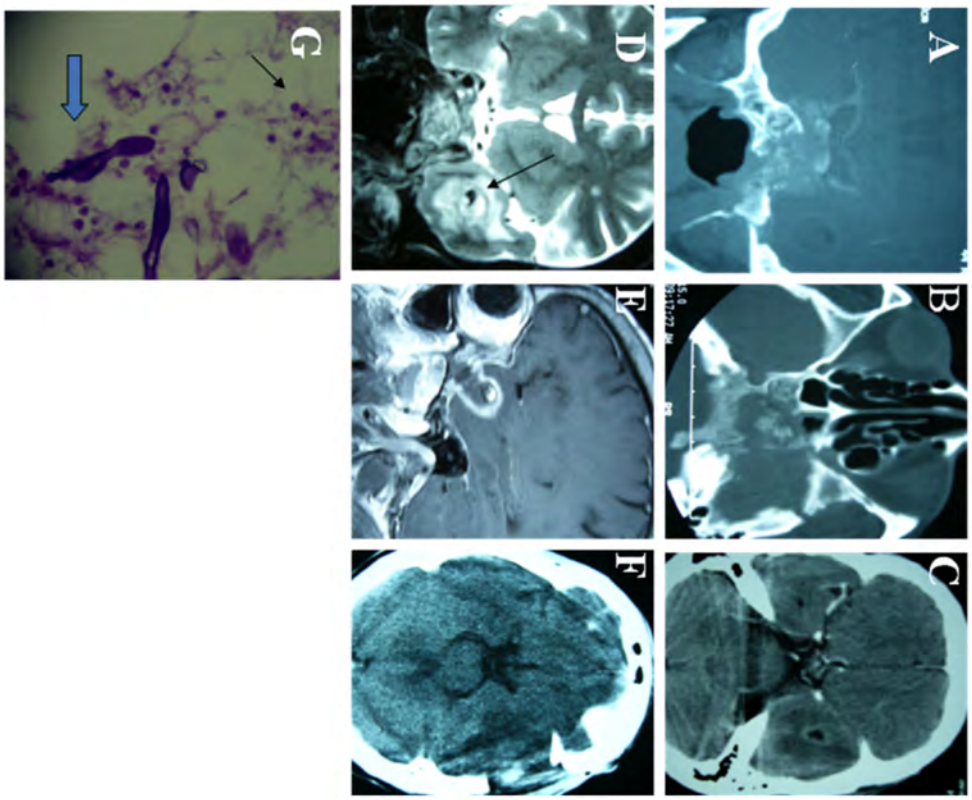

Figure 4 : (A) Coronal and (B) axial CT (bone window) images in this patient showed isolated sphenoid fungal sinusitis with destruction of the body and left greater wing of sphenoid. (C) CT with contrast showed a small left temporal cystic lesion. (D) Signal void in the coronal T-2 weighted MR image strongly suggested cerebral fungal infection. (E) The ring enhancement in the sagittal T-1 weighted MR image suggested an abscess. This patient was operated by right temporal craniotomy to remove the fungal granuloma and to drain the abscess. (F) Three months post-operative CT follow-up showed disappearance of the abscess. (G) Histopathology showed the fungal (mucormycosis) hyphae and spores (small arrow) and granulomatous inflammation with multinucleated giant cells (large arrow) (PAS, x 400).

Discussion

Several authors have reported on the aggressive and lethal nature of invasive fungal sinusitis in immunocompromized patients[13,14]. Although the lungs are the most common site of fungal infection in those patients, the paranasal sinuses and brain are the next most common sites[15]. Aspergillus was the most commonly reported organism[8,13,16,17,18]. However, mucormycosis, candidiasis, and other opportunistic fungal infections were also reported[19]. Despite the fact that invasive fungal disease is relatively rare, it is significant because of its rapidly progressive and lethal nature[20,21].

Fungal sinusitis has been classified into five clinicopathologic forms: the three invasive forms include acute fulminant, chronic (indolent) and chronic sinusitis; and the two noninvasive forms include the fungal ball (sinus mycetoma) and the allergic fungal sinusitis[22].

The acute fulminant form occurs mainly in immunocompromized patients with prolonged neurtopenia. The chronic invasive form usually appears in immunocompetent hosts, and is characterized by an indolent clinical course. Most cases with the chronic invasive form were reported in the Sudan, India, and Pakistan and was caused by Aspergillus and dematiaceous fungi. Histopathologic evidence of soft-tissue invasion by fungal hyphae is required to make the diagnosis, which is often delayed. The granulomatous invasive form is characterized histologically by: a profuse fungal growth with tissue invasion, non-caseating granulomas with multinucleated giant cells, plasma cells, and fibrinoid necrosis. The invasive granulomatous form is usually caused by Aspergillus flavus[22,23].

The fungal ball (sinus mycetoma) is characterized by a fungal ball within a single sinus. A dense collection of hyphae is observed microscopically with only a mild inflammatory response. Its growth pattern is noninvasive with sclerosis of the surrounding bone. However, an invasive variant has been reported. This invasive variant is characterized by profuse growth

patterns and invasion of the surrounding structures[5,24,25,26]. The typical intraoperative finding is a purulent mass. The sphenoid sinus represents the primary site for intracranial or orbital invasion. This type is characterized by aggressive destruction of surrounding bony structures. It may also extend into the cavernous sinus and cause thrombosis[27,28]. All reported operative patients died from fungal meningoencephalitis[22,23].

The allergic fungal sinusitis occurs in atopic, mostly young, immune-competent adults and presents clinically with a chronic infection[29,30]. It is the most common form of fungal sinusitis. Patients will have nasal polyps and chronic allergic rhinosinusitis, often produce nasal casts, and may occasionally present with proptosis from orbital extension of disease. The histopathology shows extramucosal allergic mucin that stains positive for scattered fungal hyphae and eosinophilic-lymphocytic sinus mucosal inflammation[22,23].

According to histopathological results of our study, 5 patients with fungal pansinusitis can be classified as allergic fungal sinusitis and the other three patients with fungal pansinusitis as chronic invasive sinusitis. On the other hand, one of the two patient with isolated sphenoid sinusitis had granulomatous reaction and can be classified as invasive granulomatous fungal sinusitis. The other patient with sphenoid fungal sinusitis had a purulent fungal mass and bone destruction, a finding typically found in the invasive variant of the sinus mycetoma.

Only 14 cases of isolated fungal infection of the sphenoid sinus have been reported in the literature[10,20,26,27,29,31]. Eight out of those 14 patients were examined in neurosurgical departments because of neurological symptoms and a mass in the sella and sphenoid sinus. Other patients were treated in ENT departments because they presented with nasal symptoms[10,14,26]. All 14 patients were immune-competent and had asprigellosis infection. In our study, the 2 patients with isolated fungal sinusitis were

admitted in neurosurgical ward and both were immune-competent. One patient was referred as a case of pituitary apoplexy and the other as a left temporal abscess. In contrary to reported patients, isolated sphenoid sinus infection in our two patients was caused by mucormycosis.

Siddiqui et al[6] bserved three patterns of presentation in immune-competent patients with craniocerebral aspergillosis : (Type 1) intracerebral aspergillosis which was associated with the worst clinical outcome, (Type 2) intracranial extradural aspergillosis that had an intermediate outcome, and (Type 3) orbital and cranial base aspergillosis that had good recovery. They also observed that preoperative orally administered itraconazole could improve clinical outcome in patients with intracerebral aspergillosis. In our study, the 2 patients with temporal brain abscess would be classified as type 1, and the remaining patients as type 2. The results of our study indicate that combined oral antifungal medication and surgical eradication of the granuloma in immune-competent patients having fungal sinusitis with intracranial extension would be associated with excellent outcome.

Fungal mycotic cerebral aneurysms are rare. As a result, the ideal way of management is not clear. Chun et al[9] reviewed the current multimodality of infectious intracranial aneurysms in general. They concluded that endovascular therapy would be the first option for patients in stable condition with ruptured aneurysms; surgical therapy would be the first option for patients in unstable condition with ruptured aneurysms and the second option for patients in stable condition who experience failure of endovascular therapy. On the other hand, patients with unruptured aneurysms should initially be treated medically and followed up by serial angiography. Medically treated patients with enlarging or dynamic unruptured aneurysms will also require direct surgical or endovascular intervention. We believe that this multimodality management can also be followed in cases with fungal mycotic aneurysms.

The exact mechanisms of fungal sinusitis are not clear, but the environmental load of the fungus and certain host conditions appear to be associated with its invasiveness and disease progression. Immunodeficiency and local tissue conditions, such as allergic mucosal hypertrophy and chronic bacterial sinusitis, can create an obstruction of the ostiomeatal unit and provide favorable conditions that allow the fungus to proliferate and invade[32]. Fungal sinusitis is characterized by invasion of the organism into the vascular endothelium, which leads to subsequent tissue ischemia and necrosis. Fungal extension from the paranasal sinuses into adjacent structures such as the orbit and the intracranial cavity can occur with direct local extension or hematogenous spread[22].

Once the diagnosis of invasive fungal sinusitis is suspected and an otolaryngologic evaluation is obtained, anterior rhinoscopy can be performed to detect any necrosis or ulceration in the mucosa. CT is the best means to assess soft tissue extension and bony erosion, and it should be performed prior to surgery and again afterward as a follow up measure[10,11] Magnetic resonance imaging (MRI), with or without gadolinium, is better at defining intracranial extension, particularly cavernous sinus involvement. A decrease in signal intensity on T1- and a marked decrease in signal intensity on T2-weighted MRI is characteristic of fungal disease[10,11,12]. Although CT and MRI can suggest fungal disease and might alert the physician, a definitive diagnosis can be made only after histological confirmation of the operative specimens.

The choice of surgical approach depends on the site and extent of the involvement. The choice of options is guided by preoperative CT and MR findings, and options vary considerably. In our work we followed a team ENT and neurosurgical approach. Surgical approach was decided according to the relation of the fungal granuloma to the eloquent surrounding neurovascular structures namely; the optic nerves, ICA and cavernous sinus. Endonasal approach alone can be used for lesions not inti-

mately related to these structures. Combined transcranial and endonasal approach has to be used for save eradication of fungal pansinusitis with intracranial extension displacing or surrounding any of these structures. Transcranial approach alone has to be employed for isolated fungal infection of the sphenoid sinus causing major intracranial complications like mycotic aneurysm or brain abscess. No surgical morbidity or mortality was reported in our small group of patients using this surgical protocol.

The combination of aggressive surgical debridement and antifungal therapy has been the cornerstone of treatment of invasive variants of fungal sinusitis[25]. However, debridement carries the risk of inadvertent damage to the orbit, lacrimal system, dura, and brain, which can lead to a loss of vision, injury to the ICA, epiphora, cerebrospinal fluid leakage, and meningitis[30]. As indicated from our study, team approach and selection of surgical approach that will allow debridement under direct vision can minimize these complications. Aphotricin, ketoconazole and itraconazole have been used in the management of fungal sinusitis[19]. We used aphotricin in 2 patients with evidence of meningitis. Ketoconazole was used in the other patients. All survivors had no evidence of recurrence of fungal infection. We think that the use of amphotericin B should be reserved for patients with fungal meningitis to avoid its dose-related renal toxicity.

Conclusion

In summary, team ENT and neurosurgical approach is mandatory for the surgical management of fungal sinusitis having intracranial extension. Safe eradication of this type of fungal infection dictates selection of the surgical approach according to the relation of the granuloma to the optic nerve, ICA and cavernous sinus and according to the presence of associated intradural fungal infection. Fungal granuloma should also be included in the differential diagnosis of lesions in the sellar and parasellar regions.

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SURGICAL PLANNING OF FUNGAL SINUSITIS WITH INTRACRANIAL EXTENSION IN IMMUNE-COMPETENT PATIENTS ACCORDING TO RELATION TO IMPORTANT NEURO VASCULAR STRUCTURES

Montasser A. Foda MD, Kamal Balkheur FRCS(C), Yasser Haroon MD, Mamdouh M. Fadel MD, Ahmad Haroon MD and Hassan Othman MS

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NORMAL D-DIMER COMBINED WITH LOW WELLS SCORE FOR EXCLUSION OF DEEP VENOUS THROMBOSIS: IS IT SAFE?

Khaled El-Alfy MD, Hosam Roshdy MD, Samer Regal MD, Hesham Abd Alla MD and Zeyad Tawheed MD*

*Departments of General & vascular Surgery,*Clinical Pathology,*
Faculty of Medicine, Mansoura University, Egypt

Abstract

Background : *Deep Venous Thrombosis (DVT) is common condition at emergency department and outpatient clinic. The diagnosis is often a challenge because clinical signs and symptoms of DVT are difficult to interpret. A simple Wells pretest probability (PTP) in combination with D-dimer test can eliminate the need for radiological testing or anticoagulant therapy. The aim of this study is assess the effectiveness of low Wells score in combination with normal D-dimer level to exclude DVT in suspected patients at emergency department or outpatients clinics.*

Patients and Methods : *This study was performed between December 2007 and December 2010. at Vascular Surgery Unit, Emergency Hospital, Mansoura University. All patients with a suspected first episode of DVT were potentially eligible. All patients were evaluated using clinical checklist based on the Wells PTP score. Patients were considered to have a High probability if they have 3 or more points, moderate probability: 1-2 points and Low probability: 0 or less points .*

D-dimer tests (Remel, USA, Catalog Number R 30852501) were done to patients with low score, if D-dimer level were normal (less than 1mg/L) no further investigation was performed and patients were discharged from the hospital. Patients with moderate or high score are excluded from the study in which duplex scanning was done. The patients were followed for three months.

Results: *During the period of the study, 1047 patients were consulted for suspicious of DVT. 218 patients (20.8%) were excluded from the*

start according to the exclusion criteria and the remaining 829 patients (79.2%) Wells score done for them. There were 502 females (60.6%) & 327 males (39.4%) with mean age 36 years (28-67 years). Patients with low score are 285 patients (34.48%). There were 198 females & 87 males. In which D-dimer was done. Patients with low score and D-dimer level < 1 mg/L (normal D-dimer) are 75 patients out of 285 patients (26.3%) there were 53 females & 22 male with mean age 42 years.

During 3 months follow up period none of these 75 patients developed DVT.

Conclusion: *A low Wells pre-test probability score in combination with a normal D-dimer (< 1 mg/L) safely excludes Deep Venous Thrombosis at the emergency department and outpatients' clinic.*

Key words: *Deep Venous Thrombosis, D-dimer, Wells Score.*

Introduction

Deep Venous Thrombosis (DVT) is common condition at emergency department and outpatient clinic. The diagnosis is often a challenge because clinical signs and symptoms of DVT are difficult to interpret [(1)].

Deep Venous Thrombosis can be diagnosed accurately by serial duplex ultrasongraphy; however, normal initial duplex ultrasongraphy can not exclude DVT and only 17% to 24% of patients suspected to have DVT actually have the disease so it is not a practical procedure to do serial duplex ultrasongraphy in all patients with suspected DVT [(2)].

The level of D-dimer, a breakdown product of cross-linked fibrin, is elevated in venous thromboembolism (VTE), but it is also elevated in other conditions such as infection, malignancy, and pregnancy. [(3)].

Clinical prediction models combine components of the history and physical examination to categorize a patient's probability of having a disease. The Wells pretest probability (PTP) model is well established for evaluating suspected DVT in symptomatic patients and identifying patients at low risk of DVT. This model originally stratified patients as having a low, moderate, or high

risk to DVT but subsequent analyses showed that the model could be simplified by classifying patients into likely or unlikely risk groups [4].

A simple PTP in combination with D-dimer test can eliminate the need for radiological testing or anticoagulant therapy, So low PTP in combination with negative D-dimer can be safely exclude DVT [5].

However, the algorithm fails when low scores are assigned to patients with VTE or when tests give false-normal D-dimer levels [6]. The latter may be the result of a small thrombus, reduced fibrinolysis, or D-dimer testing at a very early or late stage of the disease [7].

The prevalence of VTE in patients with a low clinical probability according to the Wells score is variable in the literature between is [5-6% or 0.6-1.8%] [8,9]. The reliability of the PTP score also depends on the personal skills of the user, which may reduce its usefulness [10].

The aim of this study is assess the effectiveness of low Wells score in combination with normal D-dimer level to exclude DVT in suspected patients at emergency department or outpatients clinics.

Patients and Methods

This study was performed between December 2007 and December 2010. All patients with a suspected first episode of DVT were potentially eligible for inclusion at Vascular Surgery Unit, Emergency Hospital, Mansoura University.

The exclusion criteria were, pregnancy, life expectancy less than 3 months, history of thrombophilia, ongoing anticoagulation treatment in the past month, previous VTE, duration of symptoms >10 days, symptoms suspicious of pulmonary embolism inability or unwillingness to provide informed consent, Geographic impossibility for follow up

The local ethic committee approved the study.

All patients were evaluated us-

ing clinical checklist based on the Wells PTP score by attending physician. Patients were considered to have a High probability if they have 3 or more points, moderate probability: 1-2 points and Low probability: 0 or less points [11]. (Table 1) .

D-dimer tests (Remel, USA, Catalog Number R 30852501) were done on samples obtained from patients with low score, if D-dimer level were normal (less than 1mg/L) no further investigation was performed and patients were discharged from the hospital. Patients with moderate or high score or a D-dimer level of 1mg/L or higher were not included in the study and further investigations were made according to routine procedure.

Follow Up :

The patients were followed for three months, and the data during this period were obtained by visit or telephone contact with the patient, and instruct the patients to contact their physician when symptoms of their leg worsened or symptoms of pulmonary embolism occurred.

Results

During the period of the study, 1047 patients were consulted for suspicious of DVT.

218 patients (20.8%) were excluded from the start according to the exclusion criteria (Table 2) and the remaining 829 patients (79.2%) Wells score done for them

There were 502 females (60.6%) & 327 males (39.4%) with mean age 36 years (28 - 67 years). Patients with high or intermediate probability are excluded from the study in which duplex scanning was done.

Patients with low score are 285 patients (34.48%). There were 198 females & 87 males. In which D-dimer was done

Patients with low score and D-dimer level < 1 mg/L.(normal level) are 75 patients out of 285 patients (26.3%) there were 53 females & 22 male with mean age 42 years.

During 3 months follow up period none of these 75 patients developed DVT.

Table (1): Pretest clinical probability (Wells' score).

Active cancer	1
Paresis, paralysis or recent plaster or immobilization of lower limb	1
Bedridden > 3 days or major surgery < 4 weeks	1
Localized tenderness	1
Entire leg swollen	1
Calf swelling > 3 cm compared with asymptomatic leg	1
Pitting oedema	1
Collateral superficial veins	1
Alternative diagnosis as likely or greater than DVT	- 2

High probability: 3 or more points. moderate probability: 1-2 points. Low probability: 0 or less points.

Table 2: Reasons for Exclusion in 218 patients.

Reason for Exclusion	**Number**
Pregnancy	23
Previous history of VTE	49
History of thrombophilia	5
The use of anticoagulant in the past months	74
Symptoms suspicious of pulmonary embolism	12
Life expectancy less than 3 months	7
Geographic impossibility for follow up	3
No informed consent obtained	45

Discussion

DVT is a common disease at hospital emergency departments and, if left untreated, has a high complication rate. The symptoms are diffuse and several other diseases have identical clinical signs. Diagnostic exclusion of DVT usually necessitates radiological tests, some of which increase the risk of complications or are not available 24 h a day. Patients sometimes have to return the following day for diagnostic testing, which is time-consuming for the patient and for the emergency departments [12].

Assessment of clinical preprobability scores and the use of D-dimer testing has simplified the diagnostic strategies for DVT and reduced the need for diagnostic imaging. Implementing these strategies into the diagnostic workup lowers costs, reduces inconvenient for the patients and is time-saving for both staff and patients at the emergency departments [13].

The effect of clinical experience on the predictive power of the Well's score in the diagnosis of DVT is a matter of debate. In our study, the diagnostic strategy was handled by physicians only briefly introduced to the Wells clinical assessment score. No specialized research staff or vascular experts were involved in the diagnostic work up of these patients. In spite of this non of our patients was diagnosed with a DVT during follow up. These results are comparable with the results reported by Duoketis 2005 [14].

Another shortcoming of the algorithm is the possibility of false-negative D-dimer test results, which can occur when the testing is performed at an early stage of the disease or when fibrinolysis is decreased [6,8].

Our results show that this simple algorithm is very useful for making a primary decision on whether further investigations are required or not. The probability that patients with a low PTP and normal D-dimer levels have VTE is very low. In our study, none of these patients developed symptomatic VTE within 3 months. Thus, the use of a PTP score and D-dimer levels instead of radiological

tests minimizes costs and inconvenience for both patients and providers of medical services.

The value of clinical assessment and specifically wells score over other clinical scoring systems has been demonstrated by Goodacre et al.[15] in a systematic review.

Other studies also emphasized on usefulness of low Wells score combined with normal D-dimer for exclusion of DVT [16,17], however some studies have widened the exclusion range for low and moderate wells score combined with normal D-dimer to exclude DVT [11].

Conclution

A low Wells pre-test probability score in combination with a low D-dimer (<1mg/L) safely excludes venous thromboembolism at the emergency department and outpatients' clinic.

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NORMAL D-DIMER COMBINED WITH LOW WELLS SCORE FOR EXCLUSION OF DEEP VENOUS THROMBOSIS : IS IT SAFE?

Khaled El-Alfy MD, Hosam Roshdy MD, Samer Regal MD, Hesham Abd Alla MD and Zeyad Tawheed MD

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EFFECT OF ANTIOXIDANT (N-ACETYLCYSTEINE) ADMINISTRATION ON CYCLOSPORINE A-INDUCED RENAL DYSFUNCTION IN RATS

Amr M. Abbas Ph.D and Ayman Z. Elsamanoudy Ph.D*

Departments of Medical Physiology and Medical Biochemistry, Faculty of Medicine, Mansoura University, Egypt*

Abstract

Background and Aim of Work: *Cyclosporin A (CsA) has played an important role in the improvement of patient and graft survival rate in solid organ transplantation. However, nephrotoxicity due to CsA treatment is an important clinical challenge. N-acetylcysteine (NAC) is a potent antioxidant and has been shown to reduce free radical injury. Therefore, the aim of this study was to investigate the effect of NAC treatment on cyclosporine-induced alterations in renal functions, lipid peroxidation, glutathione (GSH) level, and both glutathione peroxidase (GPx) and glutathione-s-transferase (GST) levels and gene expression in adult male rats.*

Material and Methods: *Forty adult male Sprague Dawley rats, weighing 220-240 g, were randomly divided into four groups, 10 animals each: control (olive oil treated), CsA (20 mg/kg, orally), NAC alone (20 mg/kg, intraperitoneally) and CsA + NAC groups (NAC was administered intraperitoneally 30 min before CsA administration). The four groups received the appropriate treatment every day for three weeks. Twenty-four hours after the last treatment, rats were sacrificed and serum was analyzed for urea, uric acid, and creatinine levels. In addition, lipid peroxidation product (malondialdehyde, MDA), and GSH levels, as well as both GPx and GST levels and gene expression in*

kidney samples were determined.

Results: *Cyclosporine treatment produced a significant increase in serum creatinine, urea, and uric acid indicating a cyclosporine-induced impairment of renal functions. Treatment with NAC significantly reduced these changes. GSH level, and both GPx and GST levels and gene expression decreased significantly in kidneys after cyclosporine. Administration of NAC significantly prevented these changes. Lipid peroxidation product (MDA) level in the kidney increased significantly with cyclosporine and these changes were reduced when supplemented with NAC.*

Conclusions: *Increased renal oxidative stress, due to depletion of antioxidants and increased lipid peroxidation, plays an important role in cyclosporine-induced renal damage. N-acetylcysteine supplementation significantly reduced cyclosporine-induced functional impairment of the kidneys. Therefore, concurrent use of antioxidant NAC may be of therapeutic value to minimize cyclosporine-induced nephrotoxicity.*

Key words: *cyclosporine, N-acetylcysteine, nephrotoxicity, glutathione, lipid peroxidation.*

Introduction

Cyclosporine A (CsA) is the most common immunosuppressive drug used for the prevention of allograft rejection[1]. Cyclosporine has improved patient and graft survival rate in solid organ transplantation and has been increasingly applied with considerable clinical benefits in the treatment of autoimmune diseases[2]. Therapeutic benefits of CsA are limited by the occurrence of chronic nephrotoxicity which continues to be a major problem. Acute renal dysfunction with CsA therapy was recognized at the time of its first use in clinical renal transplantation [3]. The exact mechanism of CsA-induced nephrotoxicity remains obscure. However, clinical and experimental studies revealed that several mechanisms may be involved [3,7]. Both in vivo and in vitro studies indicated that the predominant mediators are an altered release of vasoactive sub-

stances such as angiotensin II, endothelin-1, prostaglandins and thromboxanes, nitric oxide, increased sympathetic tone, as well as stimulation of cytokines and growth factors such as transforming growth factor b1 (TGF-b1) and osteopontin, all of which have been implicated in adverse renal effects[5,8]. Cumulative data suggested a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of cyclosporine nephrotoxicity[9,10,11]. Cyclosporine A nephrotoxicity is characterized by progressive renal dysfunction, afferent arteriolopathy and inflammatory cell influx [4]. Several lines of evidence suggest that CsA increases hypoxia, decreases glomerular filtration rate and increases intra-renal vascular resistance [5]. Endothelial dysfunction and reduced activity of endothelial derived nitric oxide might be one of the mechanisms underlining the functional effects of CsA on glomerular filtration [6].

N-acetylcysteine (NAC), introduced as a mucolytic agent for chronic pulmonary diseases[12], is a thiol-containing antioxidant agent. N-acetylcysteine scavenges oxidants directly and replenishes intracellular glutathione[13]. Stimulation of glutathione following administration of NAC results in greater supply of glutathione for detoxification of oxygen free radicals and other foreign substances[14]. There are few reports showing the protective effect of NAC against oxygen free radical mediated injuries in the liver, heart and lungs[12]. N-acetylcysteine has proven to be renoprotective in toxic and ischemic acute renal failure, although results have not been conclusive. It has been reported that antioxidant effects of NAC are able to prevent the increase in plasma peroxynitrite after ischemia and also NAC ameliorates the renal failure induced by inferior vena cava occlusion[15]. N-acetylcysteine is reported to enhance the biological effects of nitric oxide and is known to have positive effects in reversing the haemodynamic disturbances in the renal circulation in acute renal failure [16]. Although there are a large number of antioxidants, including methionine and amifostine, NAC is routinely used in clin-

ical practice in patients with acetaminophen overdose[14,17].

Therefore, the aim of this study was to investigate the effects of NAC on the cyclosporine-induced alterations in renal functions, lipid peroxidation, glutathione (GSH) level, and both glutathione peroxidase (GPx) and glutathione-S-transferase (GST) levels and gene expression in adult male rats.

Material and Methods

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 experimental procedures of the present study were approved by the Medical Research Ethics Committee of Mansoura University, Egypt.

Experimental groups: The rats were randomly divided into four groups of 10 animals each. ***Group I*** (control group) included rats that received vehicle of CsA, (i.e. olive oil) orally for 21 consecutive days. ***Group II*** included rats that were treated with CsA dissolved in olive oil orally (20 mg/kg body weight) for 21 consecutive days [18]. ***Group III*** included rats that received NAC alone intraperitoneally (20 mg/kg body weight) for 21 consecutive days[18]. ***Group IV*** included rats that were treated with CsA (20 mg/kg body weight) and NAC (20 mg/kg body weight) for 21 consecutive days. In group IV, NAC was administered intraperitoneally 30 minutes before CsA administration. Cyclosporine A (Sandimmune from Novartis, Switzerland) was dissolved to give a final concentration of 10 mg/ml. N-acetylcysteine (Sigma-Aldrich, USA) was dissolved to give a final concentration of 10 mg/ml. The drugs were freshly prepared for administration.

Sampling protocol:

Blood samples: Rats were sacrificed by decapitation 24 h after the last dose (on the 22nd day) and blood samples were collected

without anti coagulant, left for 10 minutes, then centrifuged for 10 minutes at 4000 rpm to obtain serum which was stored at -20°C until further analysis for determination of serum urea, creatinine, and uric acid.

Renal sampling: A midline abdominal incision was done and both kidneys were removed.

Biochemical investigations :

Estimation of malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx) and glutathione - s - transferase (GST) in kidney tissue homogenate:

Tissue Homogenate preparation: Renal tissue was perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg / ml heparin to remove any red blood cells and clots. Then, kidney was homogenized in 5 - 10 ml cold buffer (i.e. 50 mM potassium phosphate, pH 7.5. 1 mM EDTA). Homogenates were centrifuged at 10000 x g for 15 minutes at 4°C and the supernatant was kept at -80°C till used for analysis of lipid peroxidation product (MDA), GSH, GPx, and GST levels.

Reduced glutathione (GSH) was determined using a kit supplied by Cayman (Cat. No. 703002, Cayman, USA) according to the manufacturer's instructions. Glutathione peroxidase (GSH-Px) was determined using a kit (Cat. No. NWK-GPX01) purchased from Northwest Life Science Specialties (NWLSS^{TM}) according to the manufacturer's instructions. Glutathione S- transferases (GST) was determined by the kit (Cat. No. # GT 25 18) provided by Bio-Diagnostics, Dokki, Giza, Egypt, according to the manufacturer's instructions. Malondialdehyde (MDA) was analyzed by measuring the production of thiobarbituric acid reactive substances (TBARS) using TBARS assay kit (Cat. No. 10009055, Cayman, USA) according to the manufacturer's instructions.

Estimation of urea, uric acid and creatinine in serum: creatinine, urea and uric acid assays were done by colormetric methods using QuantiChrom Assay Kits (BioAssay Systems. USA) accord-

ing to manufacturer's instructions.

Semiquantitative Reverse transcriptase-PCR

Total RNA extraction from the kidney of rats:

Total RNA was extracted from renal tissue, after homogenization, using TriFast^{TM} reagent (PeqLab. Biotechnologie GmbH, Carl-Thiersch St. 2B 91052 Erlongen, Germany, Cat. No. 30-2010) according to the manufacturer's instructions. The remaining DNA was removed by digesion with DNase I (Sigma). The concentration of isolated RNA was determined spectrophotometrically by measuring the optical density (OD) at 260 nm (Jenway, Genova Model, UK). 10ul of each sample was added to 990ul of DEPC treated water and quantified by measuring the absorbance at 260nm as RNA yield (ug/ml) = A260 X 40 X 100 (dilution factor) [19]. The purity of RNA was determined by gel electrophoresis through agarose gel electrophoresis and ethidium bromide staining to show 2 sharp purified bands, these two bands represented 28S and 18S ribosomal RNA.

RT-PCR for extracted RNA:

RT-PCR was performed using Ready-to-Go. RT-PCR beads for first cDNA synthesis and PCR reaction provided by Amersham Biosciences, England. Cat. No. 27-9266-01, according to the method of Berchtold [20].

Ready-to-Go RT-PCR beads utilize Moloney Murine leukemia virus (M-MuLV) reverse transcriptase and Taq polymerase to generate PCR product from RNA template. Each bead is optimized to allow the first strand cDNA synthesis and PCR reaction to proceed sequentially as a single tube, single step reaction. The reaction passed as follow:

A) Synthesis of cDNA:

The followings were added to each tube containing the beads:

2 µl of first strand primer, provided by the kit, 3 µl containing 30 pmol of PCR gene-specific primer (sense), 3µl containing 30 pmol of PCR gene-specific primer (anti-sense), 25 µl of total template RNA containing 1ug and 17µl of DEPC-treated water to obtain a total volume of 50 µl. One

tube was prepared as a negative control reaction to test for DNA contamination.

The dehydrated bead (without template and primers) was incubated at 95°C for 10 minutes to inactivate the M-MuLV reverse transcriptase. 50 ul mineral oil were added to overlay the reaction. The reactions were transferred to the thermal cycler and incubated at 40°C for 30 minutes for synthesis of cDNA followed by incubation at 95°C for 5 minutes to inactivate the reverse transcriptase and completely denature the template.

Gene specific primers used were:

Gene specific primers were purchased from Biolegio. BV, PO Box 91, 5600 AB Nijmegen, Netherlands.

Gene	Primer	Reference
Glutathione-s-transferase	Forward: 5'-CTGGGCATGATCTGCTACAATC-3' Reverse: 5'-CAAAAGTGATCTTGTTTCCTGCAA-3'	21
Glutathione peroxidase	Forward : 5′ -CTCTCCGCGGTGGCACAGT-3' Reverse : 5′-CCACCACCGGGTCGGACATAC -3'	22
Internal house keeping (control) gene (GAPDH)	Forward: 5`- ACCACAGTCCATGCCATCAC-3`. Reverse: 5`- CACCACCCTGTTGCTGTAGCC-3`.	23

B) Amplification of cDNA by PCR:

Thermal cycling reaction was performed using thermal cycler (Minicycler PTC-150 with the following program:

	GST	GPx	GAPDH
Initial denaturation	94◦C for 5 minutes	95◦C for 3 minutes	94◦C for 5 minutes
Cycles			
Number	30	30	25
-denaturation	94 ◦C for 30 seconds	94 ◦C for 30 seconds	94 ◦C for 1 minute
-primer annealing	60◦C for 1 minute	58◦C for 30 seconds	55◦C for 1 minute
-Extension	68 ◦C for 2 minute	72 ◦C for 1 minute	72 ◦C for 1 minute
Final extension	68 ◦C for 7 minutes	72 ◦C for 3 minutes	72 ◦C for 7 minutes
Product size	136 bp	290 bp	577 bp

C) Detection of amplified RT-PCR products:

For semiquantitative RT-PCR, the products of amplification were subjected to agarose gel electrophoresis using 2% agarose stained with ethidium bromide and visualized via light UV Transilluminator (Model TUV-20, OWI. Scientific, Inc. 800 242-5560) and photographed under fixed conditions (the distance, the light and the zoom).

The results photos were analyzed with scion image® release Alpha 4.0.3.2. software for windows® which performs bands detection and conversion to peaks. Area under each peak were calculated in square pixels and used for quantification. Gene expression levels were determined by calculating the ratio between the square pixel value of the target gene in relation to the internal house keeping control gene (GAPDH).

Minus RT controls permitted to rule out genomic contamination. Similarly, no products were detected when the RT-PCR step was carried out with no added RNA, indicating that all reagents were free target sequence contamination.

Statistical analysis:

The data were expressed as mean ± standard deviation of mean (Mean±SDM). Data were processed and analyzed using the Stastical 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

There was a significant increase in serum creatinine, urea and uric acid levels in rats treated with CsA alone for three weeks compared to control or NAC alone groups ($p < 0.05$) (Table 1). Treatment with NAC alone did not cause any significant change in serum creatinine, urea and uric acid levels compared to control groups ($p>0.05$) (Table 1). When NAC was treated with CsA it produced a significant decrease in these blood parameters, compared to the CsA alone group ($p < 0.05$). However, the level of these parameters remained significantly higher

than the control and NAC alone groups ($p < 0.05$) (Table 1).

Renal GSH level (Table 2), as well as both GPx, and GST levels (Table 2) and gene expression (Table 3, Figures 1,2) were significantly decreased in the CsA alone group compared to the control and NAC alone groups ($p < 0.05$). N-acetylcysteine treatment alone significantly increased ($p < 0.05$) the renal GSH level (Table 2) and both GPx and GST levels (Table 2) and genes expression (Table 3, Figures 1,2) when compared to the control group. Treatment by NAC with CsA significantly increased the renal GSH level (Table 2) and both GPx and GST levels (Table 2) and genes expression (Table 3, Figures 1,2) compared to the CsA alone group ($p < 0.05$) and the increase was significantly more than the control group levels ($p < 0.05$). No significant difference in the renal GSH level (Table 2) and both GPx and GST levels (Table 2) and genes expression (Table 3, Figures 1,2) was seen when the NAC with CsA treated group was compared with the NAC alone group ($p>0.05$). Compared to CsA alone, the significant increase in the renal GSH level (Table 2) and both GPx and GST levels (Table 2) and genes expression (Table 3, Figures 1,2) in the NAC with CsA group, indicates the significant antioxidant effect of NAC.

Renal Malondialdehyde (MDA) level increased significantly with CsA treatment when compared to control and NAC alone groups ($p < 0.05$) (Table 2). Treatment with NAC alone was able to reduce the MDA compared to control rats ($p < 0.05$) (Table 2). N-acetylcysteine treatment along with CsA decreased the high renal MDA level significantly ($p < 0.05$). However, MDA in the kidneys of rats treated by NAC with CsA remained significantly higher than in the NAC alone group ($p < 0.05$) but there was no statistically significant difference in renal MDA level between the control and the NAC with CsA groups, indicating the nullifying effect of NAC on CsA toxicity (Table 2).

Table (1): Effect of N-acetylcysteine (NAC) on serum urea, uric acid and creatinine in cyclosporine A (CsA) treated rats:

	Serum urea (mg/dl)	Serum uric acid (mg/dl)	Serum creatinine (mg/dl)
Control	12.11±0.58	3.11±0.12	1.86±0.1
CsA	27.55±1.71[a]	7.32±0.21[a]	3.98±0.12[a]
NAC	10.93±0.61[b]	2.83±0.21[b]	1.62±0.1[b]
NAC+CsA	16.15 ±0.5[abc]	4.51±0.15[abc]	2.51±0.11[abc]

All values were expressed as mean ± SD (n = 10). Statistical significance between means was performed using one-way ANOVA followed by Tukey´s post hoc test.

a: significant ($p < 0.05$) compared with control group, b: significant ($p < 0.05$) compared with CsA group, c: significant ($p < 0.05$) compared with NAC group.

Table (2): Effect of N-acetylcysteine (NAC) on malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), glutathione-s-transferase (GST) in kidney of cyclosporine A (CsA) treated rats:

	MDA (nmol/g tissue)	GSH (nmol/g tissue)	GPx (mol/g tissue)	GST (U/g tissue)
Control	1.4 ± 0.3	245.4±4.2	0.91 ±0.03	221.5±5.5
CsA	4.2 ± 0.2[a]	57.6±2.8[a]	0.31 ± 0.02[a]	71.7±3.1[a]
NAC	0.7±0.1[ab]	392.4±5.1[ab]	1.9±0.1[ab]	375.3±4.7[ab]
NAC+CsA	1.9±0.3[bc]	381.2±5.2[ab]	1.7±0.1[ab]	361.4±4.5[ab]

All values were expressed as mean ± SD (n = 10). Statistical significance between means was performed using one-way ANOVA followed by Tukey´s post hoc test.

a: significant ($p < 0.05$) compared with control group, b: significant ($p < 0.05$) compared with CsA group, c: significant ($p < 0.05$) compared with NAC group.

Table (3): Effect of N-acetylcysteine (NAC) on GPx, and GST genes expression in kidney of cyclosporine A (CsA) treated rats:

	GPx/GAPDH mRNA	GST/GAPDH mRNA
Control	1.2±0.06	1.8±0.07
CsA	0.3±0.02[a]	0.6±0.03[a]
NAC	3.4±0.1[ab]	5.1±0.2[ab]
NAC+CsA	3.2±0.1[ab]	4.9±0.2[ab]

All values were expressed as mean ± SD (n = 10). Statistical significance between means was performed using one-way ANOVA followed by Tukey´s post hoc test.

a: significant ($p < 0.05$) compared with control group, b: significant ($p < 0.05$) compared with CsA group, c: significant ($p < 0.05$) compared with NAC group.

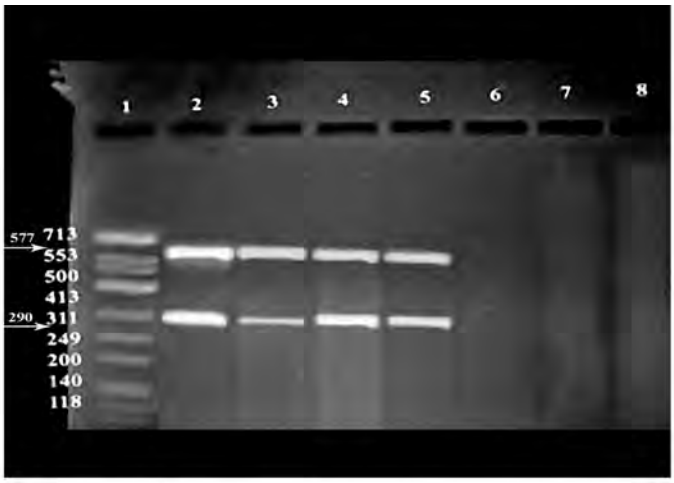

Figure (1): RT-PCR product of glutathione peroxidase (GPx) gene expression in relation to GAPDH in rats. Lane 1: DNA marker, **Lane 2:** control rats, **lane 3:** cyclosporine A(CsA)-administered rats, **Lane 4:** N-acetylcysteine (NAC)-treated rats, **lane 5:** rats treated with CsA and NAC, **lane 6:** negative control.

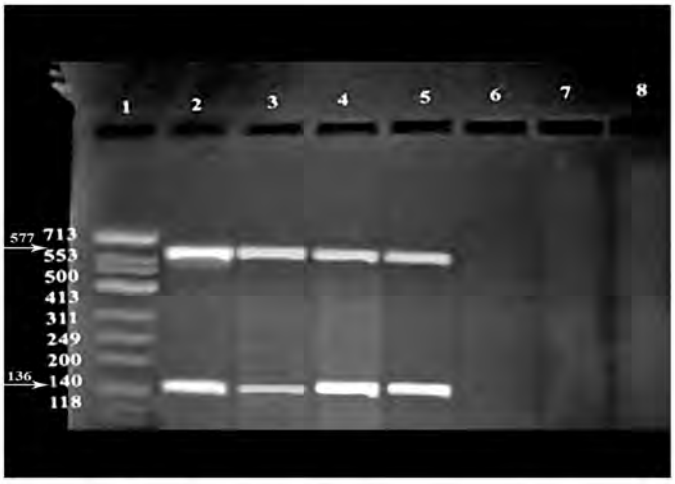

Figure (2): RT-PCR product of glutathione-S-transferase (GST) gene expression in relation to GAPDH in rats. Lane 1: DNA marker, **Lane 2:** control rats, **lane 3:** cyclosporine A (CsA)-administered rats, **Lane 4:** N-acetylcysteine (NAC)-treated rats, **lane 5:** rats treated with CsA and NAC, **lane 6:** negative control.

Discussion

This study confirms the protective effect of NAC, through its antioxidant activity, against renal impairment caused by administration of CsA in rats.

In the present study, treatment of rats with CsA for a period of three weeks resulted in a significant increase in serum urea, creatinine, and uric acid levels (Table 1), suggesting significant functional impairment in the kidneys. These observations are in agreement with earlier studies where significant alterations in the level of blood urea nitrogen (BUN) and creatinine in patients and experimental animals were reported following chronic CsA treatment [10,24].

The current work showed that treatment of rats with NAC alone did not cause any significant change in serum urea, creatinine and uric acid levels as compared to control group (Table 1). Moreover, when NAC was administered to the CsA-treatment group, there was a significant decrease in the level of serum urea, uric acid and creatinine compared to the CsA treatment group suggesting a significant improvement in renal function in those rats by NAC administration (Table 1). However, the level of these substances remained higher than the normal control and NAC alone groups (Table 1), indicating that NAC could not totally nullify the effect of CsA on renal function. Therefore, concomitant treatment of CsA administered rats with NAC attenuated the CsA-induced functional changes in the kidneys. These results are in agreement with those of previous studies [18, 25].

Also, treatment of rats with CsA for 3 weeks produced a significant increase in lipid peroxidation product (MDA) as well as decrease in GSH level (Table 2) and both GPx and GST levels (Table 2) and gene expression (Table 3, Figures 1,2) suggesting the role of oxidative stress in CsA-induced nephrotoxicity. Our results are in accord with those reported by other studies[11,26,27,28]. In addition, Zhong et al, [29] demonstrated that free radicals are dramatically increased in rat kidney after CsA treatment. Hagar[30] reported that treatment with CsA increased

oxygen free radical production. Furthermore, it has been reported that the drug induces membrane lipid peroxidation in several experimental models[31], as well as in transplanted patients[32]. Moreover, Haleagrahara et al[18] and Tariq et al[33] demonstrated that CsA-induced oxidative stress could play pivotal role in producing functional impairment of kidney. The decrease in renal GSH level (Table 2) and both GPx and GST levels (Table 2) and gene expression (Table 3, Figures 1,2), with increased renal MDA (Table 2), serum urea, uric acid and creatinine (Table 1) following CsA, observed in this study, greatly supported this hypothesis. Oxygen radicals are considered as important modulators of renal blood flow and glomerular filtration rate [34].

To explain a link between CsA treatment and production of reactive oxygen species (ROS), several hypotheses have been proposed [35] and these include (i) CsA-induced upregulation of the cytochrome P450-dependent system in the kidneys [36]; (ii) CsA may uncouple cytochrome P-450, directing electron flow to molecular oxygen and thereby generating free radical [33]; (iii) A possible direct interference of the drug with the intracellular homeostasis of glutathione has been suggested[31,32,35]. In vitro and in vivo studies indicate that CsA reduces renal reduced / oxidized glutathione ratio in the kidneys[18,35]. This is confirmed by our results that showed a significant decrease in GSH level in CsA treated rats (Table 2); and (v) Perturbation of the balance between vasodilation and vasoconstriction.[31,32]. Studies in CsA-treated animals and patients suggest that CsA-induced vasoconstriction is the underlying cause of its nephrotoxicity[37]. The CsA-induced renal vasoconstriction can be explained by several mechanisms; (i) An increased sympathetic activity [38], (ii) Stimulation of the renin-angiotensin system with increased angiotensin II production[9], (iii) increased release of the potent vasoconstrictor endothelin (ET-1) from the vascular endothelium[39] (iv) An increased expression of angiotensin II and endothelin receptors [40] in vascular tissue of CsA-treated rats, (v)

Increased renal thromboxane A2 formation [32], (vi) Decreased production of vasodilator agents such as prostacyclin and nitric oxide [27]. In addition, CsA brings about vasoconstriction in isolated renal arterioles by direct actions[41]. CsA-induced renal vasoconstriction could lead to renal hypoxic injury and production of reactive oxygen free radicals[26,37,42]. In agreement with these findings, the results of the current paper showed that renal MDA level was significantly increased in rats treated with CsA (Table 2).

Free radicals and other oxygen-derived species are constantly generated in vivo and cause damage to DNA, lipids, proteins, and other biomolecules[43]. Normally, tissues have defense antioxidant mechanisms that protect them against the toxic effects of free oxygen radicals[43,44]. Endogenous sulph-hydryl compounds play an important role by acting as a metabolic buffer against oxidant species and serve as a natural reservoir of reducing power which can be quickly used in the cell as a defense against oxidative stress[45]. Glutathione, sulphur-containing nucleophilic substance, is considered to be the most important water soluble antioxidant within cells which is critical to normal cellular physiological processes [43,46]. It is found in high concentration in kidney. It plays a central role in protecting cells from oxygen-derived free radicals injury and other activated toxic compounds[35,47]. Tariq et al,[33] reported that renal GSH decreased following CsA administration. In addition, decreased total antioxidants after CsA treatment proves that there is increased production of oxygen free radicals and endogenous antioxidants are being used up for scavenging the wide variety of these free radicals including O_2^-, H_2O_2 and OH^- [48]. These studies are confirmed by our results which showed that renal MDA significantly increased whereas GSH level (Table 2), as well as both GPx and GST levels (Table 2) and gene expression (Table 3, Figures 1,2) decreased after CsA administration, therefore rendering the renal cells more susceptible to the oxidative stress caused by CsA.

In previous work with cultured

hepatocytes, Vitamin E, an effective antioxidant, totally prevented CsA cytotoxicity as well as lipid peroxidation and increased the activity of several endogenous antioxidant enzymes[32]. In addition, vitamin E has been shown to protect rats against CsA-induced nephrotoxicity[31]. Also, in a study by Chen et al[49], they demonstrated that NAC prevented ifosfamide-induced nephrotoxicity. Consistent with these findings, our study showed that treatment of CsA administered rats with NAC significantly decreased serum urea, creatinine and uric acid as compared with CsA alone group ($p< 0.05$) (Table 1), suggesting that NAC attenuated the CsA-induced functional impairment of the kidneys. The mechanism of action of NAC in reducing renal damage caused by CsA is not clear[50]. Protective effects of NAC against oxidative stress induced renal damage might involve various chemical mechanisms. N-acetylcysteine directly scavenges oxygen free radicals as superoxide radicals [14,49]. As a precursor of glutathione synthesis NAC is an ideal candidate for replenishing tissue GSH levels[14,15,45]. These observations were confirmed by the results of the current study which demonstrated that treatment by NAC along with CsA increased the GSH level (Table 2) and both GPx and GST levels (Table 2) and gene expression (Table 3, Figures 1, 2) in the kidneys. Also administration of NAC with CsA decreased the lipid peroxidation product (MDA) level in the kidney (Table 2). Moreover, treatment of rats with NAC alone caused a significant increase in the GSH level (Table 2) as well as both GPx and GST levels (Table 2) and gene expression (Table 3, Figures 1, 2) in the kidney compared to control rats. In addition, NAC alone decreased the lipid peroxidation product (MDA) level in the kidney (Table 2) compared to control rats. Therefore, marked protective effects of NAC administration against CsA-induced renal damage may be strongly associated with amelioration of the effects of oxidative stress. Our results were in accord with those of Efrati et al,[16] and Haleagrahara et al.[18].

On the other hand, NAC has

been shown to decrease renal vascular resistance and enhance tissue perfusion [51]. The vasodilating effects of NAC may be attributed to its direct relaxing action on vascular smooth muscle and its ability to inhibit angiotensin-converting enzym [52]. In addition, NAC stimulates the release of NO, which in turn may decrease CsA - induced vascular resistance[53]. This decrease in vascular resistance with enhanced tissue perfusion, by NAC, reduces the formation of oxygen free radicals and minimizes the CsA-induced cellular damage in renal tissues[51]. In agreement with these findings, our results demonstrated reduced serum urea, creatinine, uric acid (Table 1), renal MDA (Table 2) and increased the GSH level (Table 2) as well as both GPx and GST levels (Table 2) and gene expression (Table 3, Figures 1, 2) in the kidney after NAC treatment of CsA administered rats. Furthermore, CsA-induced nephrotoxicity is associated with accumulation of cellular calcium, and calcium channel blockers have been shown to reduce CsA-induced kidney damage. N-acetylcysteine have also been shown to block calcium channels, maintain calcium homeostasis and improve renal function [54].

In conclusion, NAC could prevent the functional renal impairment of CsA by increasing the GSH level as well as both GPx and GST levels and gene expression and reducing the lipid peroxidation product (MDA) in the kidney. In addition, our study confirms the hypothesis that oxidative stress is the main cause of toxic renal damage induced by CsA, and NAC, through its marked antioxidant activity, significantly reduces this renal damage.

Further studies are still required for N-acetylcysteine to be considered for pharmacological therapy for cyclosporine-induced nephrotoxicity in humans.

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EFFECT OF ANTIOXIDANT (N-ACETYLCYSTEINE) ADMINISTRATION ON CYCLOSPORINE A-INDUCED RENAL DYSFUNCTION IN RATS

Amr M. Abbas Ph.D and Ayman Z. Elsamanoudy Ph.D

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EARLY OUTCOME OF MOBILE-BEARING VERSUS FIXED-BEARING TOTAL KNEE ARTHROPLASTY A PROSPECTIVE RANDOMIZED STUDY

Mostafa A. El-Khalik MD, Yehia E. Bassiony MD, Akram A. Hammad MD and Mahmoud A. El-Ghafar MSc.

Departments of Orthopaedic

Faculty of Medicine, Mansoura University, Egypt

Abstract

Background: *Proponents of mobile-bearing total knee arthroplasty believe that it has potential advantages over a fixed bearing design in terms of diminished wear, aseptic looseness, and improved motion and/or function, but these advantages have not been demonstrated in a randomized clinical comparison. We conducted a patient-blinded, prospective, randomized clinical trial to compare mobile-bearing and fixed-bearing designs.*

Methods: *one hundred twenty knee arthroplasty were prospectively randomized to receive a mobile bearing design or a fixed-bearing design. There were no significant differences in the demographic characteristics (mean age, 56.93 years; mean body mass index, 33.96 kg/m2, The preoperative diagnosis was osteoarthritis in 77.5%,and inflammatory arthritis in 21.2%) or preoperative clinical or radiographic measures between the groups. Routine clinical and radiographic follow-up measures included the Knee Society score (KSS) outcome measures.*

Results: *The results of 120 arthroplasties (60 with a fixed bearing and 60 with mobile-bearing designs) with follow up of two minimum years (mean, thirty months) postoperatively. Although there was significant improvement in both groups, there was no significant difference between the groups with regard to the mean postoperative range of motion (111.9 and 110.4,; p = 0.08), the mean KSS clinical score (91.50 and 88.35 points; p = 0.12), or the mean KSS pain score (76.50 and*

73.42 points; p = 0.19) respectively at this follow-up point. There were three revisions: two because of infection, and one because of aseptic loosening.

Conclusions: *Neither design showed a clear superior advantage. No significant difference between fixed-bearing and a mobile-bearing design with regard to the range of motion, KSS clinical or functional scores, radiographic parameters, or revisions due to aseptic loosening in the reported time frame.*

Introduction

Indications of total knee arthroplasty continue to expand as advances in implant design and surgical technique have improved the outcomes. The ability to predictably achieve excellent 10- to-15 year outcomes with fixed bearing TKA[1,2,3,4] has encouraged many surgeons to consider performing TKA on younger patients with high activity level. Patient expectations for a more functional and long lasting result following TKA continue to drive advances in both surgical technique and component design.

Current total knee prosthesis devices can be subdivided into two groups based on different fundamental design principles: fixed-bearing knees (FBK), where the polyethylene tibial insert locked with tibial tray, and mobile-bearing designs (MBK) which facilitate movement of the insert relative to the tray [5].

Implant loosening and polyethylene wear in fixed-bearing knee prostheses were recognized as major causes of late failure. Fixed-bearing prosthesis with a high conformity bearing surface provides low contact stress, but produces high torque at the bone-implant interface predisposing to component loosening. Conversely, prosthesis with a low conformity bearing surface produces less constraint force that decreasing component loosening, but generates high contact stress leading to early failure of the polyethylene [6,7]. Furthermore, the kinematic conflict between low-stress articulations and free rotation cannot be solved by any fixed-bearing knee design [8].

Mobile bearing total knee arthroplasty systems is emerging as the next wave of development in knee joint prosthetic reconstruction, and as a new concept that progressed over the last three decades to address the demands of improved function and longevity for the younger patient. Mobile-bearing knee prosthesis was introduced with the aim to reduce polyethylene wear and component loosening[9]. The mobile-bearing design provides both congruity and mobility in the tibiofemoral bearing surface. This allows low contact stress and low constraint force to improve wear resistance and, theoretically, to minimize loosening[7]. In addition, the mobile-bearing knee also solves the kinematic conflict of fixed-bearing knee because a high conforming articular surface can now coexist with free rotation[7].

Additional theoretical advantages to a rotating-platform design include the ability to compensate for small errors in the rotational alignment of the components, an increase in the range of motion of the knee resulting from the tibia being allowed to rotate during higher degrees of flexion, and the possibility of reducing nonarticular surface wear by using a polished forged cobalt-chromium baseplate rather than locking an insert to a baseplate made of cast titanium or other materials[10,11]. Disadvantages of the mobile-bearing design include bearing dislocation[12], higher cost, and higher gravimetric wear in simulator studies?[13].

This randomized prospective comparison of a mobile-bearing design with a fixed-bearing design was initiated to answer important questions about clinical and radiological results and value after total knee arthroplasty. Does a mobile-bearing design offer improvement over a fixed-bearing design with regard to clinical and radiological characteristics?

Materials and Methods

The present study included 88 patients (120 arthroplasties) suffering of painful arthritis of the knee joint with osteoarthritis and rheumatoid arthritis in the period between November 2006 and July 2009. They scheduled for primary TKA, for participation in a pros-

pective, patient blinded, randomized clinical trial, to compare between mobile bearing and fixed bearing TKA. Patients with substantial angular deformity that clearly required an osteotomy or use of a more constrained design were excluded, as were those in whom bone loss necessitated structural grafting or modular augmentation. If it was unclear whether these options would be necessary, the patient was routinely randomized to receive one of the operations under study and then excluded from the analysis only if the intraoperative findings dictated the use of a procedure other than the one to which the patient had been assigned. All patients provided informed consent. No patient refused to participate. Patient randomization was performed one day before surgery and was accomplished with use of a randomized numbers table. Patients with even numbers were assigned to the fixed design and patients with an odd number were assigned to receive the mobile-bearing design. Fixed bearing total knee prosthesis (FBK) was implanted in 60 knees and mobile-bearing total knee prosthesis (MBK) was implanted in 60 knees.

The mean age of the patients at the time of the index surgery was 56.93 ± 6.99 years (range of 25-70 years), and the mean body mass index was 33.96 ± 4.58 kg/m^2 (range, 26 to 50 kg/m2). The preoperative diagnosis was osteoarthritis 93 (77.5%) cases of the 120 knees, inflammatory arthritis in 25 (21.2%) cases, and posttraumatic arthritis in 2 (1.7%) cases. Preoperatively, there was no significant difference between the two populations regarding age, body mass index, sex, comorbidities, diagnosis, range of motion, radiographic alignment, knee society score (KSS) (Tables 1 through 3) All the procedures were performed through a standard medial para-patellar approach. The prosthesis used was either posterior cruciate retaining (PCR) or posterior stabilized prosthesis (PS) in either design of study. Tabial and femoral components were cemented, and no patellar components were implanted. All cases were staged and not simultaneously done. The patients were blinded to the implant used at the surgery.

Standard operative techniques were used; the femoral component was aligned in 3° of external rotation relative to the posterior condylar axis or in neutral rotation relative to the transepicondylar axis with use of an intramedullary guide, and the tibial cuts were aligned with an extramedullary system with 0 slope. All operations were performed by senior authors. Perioperative antibiotic prophylaxis, prophylaxis against deep venous thrombosis, postoperative nursing care, and rehabilitative protocols were identical for the two groups.

Patients were evaluated clinically and radiographically according to knee society clinical and radiological rating system[14,15]. A knee society system (KSS) was assigned preoperatively; at six weeks, six months, and twelve months; and annually thereafter. The mean duration of FU was 30 months (range, 24-42 months), with patient's scores at 24 months follow up were used for comparison between two groups. The range of motion of the knee was usually recorded by using a goniometer and referencing standard landmarks with the patient seated. Anteroposterior and lateral radiographs were made with the patient standing and were evaluated for anatomical alignment, and the presence and size of any radiolucent lines. These radiographs were not made with fluoroscopic guidance, and as a result radiolucent zones were probably underestimated.

Statistical Methods

Statistical evaluation was performed using independent sample t test or chi-square test. Statistical significance was considered for P values less than 0.05.

Results

Clinical Results

Results for each parameter of the Knee Society scoring system for both groups obtained from the clinical evaluation at two years follow up shown in table 2.

Neither design showed a clear clinical advantage. The KSS pain and clinical scores did not differ significantly ($p = 0.34$ and $p = 0.12$) between the groups, although both improved markedly

($p < 0.0001$) from the preoperative values. The KSS functional score also did not differ significantly ($p=0.19$) between the groups, although it improved significantly compared with the preoperative value ($p<0.001$). Either design demonstrated a superior postoperative range of motion as measured with a goniometer with no significant difference between them. The preoperative flexion contracture (mean, 3.88) improved significantly (to a mean of 1, $p=0.001$) in both groups.

Fixed bearing group had 98.3% Success rate with 95% excellent and good results (48.3% excellent, 46.7% good) and 3.3% fair results. Poor result was 1.7%. Mobile bearing group had 95% Success rate with 93.3% excellent and good results (35% excellent, 58.3% good) and 1.7% fair results. Poor result was 5%. Neither design demonstrated a superior Success rate and no significant difference between them ($p=0.33$). Three cases were candidates for revision (2.5%); two cases due to deep infection that required a two-stage reimplantation and one case failed by aseptic implant loosening.

Radiographic Results

Radiographic parameters that were measured did not vary significantly between the fixed bearing and mobile bearing designs (table 3).

Preoperative anatomic alignment ranged from 40 anatomical varus to 30 anatomical valgus (mean, 10.5^{o} of varus), this improved post operatively to an average of 5.63 degrees of anatomical valgus, alignment of the femoral component in the coronal and sagittal plane, alignment of the tibial component in the sagittal and coronal planes, and patellar tracking were similar between the two designs. Fixed bearing group had 18 cases (15.3%) with tabial component radiolucencies and 3 cases (2.5%) with femoral component radiolucencies. Mobile bearing group had 22 cases (17%) with tabial component radiolucencies and 3 cases (2.5%) with femoral component radiolucencies. radiolucent lines were similar between either designs.

Table (1): patient's characteristic.

Patent characteristic	Fixed bearing group (N. 60 cases)	mobile bearing group (N. 60 cases)	P value*
Age (yrs)	*56.35 ± 6.85*	*57.52 ± 7.15*	*.36*
Sex (male/female %	*18 / 82*	*10 / 90*	.19
Side (right /left %)	*57 / 43*	42 /58	.10
Body mass index(kg/m2)	*33.55 ± 4.36*	*34.37 ± 4.79*	.33
Underlying diagnosis (%)			
✓ Primary osteoarthritis	*76*	78	.76
✓ Inflammatory arthritis	*22*	20	
✓ Post traumatic O.A.	*2*	2	
Duration of symptoms (yrs)	*7.55 ± 4.42*	*9.95 ± 4.49*	.01
Activity level (%)			.50
✓ Bedridden	18	22	
✓ Semi-sedentary	5	10	
✓ Sedentary	60	58	
✓ Light labor	10	6.7	
✓ Moderate labor	7	1.7	
✓ Heavy labor	0	1.7	
Medical co morbidity (%)			,97
✓ No	43	42	
✓ Mid	35	35	
✓ moderate	22	23	
Patient category (%)			.41
✓ unilateral	*28*	20	
✓ bilateral	*52*	63	
✓ c -multiples	*20*	17	

*The comparisons were made with use of the independent sample t test or chi-square test as appropriate. †The values are given as the mean and standard deviation.

Table (2): ***Preoperative and postoperative clinical evaluation.***

Knee score		**Fixed bearing group (N. 60 cases)**	**Mobile bearing group (N. 60 cases)**	**P value***
Pain				
✓ *Preoperative*		*17.67 ± 5.63*	*17.83 ± 5.55*	*0.87*
✓ *postoperative*		46.92 ± 2.62	45.92 ± 7.62	*0.*34
Range of motion		*18.97 ±*		
✓ *Preoperative*		*18.4± 4.50*	*18.40 ± 4.17*	*0.48*
✓ *postoperative*		22.03± 1.57	21.40 ± 2.29	0.08
Stability:				
✓ Anteroposterior				
✓ *Preoperative*		*5.26 ± 2.67*	*4.50 ± 2.72*	*0.13*
✓ *postoperative*		9.91 ± 0.64	9.33 ± 1.95	030
✓ Mediolateral				
✓ *Preoperative*		*6.08 ± 3.81*	*5.42 ± 4.04*	*0.35*
✓ *postoperative*		14.67 ± 1.26	14.25 ± 2.02	0.18
deduction	*Flexion contracture*			
	• *Preoperative*	*3.88 ± 5.68*	*3.85 ± 5.35*	*0.97*
	• *postoperative*	.82 ± 2.89	.73 ± 2.42	0.86
	Extension lag			
	• *Preoperative*	*.67 ± 2.34*	*.67 ± 2.83*	*1.00*
	• *postoperative*	.50 ± 2.72	.08 ± .65	0.25
	malalingment			
	• *Preoperative*	*17.03 ± 6.84*	*14.40 ± 8.75*	*0.07*
	• *postoperative*	.50 ± 2	.08 ± .65	0.25
Total knee scoring				
• *Preoperative*		*27.23 ± 14.97*	*27.65 ± 15.56*	*0.88*
• *Postoperative*		91.50 ± 6.52	88.35 ± 14.15	.012
• *P. value*		0.00	0.00	
Function score	Walking distance			
	• *Preoperative*	18.00 ± 9.35	16.67 ± 8.96	0.43
	• *postoperative*	42± 5.46	39.83 ± 8.13	0.09
	Stair climbing			
	• *Preoperative*	19.58 ± 14.59	19.50 ± 13.89	0.94
	• *postoperative*	37.17± 7.83	35.67 ± 6.98	0.27
	Deduction			
	• *Preoperative*	5.08 ± 5.93	4.33 ± 4.91	0.45
	• *postoperative*	2.58 ± 2.52	2.42 ± 3.38	0.76
	Total function score			
	• *Preoperative*	33.42 ± 23.44	32.17± 21.20	0.76
	• *Postoperative*	76.50 ± 11.91	73.42± 13.92	0.19
	• *P. value*	0.00	0.00	

*The comparisons were made with use of the independent sample t test or chi-square test as appropriate. †The values are given as the mean and standard deviation.

Table 3: Preoperative and postoperative radiological evaluation.

Radiographic parameters		Fixed bearing group (N. 60 cases)	mobile bearing group (N. 60 cases)	P value
axial	preoperative	10.6°of varus (SD±14.6)	10.5 °ofvarus(SD±14.6)	0.96
	Postoperative **(angle omega Ω)**	6 °of valgus (±1.56)	5.27 ° of valgus (±2.85)	0.84
Femoral component	Coronal **(alpha angle, ά)**	96.62°(± .76).	96.50°(± 1.05)	0.49
	Sagittal **(angle gamma γ)**	.18 ° (±SD .62)	.43 °(± 1.36	0.19
Tabial component	coronal **(angle beta β)**	89.37 ° (±SD 1.57)	88.58 °(±SD 2.81)	0,06
	Sagittal **(angle sigma σ)**	89.8 ° (±SD .75)	89.92° (±SD .93)	0.32
Patellar tracking	✓ normal ✓ Tilt ✓ sublaxation	55 cases (8.33%) 2cases (3.33%) 3 cases (5%)	54 cases (90%) 2 cases (3.33%) 4 cases (6.7%)	0.76
radiolucent line	✓ tabial components ✓ femoral components	15 .3% 17 %)	2.5% 2.5%	0.43

Discussion

The results of fixed-bearing TKA have been successful with long-term survival rates of approximately 95% at 10 to 15 years of FU 1, 2. These results were obtained in an elderly population with low activity levels. Problems of wear and loosening become an important concern especially when the indication to TKA was expanded to a younger, more active population[16,17]. Mobile-bearing knee arthroplasty was introduced in the late 1970s encouraged by several potential advantages compared with fixed-bearing TKA[18,19]. This knee arthroplasty design aimed to reduce surface and undersurface bearing stresses and at bone-implant surfaces by maximizing the conformity between the tibial and femoral surfaces and allowing mobility of the bearings [16,15]. Long-term results of mobile-bearing TKA are now available for few designs with similar survivorship rates to that of the best fixed-bearing series [9,20,21,22].

This randomized prospective comparison of a mobile-bearing design with design was initiated to answer important questions about function, and value after total knee arthroplasty. Does a mobile-bearing design offer improvement over a fixed-bearing design with regard to the clinical characteristics and Radiographic parameters? Although we believe that a randomized, prospective clinical trial of the two designs is the best pos-

sible way to answer such questions, this study did have two limitations; the first was the short follow-up period (mean, thirty months) does not permit meaningful survival analysis or provide perspective on the longterm wear or osteolysis rate. The second limitation was using only one scoring systems in our study that perhaps not ideal to investigate performance of modern TKA and with their use could be difficult to show significant differences between well performing implants. Our study also, was undertaken to assess the safety and efficacy of a new mobile-bearing device. For this purpose we compared it to an established fixed-bearing TKA that in our opinion represents the modern TKA gold standard.

In Our study, neither design showed a clear superior advantage. Clinical, functional, and radiological results of mobile bearing designs were equivalent to that of the well established mobile bearing designs and demonstrating the efficacy of the mobile bearing prosthesis in improving the performances of the replaced knee. Both designs had a success rate at two years follows up of 96.7% with 95.2 excellent and good results. TKAs Results at short-term FU were excellent for both groups with a relatively high percentage of patients not having significant pain at the final FU (86%). Absence of significant pain at 12 months after TKA is reported in the literature to be in around 87% of the patients [23].

Various studies[24-34] recently tried to compare clinical results of mobile-bearing and fixed-bearing. Smith et al, 2011[24] conducted a meta-analysis and systematic review of randomized controlled trials comparing outcomes of mobile and fixed bearing total knee arthroplasty. They identified[14] studies reporting outcome of Knee Society Scores (KSS), postoperative range of motion (ROM) and Hospital for Special Surgery scores (HSS). The standard difference in mean outcome scores for KSS, ROM, HSS demonstrated no difference between groups (P = .902, P = .265 and P = .426 respectively). The results of this study found no significant differences between clinical outcomes of MB and FB TKA. Lädermann et

al, 2008[25] reported that the clinical results were similar for mobile-bearing and fixed-bearing in terms of function, pain, general status and complications. The outcome at 36 months was considered excellent or good in 90% of the fixed-bearing group and 88% in the mobile-bearing group. Oh et al, 2009[26] did meta-analysis trials comparing outcomes from using the mobile-bearing and fixed-bearing designs. The meta-analysis did not find a significant difference in the American Knee Society Knee Scores, Knee Society Functional and Pain Scores, range of motion, occurrence of radiolucent lines, prosthesis-related complications, and participant preference. Gioe et al, 2009[27] concluded that the two designs functioned equivalently at the time of early follow-up in their low-to-moderate-demand patient group. The rotating-platform design had no significant clinical advantage over the design with the all-polyethylene tibial component

Kim et al, 2007[28], Contrary to expectations, they found worse early clinical outcomes, smaller ranges of knee motion, less patient satisfaction, and a higher complication rate for fixed-bearing prosthesis than for the mobile-bearing prosthesis. Price et al[29] in a prospective multicentric trial compared the results at 1 year FU of fixed-bearing prosthesis to the mobile-bearing TMK prosthesis performed in the same patients in 39 simultaneous bilateral procedures. They found significantly better results in the mobile-bearing group for Knee Society pain scores and subjective preference although there was one revision in mobile-bearing group because of bearing dislocation

Our study has shown that using a fixed-bearing or a mobile-bearing design, when all the other variables are controlled, did not seem to influence the outcome in short-term FU. The strength of the present study is that it was a prospective controlled randomized trial enrolling a homogenous population for both groups with no differences in any preoperative parameter with the FU examinations performed in a patient-blinded fashion. The number of patients enrolled. Surgical procedures were standardized being performed all

by senior author and using the same instruments. Postoperative recovery, rehabilitation protocol, and medical prophylaxes were the same for both groups. All the patients were evaluated for each FU without any patient lost to FU.

Our study concluded that there was no significant difference between fixed-bearing or a mobile-bearing design with regard to the range of motion, KSS clinical or functional scores, radiographic parameters, or revisions due to aseptic loosening of the tibial component in the reported time frame. We believe that our results justify the continued use of both designs in this population. No benefit of one design over the other could be demonstrated in this patient population but with longer follow-up of this population we may be able to address the wear issue, and similar randomized prospective studies of populations with different demands would be helpful to determine if either design confers a clinical advantage over time.

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EARLY OUTCOME OF MOBILE-BEARING VERSUS FIXED-BEARING TOTAL KNEE ARTHROPLASTY A PROSPECTIVE RANDOMIZED STUDY

Mostafa A. El-Khalik MD, Yehia E. Bassiony MD, Akram A. Hammad MD and Mahmoud A. El-Ghafar MSc.

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OUTCOME OF ENDOVASCULAR COILING OF SMALL AND LARGE INTRACRANIAL ANEURYSMS IN AWAKE PATIENTS, INITIAL ANGIOGRAPHIC OUTCOME

Hany A. F. El-Dawoody MD, Mohamed S. Ibrahim MD, Abdel Wahab M. Ibrahim MD, Teiji Tominaga MD* and Tarek A. Eldiasty MD**

Neurosurgery Department, Faculty of Medicine, Mansoura University,

**Neurosurgery Department, Faculty of Medicine, Tohoku University , Japan,*

***Diagnostic Radiology department, Mansoura Urology and Nephrology center - Mansoura University, Egypt*

Abstract

The purpose of the study was to analyze the angiographic results of endovascularly treated intracranial aneurysms in awake patients.

The study population consisted of 20 consecutive patients (21 aneurysms, 2 ruptured), who were referred for endovascular treatment of intracranial aneurysm(s) between Feb 2008 and July 2008. Angiographic results were assessed using Raymond Classification. Patient, aneurysm, and procedure related variables were tested with an intention to find the predictors of the angiographic and clinical outcome.

Initially, 45 % of the aneurysms were completely occluded, 55 % had a neck remnant. The main parameter predicting the follow up angiographic result was the initial aneurysm volume (p = .016).

The present results confirmed endovascular treatment of intracranial aneurysms in awake patients is a feasible, safe, and effective method in preventing further bleeding of aneurysms. Increased experience in a single center improved the feasibility of the treatment as well as the angiographic results.

Keywords: *Intracranial aneurysm, subarachnoid hemorrhage, therapeutic embolization, awake patients, treatment outcome.*

Introduction

Intracranial saccular aneurysms represent the most common etiology of non traumatic Subarachnoid hemorrhage (SAH) with about 75- 80 % of SAH resulting from ruptured aneurysms.[1] The prevalence of unruptured intracranial aneurysms in general population have been estimated to be 2%.[2]

There are currently two major categories of endovascular treatment of intracranial aneurysm; occlusion of parent artery and aneurysm together, or selective endosaccular coil embolization of the aneurysms with parent artery preservation.[3] Endovascular detachable coil obliteration of aneurysms has been shown to be effective in the treatment of all sizes of aneurysms if they acquire narrow necks, especially in the posterior circulation that may possess significant risk in operative exposure.[4] Coil embolization should therefore be performed as early as possible after aneurysmal SAH, to prevent aneurysmal rerupture.[4] In a large multicenter series in the United States, complete aneurysm obliteration was achieved in 70.8 % of aneurysms with a small neck and 35.0 % of large aneurysms and there was an 8.9 % immediate procedural morbidity. [3]

The clinical and anatomical outcomes in patients with aneurysms treated with platinum coils have improved in the last 5 years due to the addition of several technical improvements such as 3D coils, balloon-assisted technology, and microstents. [5] However, these technical improvements may not be the fundamental solution to this problem.

Embolization of intracranial aneurysms performed using platinum coils in awake patient appears to be safe and feasible and allows intraprocedural evaluation of the patient. Potential advantages, including decreased cardiopulmonary morbidity rates, shorter hospital stay, and lower hospital costs, still require confirmation by a direct comparison with other anesthetic procedures. [6]

Materials and Methods

Prospective study included twenty patients of spontaneous subarachnoid hemorrhage or

patients with radiological evidence of unruptured intracranial aneurysms who were admitted to neurosurgery departments in Mansoura University Hospitals and/or Kohnan hospitals, Tohoku University, Sendai, Japan. Patients were subjected to the following: History taking and clinical assessment, Complete laboratory investigations; including, CBC, INR, S.Creatinine, liver function tests, blood sugar level, radiological investigations; including Computed Tomography (CT) Brain & CT angiography (in some selected cases), Brain Magnetic Resonance Imaging (MRI) & Magnetic Resonance Angiography (MRA) [in some selected cases], Diagnostic Cerebral Angiography (conventional 4 vessel angiography was done to demonstrate the etiology of SAH in all selected cases, or when CTA and/or MRA demonstrated cerebral aneurysm in unruptured aneurysm cases, Endovascular treatment; Endovascular coiling of intracranial aneurysms was done for all cases included in the study, on the angiography machine & all patients was managed awake with local infiltration anesthesia plus minimal sedation using midazolam, Statistical analysis; The statistical analysis of data done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL). N.B: P is significant if < or = 0.05 at confidence interval 95%.

Results

This study was carried out on 20 patients with 31 intracranial aneurysm(s) - including both ruptured and unruptured aneurysms - whom were admitted to the Neurosurgery and Neuroendovascular therapy departments in Kohnan Hospital, Tohoku University. This cohort of 20 patients harboring 31 aneurysms underwent 22 intervention sessions for coiling under Local anesthesia - one for another unruptured aneurysm and one as redo after recanalization - 2 sessions under general anesthesia, and the remaining 8 small unruptured aneurysms were left untreated for follow up.

In this study, 9 of the twenty patients were males with percentage of 45 % and 11 were females with percentage of 55 % with ratio of 1: 1.22. The distribution of SAH presentation among men this cohort was 3 out of 9 (33.3%), while

it was only one out of 11 women 9%, and 175 were females with percentage of 40% with ratio of 3 : 2. The age of the patients in this study ranged from 35 to 81 years, with a mean of 59.1 years (±12.05).

Out of our study group only 4 (20%) patients were presented with the picture of SAH due to aneurysmal rupture, while the remaining 16 (80%) patients were incidentally discovered by MRI & MRA on the patient own request to do imaging of his/her brain.

Aneurysm distribution by location and numbers were as follow: 6 patients had AComA Aneurysm, 1 patient has pericallosal artery aneurysm (DACA), 2 have basilar tip aneurysms, 1 had supraclinoid ICA aneurysm (IC-Anterior wall), 4 middle cerebral artery bifurcation (MCB) aneurysms, 2 Internal carotid bifurcation (ICB) aneurysms, 4 Internal carotid - anterior choroidal aneurysms (IC-Ach), 6 Internal carotid - posterior communicating artery (IC-PC) aneurysms, 4 Internal carotid - Superior hypophyseal artery (IC-SHA) aneurysms, and one Internal carotid - Ophthalmic artery (IC-Ophth.) aneurysm.

The initial angiographic results of 21 saccular aneurysms (in 20 patients) according to the location, size, and neck width of the aneurysm are summarized in Table 2. In terms of complete occlusion, no statistically significant difference was seen between ruptured and unruptured aneurysms (50% vs. 47%). Complete occlusions were 50 % among small and nil in large aneurysms (only one large aneurysm - NR). Complete occlusions among aneurysms treated with the balloon remodelling technique were less common (but not statistically significant) than among the cases where standard coiling was used (44 % vs. 50 %). The percentages of complete occlusion among the AComA aneurysms were higher than those of MCA territory than those of ICA territory aneurysms (80 %, 50 % and 35.7 % respectively). In terms of neck remnant, no statistically significant difference was detected between ruptured and unruptured aneurysms (50 % vs. 47 %).

Through the help of Neurovi-

sion 1 software (Tokyo-Japan), the aneurysm volume, volume embolization ratio could be calculated from the DICOM data of the preoperative 3D angiography of the treated aneurysms (table 11). The mean aneurysm volume was 109.5 cmm while the mean volume embolization ratio (VER) was 26.3 and the mean coil length per aneurysm treated was 36.5 cm. The main parameter predicting the follow up angiographic result was the initial aneurysm volume (P = .016); the higher the aneurysm volume, the higher the chance for development of BF and the least aneurysm volume even with body NR was associated with CO at follow up.

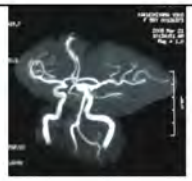
Female gender was found to be at risk of development of neck remnant after endovascular embolization (odds ratio 2.25 with 0.27-20.42) with relative risk of 1.5. Diabetic patients have a statistically significant tendency to develop NR at initial angiographic results than non diabetics (P=0.034). There was a statistically significant higher incidence of neck remnant results with advanced age patients than with younger group (p=002).

Table: Total number of aneurysms in the study group, the Number of ruptured and unruptured, treated and untreated aneurysms.

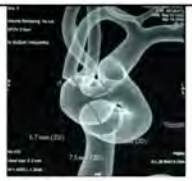
	No. of patients	No. of ANs diagnosed		No. of coiling sessions			ANs treated	Untreated ANs left for FU
				Under LA		Under GA		
		Ruptured	*Unruptured*	Denovo	Redo			
single AN	15	2	13	15	0	0	15	0
multiple ANs	5	2	14	6	1	2	8	8
Total	20	4	27	21	1	2	23	8
		31						

Case presentation:

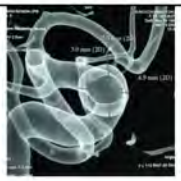
Preop. CTA



Preop. MRA

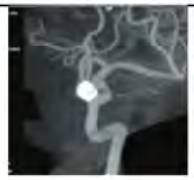


Preop. 3D DSA working angle 1



Preop. 3D DSA working angle 2

Postop. DSA (Immediate)



Postop. DSA (MIP) (12 months)

Lt. IC-SHA Aneurysm

55 Y ♀ with family history of SAH (her mother), diagnosed incidentally by MRA for the purpose of investigating mild tension type headache underwent successful coil embolization under LA with excellent angiographic results

Discussion

The present study consisted of a consecutive series of 20 patients admitted for treatment of intracranial aneurysm(s) with endovascular embolization under local. The present study included 22 aneurysm treatment sessions under local anaesthesia, all of them located in the anterior circulation. This is the first prospective single-center series of aneurysm treatment under local anaesthesia, there was only one retrospective study discussing endovascular embolization under local anesthesia. [6]

Male to female ratio in this study was found to be 1:1.22, that was in range of Harrigan and Deveikis 2010 [7], where male to female ratio was 1:1.3. Nevertheless, female gender was found to be at risk of development of neck remnant after endovascular embolization (odds ratio 2.25 with 0.27-20.42) with relative risk of 1.5. Two or more aneurysms were present in 25% of cases, a figure in range of most series; 15-30%. [8,9] In the present study, there was no statistically significant difference in angiographic outcome between patient harboring either single or multiple aneurysms groups (P= 0.5). There was a statistically significant higher incidence of

neck remnant results with advanced age patients than with younger group (p=002).

Eighty percent of our study group was incidentally discovered unruptured aneurysms and only 20 % were presented by SAH either in the acute or chronic stages. All the patients were fully conscious and cooperative enough to allow for interventional procedure under local anesthesia with minimal sedation. Many centers perform most neuroendovascular interventions only under general anesthesia, but in Kohnan hospital and Mansoura University the choice of LA/GA depends upon the patient GCS, cooperation, general health status and availability of anesthesiologist at the time of interference. Coiling with the patient awake, permits continuous neurological monitoring, eliminates the risks of general anesthesia, can shorten the length of the case, and has been shown to be safe and feasible by a British group.[8] However, if aneurysm rupture occurs during treatment it is quite difficult to continue embolization if the patient is under local anesthesia alone. [10] Even though it is not the role as the only case of intra-procedure aneurysm rupture in this study was managed successfully and continued under LA.

In this study, the prevalence of patients with positive family history are 35 %, higher than other series as this might be related to the high percentage of unruptured aneurysms in this cohort plus the wide availability and unrestricted, non invasive, investigatory tools by national insurance in Japan.

Regarding the aneurysm locations, the highest proportion of aneurysms were AComA Aneurysms (30%), and Internal carotid - posterior communicating artery (IC-PC) aneurysms (30%) a finding in range with previous studies. [1, 11]

In the present study, complete aneurysm occlusion was initially achieved in 45% of the saccular aneurysms. In terms of the percentage of complete occlusions, the initial and follow up angiographic results are in range to the majority of results from previously published endovascular series,

where complete occlusion has been achieved initially in 33-71% of the treated aneurysms and in follow-up in 35-86%. These results obtained (complete occlusion initially in 45% of saccular aneurysms) are comparable to the range of the published series (46%, Ng et al. 200212). In terms of neck remnant, the results of the present study (55 % initially) are in the range of the other published series (21-63% initially). The meta-analysis for initial and follow up angiographic complete obliteration of saccular aneurysms were (53.1 ± 13.2 % and 60.5 ± 15.9 %) respectively.

In the present study, all angiograms were retrospectively analyzed by two reviewers in consensus. Strict criteria for the classification of complete occlusion were used, and in several cases the initially reported complete occlusion was re-evaluated to have a neck remnant. Therefore, we were most likely to be even stricter than the average in accepting occlusions as complete. There were a few cases in the present series where the aneurysm neck was intentionally left open to secure the patency of the parent artery or the arterial branches originating from the aneurysm neck. Complete occlusion of the aneurysm would have resulted in ischemic complications in these cases, and partial aneurysm occlusion was considered a better option for the patient than sacrificing the branch artery.

It is usually technically easier to achieve complete occlusion of a small aneurysm, where less coils are needed, and the neck is more often suitable to retain the smaller filling coils needed to finish the coiling procedure successfully. No significant difference was detected between wide and narrow-necked aneurysms in the percentage of complete occlusions in either initial or follow-up angiography.

Unaddressed issues in previous studies, Diabetic patients have a statistically significant tendency to develop NR at initial angiographic results than non diabetics (P=0.034). Type II diabetes mellitus is associated with endothelial dysfunction that impairs eNOS secretion & expression together with

impaired local release of VEGF at the site of vascular injury augmented by diabetic vasculogenic progenitor cell dysfunction (i.e., impaired adhesion, proliferation, migration, tubule formation) impairs new blood vessel formation and in turn tissue repair in diabetic patients.[13,14,15] The relationship between diabetes mellitus and NR might be explained by the impaired tissue healing in diabetic patients in general and this impaired healing capability could include also impairment of healing at aneurysm neck. These suggestions are to be elucidated in future laboratory and/or clinical studies on wider scale to prove or disprove such postulates.

Initially, endovascular treatment was usually used only in cases that were poor candidates for surgery, especially in patients in a poor clinical condition or with posterior circulation aneurysms. By now, in many centers, including Kohnan hospital and Mansoura University Hospital, endovascular treatment is considered the primary method in most cases that would also be suitable for surgery.

As demonstrated by Wermer et al. (2005) [16], aneurysmal SAH is not a single event in the lifetime. Recurrences occur even after initially completely occluded aneurysms. In addition, up to one third of patients harbour multiple aneurysms, as confirmed in this study, and the formation of de novo aneurysms is possible. Imaging follow up is mandatory after treatment to identify the patients who should undergo further treatment and thus to minimize the risk for further bleedings. The question remains - which method should be used and for how long?

Conclusions

Endovascular coiling of intracranial aneurysms is a safe, feasible to be done under local anesthesia in well trained hands when the procedure is expected to be short. Our initial angiographic outcome is in range of most published series. We recommend performing endovascular coiling under LA in unruptured aneurysms, high volume centers, well experienced neurointerventionalist, cooperative patient, when high cardiac and/or pulmonary risk of general anesthesia exists.

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OUTCOME OF ENDOVASCULAR COILING OF SMALL AND LARGE INTRACRANIAL ANEURYSMS IN AWAKE PATIENTS, INITIAL ANGIOGRAPHIC OUTCOME

Hany A. F. El-Dawoody MD, Mohamed S. Ibrahim MD, Abdel Wahab M. Ibrahim MD, Teiji Tominaga MD and Tarek A. Eldiasty MD

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THORACOSCOPIC ANTERIOR RECONSTRUCTION COMBINED WITH POSTERIOR PEDICLE SCREW-BASED FIXATION IN MANAGEMENT OF THORACOLUMBAR JUNCTION FRACTURES

Hazem M. Adel Elkosha MD, Hesham A. Elsaghir MD*, Ashraf A. Ezz-Edeen MD, Nabil M. Ali MD and Mohamed S. Ebrahim MD

Departments of Neurosurgery, Mansoura University, Orthopedics, Alexandria University, Egypt*

Abstract

Background: *Video-assisted thoracoscopic surgery (VATS) is a minimally invasive procedure applied to the field of spine surgery since 1990s.*

Objective: *To explore the feasibility, safety, efficacy, and cosmotic results of thoracoscopy in management of thoracolumbar fractures.*

Methods: *We used both the Thoracolumbar Injury Classification and Severity (TLICS) score and the Load Sharing Classification score for selection of cases. Cases were operated on by a combined approach of posterior pedicle screw fixation and anterior thoracoscopic reconstruction and fusion with follow up of cases up to 12 months as regard clinical and radiological results.*

Results: *Patients ranged from 22-56 years, including 8 males and 7 females. Falls were the most common cause and L1 the most common level. Two cases were reconstructed by a corpectomy cage, while the rest by tricortical iliac bone graft. All except two cases showed signs of fusion at 12 months, while all cases were cosmetically satisfied. No major complications nor death.*

Conclusion: *Video-assisted thoracoscopic reconstruction of thoracolumbar fractures is an effective, minimally invasive, relatively safe technique that needs further training to widen.*

Introduction

Each year, there are approximately 5 million new vertebral fractures worldwide. [1] The thorac-

olumbar junction, being the mechanical transition zone between the rigid thoracic and the flexible lumbar spine, is the most common site of injury to the spine.[2] However, the possibility of thoracolumbar fracture cannot be ruled out with a normal neurological examination, as the majority of these injuries do not have associated neurological deficits.[3]

The proper way of management for thoracolumbar fractures is still a matter of debate. The debate includes; conservative versus surgical, anterior versus posterior, early versus delayed...etc. One of the recent debates around management of thoracolumbar fractures is whether to treat it using the recent modifications of the old conventional methods like the recently described mini-open techniques,[4-7] versus the use of the minimally invasive technology of video-assisted thoracoscopic surgery that was introduced in the nineties of the last century.[8,9]

Although many studies had reported the efficacy and safety of video-assisted thoracoscopic spine surgery,[10-17] such technique still in its infancy in our locality. It is a virgin field that is not sufficiently explored by us to find out its potential applications, and build up our own experience. In this study, we are trying to explore the feasibility, safety, and efficacy of this technique in management of thoracolumbar burst fractures that need anterior reconstruction.

Patients and Methods

This is a prospective study carried on in the Hadara Hospital, Alexandria, by a team of composed both neurological and orthopedic surgeons from both Mansoura and Alexandria universities. The study was conducted between June 2009 and July 2011, including 15 cases of thoracolumbar burst fractures admitted in Hadara Hospital. The selection of cases for surgery was based on the Thoracolumbar Injury Classification and Severity (TLICS) score, and the candidacy for anterior reconstruction was based on the Load Sharing score. All selected cases were TLICS ≥4, and have Load Sharing score ≥7. Moreover, cases with extremes of age, medical problems that preclude general anesthesia, sever psychiatric ill-

ness, or more than one level of fracture were excluded from our study. Selection criteria for eligibility are listed in (table 1).

Preoperative evaluation:

Patients who entered the study, underwent careful history taking that provides important insights into the pathomechanics of the injury, thus determining which force vectors predominates. Careful neurological examination was conducted and its results were registered. The American Spinal Injury Association (ASIA) Impairment Scale was used in documenting, monitoring, and treating neurologic injuries.[18]

Initial radiographic assessment includes anteroposterior (AP) and lateral spine films, assessed for loss of vertical body height, malalignment of vertebral bodies, widening of interspinous distance. Computerized tomography (CT) was used to reveal the degree of canal compromise, degree of separation of bone fragments, and pedicular diameter for screw selection. Magnetic resonance imaging (MRI) was used in evaluating those patients with neurologic injury that cannot be accounted for by osseous disruption on plain radiographs and a CT scan.

Table 1. Selection criteria for trial eligibility.

Inclusion:
• Age 18–60 years.
• Proved (radiologically) fracture in the thoracolumbar area from T10-L2
• TLICS score ≥5, or
• TLICS score 4 with special considerations (young, hard workers, medically fit, patient's well).
• Load Sharing score of 7 or more.
• Informed consent.
Exclusion:
• Medical co-morbidities making surgery hazardous.
• VATS: pleural symphysis, previous thoracotomy.
• More than one level fractured
• Pregnancy.
• Severe psychiatric illness.
• Planned (e)migration to another country with the 6 months following surgery.

Preoperative laboratory investigations included, routine full investigations (CBC, INR, Blood grouping, LFTs, Serum creatinine, RBS, ECG), in addition to chest x-ray and pulmonary function tests for those who undergo VATS approach.

Equipments :

All equipments used were provided by KARL STORZ®, Germany, including 10 mm portals, 36cm long rigid 45 angled telescope, telecam-C with adjustable focus, light source with xenon lamp, Sony® video monitor, and instruments especially designed for endoscopy.

Surgical technique

Cases are operated in the prone position under general anesthesia using single lumen endotracheal tube permitting double lung ventilation. All cases undergo posterior pedicle-screw based fixation first, then anterior thoracoscopic reconstruction is conducted in the same position using tricortical iliac bone graft or cage insertion. Finally, the screws become attached and tightened to the rods in some compression.

Draping is done so that the surgeon can access the midline back at the desired level, the posterior iliac crest, and the left side of the chest laterally and anterior to the anterior axillary line (Fig. 1a). A 3 cm incision for the working port is positioned directly over the target vertebra parallel to the ribs and centered over the mid-axillary line directed by fluoroscopy. Scope port should be marked two or three intercostal spaces above the working port in the posterior axillary line (Fig. 1b). We first start by the 1 cm scope port incision through which the 45º angled endoscope can be introduced. This is followed by opening of the 3 cm working port incision under direct vision through the endoscope. This 3 cm incision can be used as a working port for diaphragmatic retraction, irrigation, suction, and passage of the working instruments. We do not need more than these two incisions.

Using a broad spatula for diaphragmatic retraction, operating in the prone position, and reducing the tidal volume of ventilation, exploration of the thoracic cavity can get easier while permitting

double lung ventilation. The diaphragm usually inserts at the T12-L1 level perpendicular to the spine. The diaphragm can be opened endoscopically if surgical exposure below the insertion of the diaphragm is needed. For exposure of L1 and L2, the diaphragm is opened farther caudally for up to 5 cm at its attachment site. The incision should run parallel to and 1 to 2 cm away from the diaphragmatic insertion. This remaining diaphragmatic strip is used to close the diaphragm. This technique obviates the need for complete detachment of the diaphragmatic insertion, as required in open procedures. After the diaphragm has been split, the retroperitoneal fat and peritoneal sac are bluntly dissected away from the fascia of the psoas muscle to expose the vertebral bodies.

Once the desired segment of the spine is identified, the parietal pleura over the spine is opened using a pointed dissector and the opening is extended over the whole desired segment of the spine. Now, the vertebral body surface with the above and below intervertebral discs and the segmental blood vessels will come clear. Discectomy in the conventional method is undertaken using scalpel for annulotomy, fenestrated punch forceps, and curettage spoon. When discectomy and removal of the cartilaginous end plates are completed (figure 2a), intersomatic bone graft placement is started. The tricortical iliac bone construct is inserted in the desired disc space (figure 2b) aided by distraction of the motion segment using the posteriorly placed screws, or by gentle palmar pressure on the back of the patient to minimize the local kyphosis and open the anterior disc space. The placed bone construct is then tapped using a blunt-tipped instrument and a hummer. The reconstruction and fusion can also be done using a titanium cage filled with cancellous bone inserted in the defect (figure 2c).

At the end of procedure, the diaphragmatic split is closed when possible to avoid diaphragmatic hernia, the chest cavity is inspected for any bleeding points, and a chest tube is inserted through the working port under direct vision of the endoscope. The incisions are

closed, and extubation is usually done immediately after the procedure. Follow up x-rays are done to verify normal lung expansion. The chest tube should not be removed until air leakage ceases or until drainage is less than 150ml per 24 hours.

Postoperative assessment

(A) Clinical assessment:

[a] Neurological improvement: determined by measuring the change in ASIA impairment scores 12 months postoperative.

[b] Cosmosis: evaluated by the patient and an independent surgeon using a Visual Analogue Scale (VAS) ranging from 0 (very poor), to 10 (excellent) and the scores are calculated and their mean is obtained.

(B) Radiographic Assessment:

Radiological signs for fusion [end-point at 12 months postoperatively] are tested by an independent radiologist. Fusion is defined as contiguous bone from endplate to endplate without evidence of radiolucency along either margin and / or less than 5 degrees change in the Cobb angle on flexion / extension dynamic study.

Results

This is a prospective study that included 15 cases with thoracolumbar fractures that were in need for anterior reconstruction in addition to the posterior pedicle-screw based stabilization. The age of patients ranged from 22-56 years with a mean age of 31.06 years and a standard deviation of 9.28. Males were 8 (53.3%), and females were 7 (46.7%).

Eight of our patients (53.3%) were victims of fall from height, constituting the most common mode of trauma. The second most common cause was motor vehicle accidents which affected 7 patients (46.7%). Seven cases of fracture were at L1 level (46.7%), 4 cases at L2 (26.7%), 2 at T12 (13.3%), 1 at T11 (6.7%), and 1 case of T10 fracture (6.7%). This makes L1 vertebra the most commonly fractured among the thoracolumbar junction area.). According to the AO classification, all types of fractures in our study were burst fractures (type A), except 2 cases of rotational injury

(type C), but no distraction injury (type B).

All cases entered our study were surgical candidates according to the Thoracolumbar Injury Classification and Severity score (TLICS). This means that all patients included had at least TLICS score of 5 or a score of 4 with preference of surgery over conservative treatment. Only two cases with score 4 were included, while the rest were ≥ 5. All cases in our study were in need for anterior reconstruction (score ≥ 7 according to the Load Sharing Classification). The mean score of patients was 7.2.

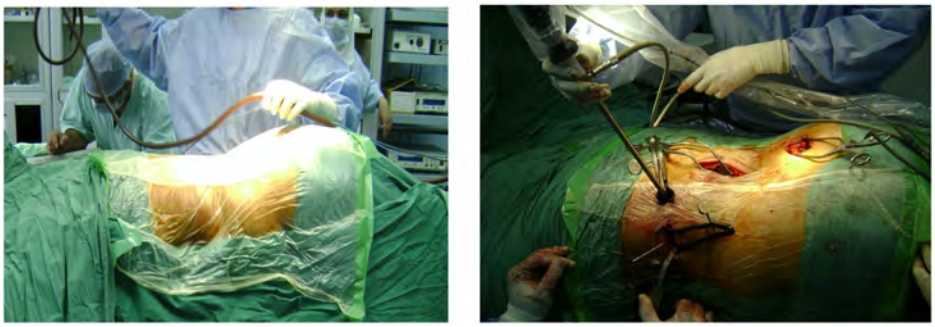
The vast majority of cases (12 cases representing 80%) underwent reconstruction using an intersomatic tricortical iliac bone graft placement combined with posterior transpedicular screws and rods fixation. Only 3 cases (20%) underwent reconstruction using an intersomatic, or corpectomy cage placement combined with the posterior screws and rods fixation. The timing of surgery varied from the 2nd, to the 7th posttraumatic day. Five cases in our study were operated on their 2nd posttraumatic day, while 2 cases were delayed up to the 7th day. The mean timing for reconstruction was 3.4 ± 1.5 days. The mean length of procedures was 5.3 ± 1.2 hours. However, when earliest and latest 3 procedures were compared there was a significant difference in length of procedures (mean of 6.2 versus 3.6 hours, respectively). The blood loss was lesser in late than early cases. The mean blood loss was 652 ± 130 ml. Intraoperative complications were nil.

When ASIA scores were compared at 12 months postoperatively with those initial scores (Table 2), we found those with score A or E remained unchanged till end of the study, while those with scores B, C, and D improved on the scale to higher scores with time. As regard cosmosis, the lowest patient VAS score for cosmosis was 7 (1 case) and the highest was 10 (1 case), while lowest physician VAS score was 7 (3 cases) and the highest was 9 (7 cases). The mean cosmosis score on the VAS (mean of patients' and physicians' scores) was 8.4 ± 0.4 (Figure 3).

Radiological follow up of cases was directed to evaluate fusion at 12 months. At the end of the 12 month-follow up period, 2 cases were found with no radiological signs of fusion. These 2 cases with failed fusion included the 4th, and the 6th cases which were early cases in our study, while the other 13 cases showed radiological signs of fusion (Figure 4) at the end of the 12 months. The rate of fusion is about 87%. The postoperative hospital stay ranged from 4 to 8 days. The mean hospital stay was 5.9 ± 0.8 days.

Table 2. ASIA scores of the study group, initially and at 12 months.

	Initial ASIA score	ASIA score at 12 months
A	*3*	*3*
	20.0%	*20.0%*
B	*1*	*0*
	6.7%	*.0%*
C	*3*	*2*
	20.0%	*13.3%*
D	*5*	*4*
	33.3%	*26.7%*
E	*3*	*6*
	20.0%	*40.0%*



(a) **(b)**

Fig 1. (a) Position and draping, **(b)** incisions and ports.

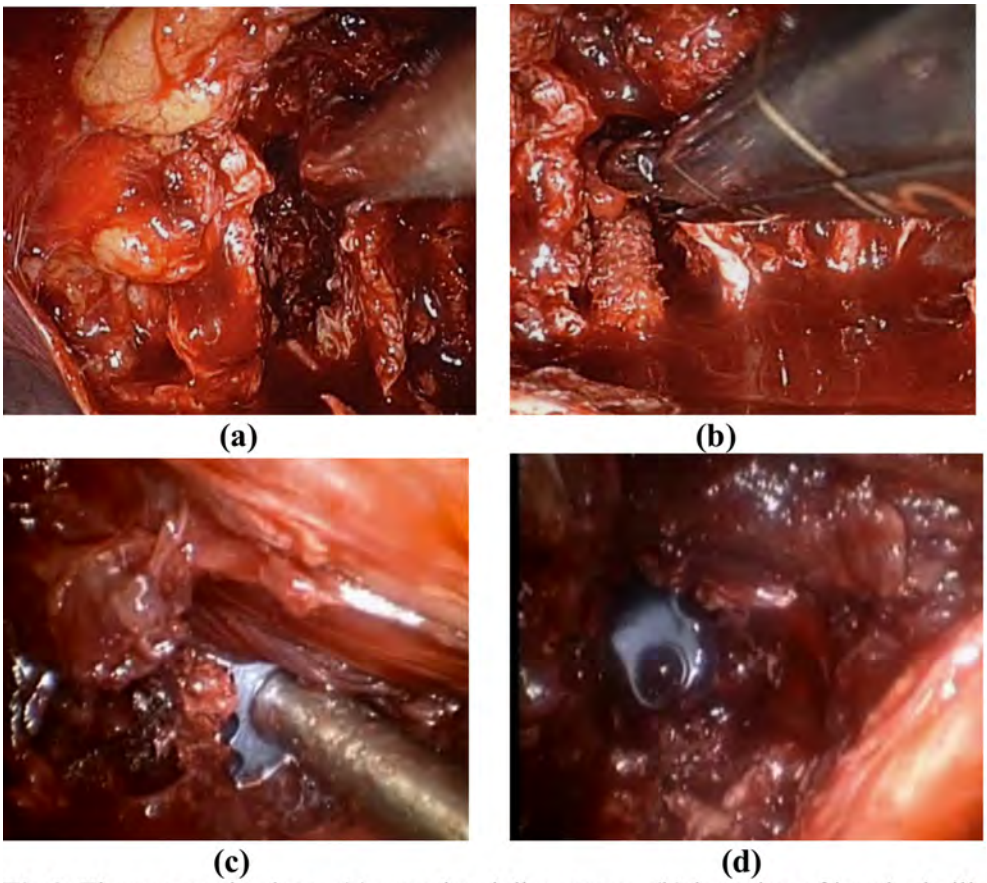


Fig 2. Thoracoscopic views. (a) completed discectomy, (b) insertion of interbody iliac bone graft for fusion, (c) insertion of interbody cage, (d) view at the conclusion of procedure before closure with cage insitu.

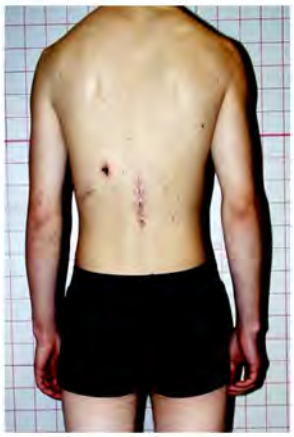
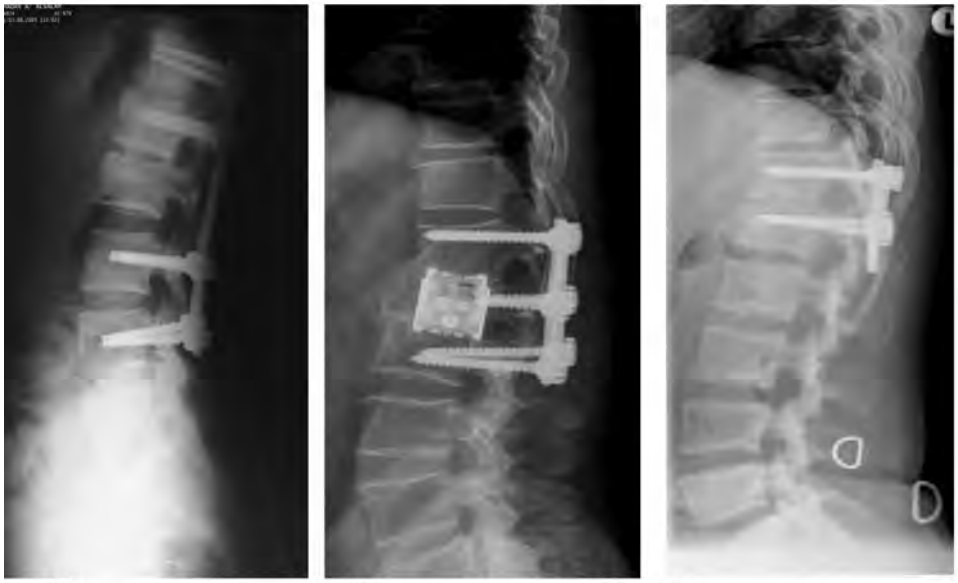


Fig 3. Acase with thoracolumbar fracture who underwent posterior stabilization using short segment pedicle screw-rod system (midline vertical wound), combined with thoracoscopic reconstruction of anterior column in the same setting (Left inclined wound). Note the good cosmosis of the wound used for thoracoscopy.



(1) (b) (c)

Fig 4. Radiography of three cases of thoracolumbar fractures constructed thoracoscopically. (a), and (c) were reconstructed using harvested iliac bone graft. (b) was reconstructed using expandable corpectomy cage filled with cancellous bone.

Discussion

With the improvements in access technology, interest in approaching the thoracolumbar junction with the aid of the endoscope and surgical microscope has evolved. Therefore, various anterolateral transthoracic and transabdominal approaches with mini-open modifications has been developed as an attempt to achieve the same efficacy and minimize the drawbacks of the invasiveness of traditional approaches.[4,6,7] In parallel to these advances towards less invasion in open approaches, there were trials and progresses in the endoscopic access to the thoracic and thoracolumbar area.[8-14]

Those who advocate video-assisted endoscopic spine surgery are still reporting feasibility and safety of the technique, with better cosmosis, less blood loss, less postoperative discomfort, shorter hospital stay, and in good hands; equivalent outcomes to open procedures.[9,15,16] Few spine surgeons have enough experience in such technique and can conduct studies for exploring its efficacy and safety.

The mean age of patients in our study is 31.06 years ranging from 22 to 56 years. This age correlates with most series dealing with vertebral fractures where mean ages lie in the first half of thirties.[8,19,20] The most common age group is that of late third and early fourth decades, as this age is the age of maximum activity in the community and practicing risky jobs and dangerous styles of driving. The male predominance in our study agrees with the literature. However, male-to-female ratio in our study differs from other studies. It is far different from Indian epidemiological studies,[21,22] where in the rural setup, the ratio of male to female ranges from 9:1 to 13.5:1. Another Indian study done by Upendra et al, [23] reported a ratio that approximates western literature where male-to-female ratio was 4.5:1.[24] Many western studies,[19,20] reported that men are 2-4 times more likely to have these fractures compared to women. Although our sample of study is too small to explain the difference, it seems that community economic and traditional characteristics are responsible for the distribution of vertebral

fractures among both sexes.

We agreed with Indian studies about fall from height being the most common mode of injury in thoracolumbar fractures.[21-23] However, several western studies reported motor vehicle accidents to be the most common.[25,26] This discrepancy may be due to the lack of safety measures for those who work at heights in our society. We found L1 vertebral level to be the most commonly affected which agree with the literature. However, L2 level was found to be the second common, which disagree with other studies that found T12 to be the second common vertebral level in thoracolumbar fractures. We explain this by the fact that those studies defined thoracolumbar fracture as being from T11-L1.

Performance of thoracolumbar reconstruction in the prone position enabled us to simultaneously access the spine from its ventral and dorsal aspects. The prone position gives us an access to fix the spine by pedicle screws, harvest a tricortical iliac graft, and in the same position we can access the spine thoracoscopically for anterior reconstruction. Moreover, the prone position helps to bring the lung away from the spine by gravity, and can help to reduce kyphosis and open the disc space anteriorly by gentle pressure applied to the back.

Although it has been reported that up to 3-8% of patients with complete spinal cord injury can reach an ambulatory status,[27] we found no improvement among those patients with ASIA score A during the period of follow up. This is due to the usual delay of referral or the inexperienced transport of such patients which results in irreversible secondary insult of the cord. However, the majority of those with partial cord injury improved and those with intact neurological status remained intact. All patients were satisfied with wound shape and length with reported excellent cosmosis. As regard fusion, we fulfilled about 87% fusion rate which correlates well with the results of others.[28]

This makes thoracoscopy as effective as open procedures in achieving spinal fusion with the

extra benefit of minimal invasion, better cosmosis, one-staged operation, and relatively shorter postoperative hospital stay. Further training and enthusiastic research can open wider field for application of thoracoscopy in the future.

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BENHA MEDICAL JOURNAL

THORACOSCOPIC ANTERIOR RECONSTRUCTION COMBINED WITH POSTERIOR PEDICLE SCREW-BASED FIXATION IN MANAGEMENT OF THORACOLUMBAR JUNCTION FRACTURES

Hazem M. Adel Elkosha MD, Hesham A. Elsaghir MD, Ashraf A. Ezz-Edeen MD, Nabil M. Ali MD and Mohamed S. Ebrahim MD

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DOES EGF HAVE A PROTECTIVE ROLE AGAINST ACETAMINOPHEN TOXICITY IN RAT KIDNEY OR LIVER?

Shereen M. Samir MD and Mie A. Mohamed MD*

Departments of 1Physiology and 2Pathology,
Faculty of Medicine, Mansoura University

Abstract

Acetaminophen (APAP) is a commonly used and effective analgesic and antipyretic agent for relief of mild and moderate pain. However, it may induce hepatotoxicity and nephrotoxicity, as the result of either deliberate overdose or accidental overdose. In vitro and in vivo evidences suggest the cytoprotective ability of EGF which is expressed in reducing lethal tissue damages and the lipid peroxidation process. So, the aim of this study was to examine the protective effect of EGF on acetaminophen induced toxicity in male rat liver and kidneys. The animals were divided into 3groups which are: A control group that received an equivalent volume of saline, a (APAP) paracetamol-only group, a group that received EGF 30 minutes before APAP-injection. After 24 h of administration, surviving animals were sacrificed. Blood, liver and kidney tissues were obtained for biochemical and pathological examination. Results showed toxic effects of acetaminophen and preadministration of EGF showed significant renal protection and mild hepatic protection. The mechanisms beneath the protective role of EGF remain to be clarified.

Keywords: *EGF (epidermal growth factor), APAP (acetaminophen), nephrotoxicity, hepatotoxicity.*

Introduction

Drug-induced toxicity is one of the major causes of clinical hepatic and renal complications associated with drug therapy[1-2]. Acetaminophen (APAP) is a commonly used and effective analgesic and antipyretic agent for relief of mild and moderate pain and is probably the most widely used drug in

the world. However, acetaminophen may induce hepatotoxicity and nephrotoxicity, as the result of either deliberate overdose or accidental overdose [3].

Harris et al. noticed that growth factors like hepatocyte growth factor, or the hepatocyte-derived insulin-like growth factor binding protein-1 display potent hepatoprotective effects, including the attenuation of apoptosis induced by Fas ligation. Epidermal growth factor (EGF) is a 53 amino acid peptide that is produced by the salivary glands and Brunners' glands of the duodenum [4]. In vitro and in vivo evidences suggest the cytoprotective ability of EGF which is expressed in reducing lethal tissue damages and the lipid peroxidation process. These findings include ozone cytotoxicity on airway epithelial cells[5], carbon tetrachloride-mediated liver necrosis[6], apoptosis by hyperoxia in cultured neurons[5], and cerebral injury by ischemia[7]. It is a potent stimulant of proliferation and healing of the gastrointestinal tract, acting as a cytoprotective agent and also stabilizing cells against noxious agents such as indomethacin[8]. Hepatotoxicity driven by acetaminophen (APAP) overdose is the most common cause of death by acute liver failure in patients upon hospitalization. Murthy et al. noticed that adenoviral delivery of ADAM17 [Adenoviruses encoding Gfp (Ad-GFP) or Adam17 (Ad-ADAM17)] is capable of protecting mice from APAP-induced liver failure. Its activity in hepatocytes is essential for EGFR ligand release and promotes their paracrine and/or autocrine survival signaling. Meanwhile, EGFR activation acts to promote cell survival by induction of antiapoptotic genes and suppression of Bim. So, we decided to examine if EGF can prevent hepatic and renal injury induced by acetaminophen (paracetamol) or not? [9]

In summary, the goals of this study were two-fold: to characterize the changes of the microscopic lesions and clinical pathology parameters that occur in the livers and kidneys of rats (a) after a single acetaminophen administration only and (b) after a single EGF administration as a protective measure.

Materials and Methods

Experimental animals

All experiments were carried out with male albino Wister rats weighing 180-240 g, obtained from the Animal House. Thirty rats were randomly divided into 3 groups (n = 10 for each group) using a computer-assisted randomization procedure to ensure similar distribution of body weights. Rats were housed in stainless steel cages under a 12 h light/dark cycle at 25°C and allowed water and food (Laboratory chow) ad libitum. Animals were fasted 12 h before experiments and allowed water ad libitum.

Experimental protocol. Acetaminophen (paracetamol) was dissolved in pathogen-free normal saline to make a concentration of 30 mg/ml. Animals were anesthetized by inhalation of halothane, and paracetamol was administered intraperitoneally in an hepatotoxic dose of 800 mg/kg as determined in Waters et al. study[10]. Epidermal growth factor (EGF) (Sigma, St. Louis, MO) dissolved in BSA was injected intraperitoneally into animals at a dose of 30 μg/kg[11].

The 3 groups are : A control group that received an equivalent volume of saline, a (APAP) paracetamol-only group, a group that received EGF 30 minutes before APAP-injection. After 24 h of administration, surviving animals were sacrificed. Blood, liver and kidney tissues were obtained from each animal for biochemical and pathological examination.

2. Biochemical measurements

Blood samples were collected into dry clean tubes. After centrifugation for 10 min, the serum samples were taken for determination of the activities of glutamyl-oxaloacetic transaminase (SGOT), glutamyl-pyruvic transaminase (SGPT) and alkaline phosphatase (SAP) as well as levels of creatinine and blood urea nitrogen.

The serum enzyme activities, creatinine and BUN levels were measured spectrophotometrically using standardized commercially available Diamond kits (Modern Laboratory Chemicals, Egypt).

3. Histopathological examination

Samples of liver and kidney tissues were obtained from animals after sacrification at the end of the specified time. Tissue blocks for light microscopy were fixed in 10% buffered neutral formalin solution. All samples were embedded in paraffin, cut in sections of 3-5 μm thickness and stained with hematoxylin and eosin (H. & E. stain). Features of cell injury and necrotic changes were scored on a semi-quantitative scale: [+, mild (less than 25% of the tissues were affected); ++, moderate (25-50% of the tissues were affected); +++, severe (more than 50% of the tissues were affected)] (12).

4. Statistical analysis

Statistical analysis was done by using SPSS (statistical package for social science) program version10, 1999.

The data were parametered by using SPSS Kclmegro-Smirnor test. The data were expressed as mean ± standard error (X ± S.E.). The significance of differences between mean values was determined using Mann-Whitney and Student's t-test and P- values were considered insignificant if they were higher than 0.05. While values less than 0.05 were significant (*), values less than 0.01 were considered highly significant (**), and values less than 0.001 were extremely significant (***).

Results

Forty per cent (4/10) of APAP received animals died within the first 24 hour period. Mortality was almost completely prevented by EGF prior to APAP administration.

Table1, 2 and figures1 (A, B&C) show extremely significant deterioration of hepatic and renal biochemical parameters in group 2 when compared with group1 proved toxic effect of APAP, but in group 3 with pretreatment of APAP rats with EGF there is extremely significant improvement of renal function (creatinine and BUN) (in table 2) from (2.1±0.1 and 144.01±0.8) to (1.05±0.04 and 36.9±0.5) respectively, with only moderate significant changes of hepatic function (sGOT, sGPT and SAP) (in table1)

from (76.5±0.9, 94.9±0.5 and 1217.1±5.6) to (72±0.9, 61.6±0.6 and1178.1±10.8) respectively.

Histopathological examination

Consecutive sections (5 μm) of paraffin-embedded liver were prepared for H&E staining. The percentage of necrosis was estimated by evaluating the number of microscopic fields with necrosis compared with the entire cross-section. In general, necrosis was estimated at low power (x100) and questionable areas were evaluated at higher magnification (x 200 or x 400). The histological sections were evaluated in a blinded fashion. Five fields were evaluated per tissue sample.

Tables (3) & (4) show the effect of EGF on APAP induced injury in both kidney and liver, the kidney shows marked improvement with EGF on APAP induced injury than the liver, the improvement is clearly observed in table (4), there is significant relationship between cloudy swelling of the proximal tubules and Vacuolar degeneration of distal tubules :

Table(1): Comparison between biochemical effects of APAP and of EGF on APAP induced injury in rat liver .

Group	N=10	sGOT	sGPT	SAP
Control (gp.1)	mean ±S.E.	12.3 0.3	9.96 0.2	143.8 1.3
APAP (gp.2)	mean ±S.E. P1	76.5 0.9 0.000	64.9 0.5 0.000	1217.1 5.6 0.000
EGF+APAP (gp.3)	mean ±S.E. P2	72 0.9 <0.01	61.6 0.6 <0.01	1178.1 10.8 <0.01

P<0.05 means significant .P1 represents significance of gp.2 as compared with gp.1
P2 represents significance of gp.3 as compared with gp.2

Table (2) : Comparison between biochemical effects of APAP and of EGF on APAP induced injury in rat kidney.

Group	N=10	Creatinine	BUN
Control (gp.1)	mean ±S.E.	0.9 0.01	33.4 0.9
APAP (gp.2)	mean ±S.E. P1	2.1 0.1 0.000	144.01 0.8 0.000
EGF+APAP (gp.3)	mean ±S.E. P2	1.05 0.04 0.000	36.9 0.5 0.000

P<0.05 means significant .P1 represents significance of gp.2 as compared with gp.1
P2 represents significance of gp.3 as compared with gp.2

Histopathological tables:

Table (3) : Effect of EGF on APAP induced injury in liver

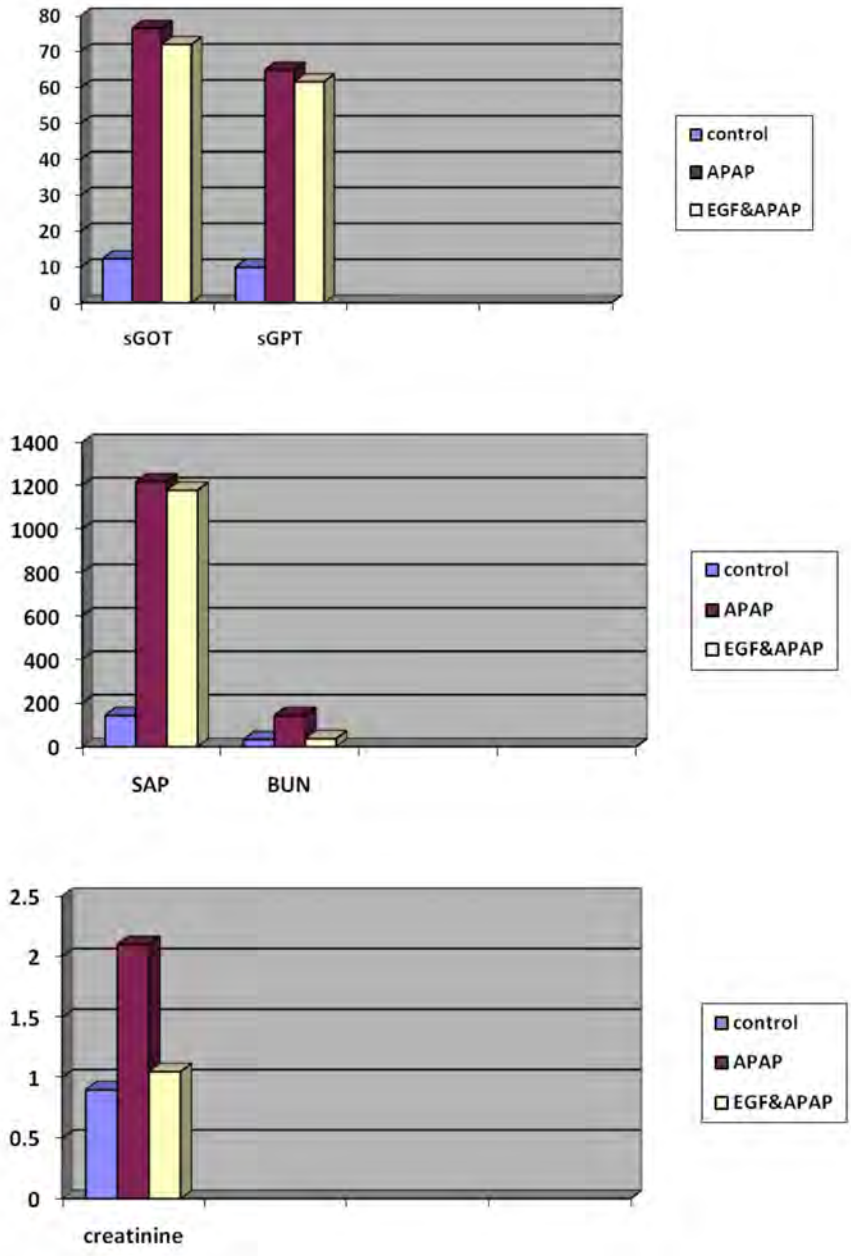
	liver					
	Vacuolar degeneration			**Centrilobular necrosis**		
	mild	moderate	severe	mild	moderate	severe
APAP Group 2	1	2	3	1	3	2
APAP + EGF Group 3	2	8	0	2	8	0

P value=000

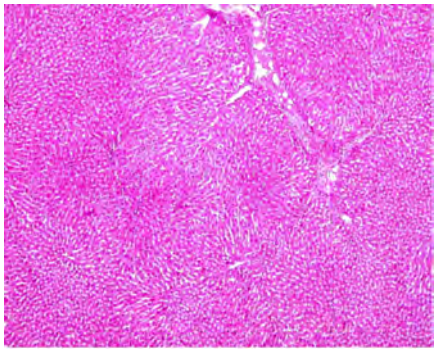
Table (4) Effect of EGF on APAP induced injury in kidney

	kidney					
	Cloudy swelling of proximal tubules			**Vacuolar degeneration of distal tubules**		
	mild	moderate	severe	mild	moderate	severe
APAP Group 2	0	4	2	0	3	3
APAP + EGF Group 3	9	1	0	8	2	0

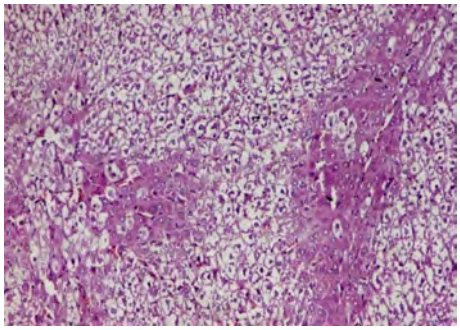
P value = 000



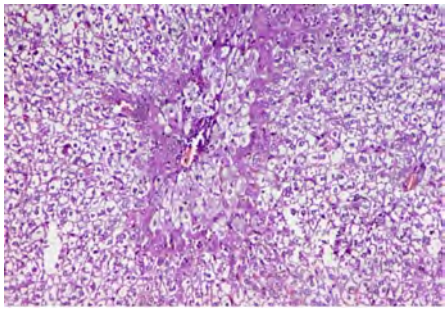
Figures1 (A, B&C) show a comparison between the effect of APAP and of EGF on APAP induced injury in liver and kidney.



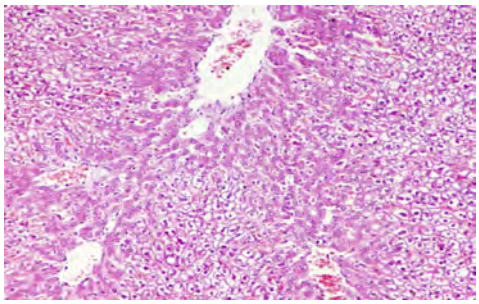
Photomicrography (1): Section in the rat liver of group I revealed normal liver architecture, normal sinusoidal pattern and normal portal tract areas.



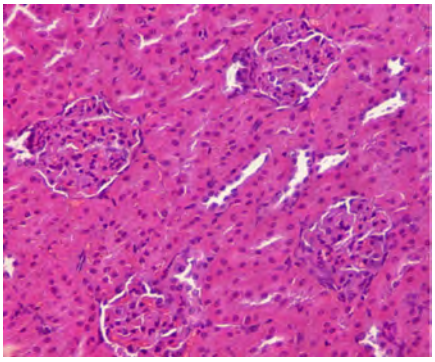
Photomicrography (3): Another section in the rat liver treated with APAP is coagulative necrosis around the central vein (zone III) with prominent hepatocellular vacuolation in between.



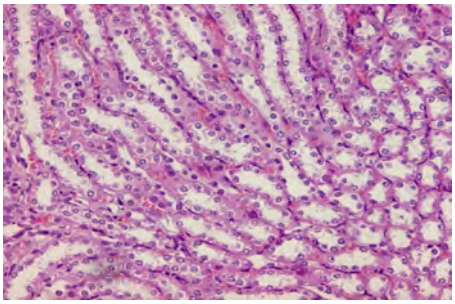
Photomicrography (2): section in the rat liver treated with APAP is coagulative necrosis around the central vein (zone III) with prominent vacuolar degeneration in the adjacent area.



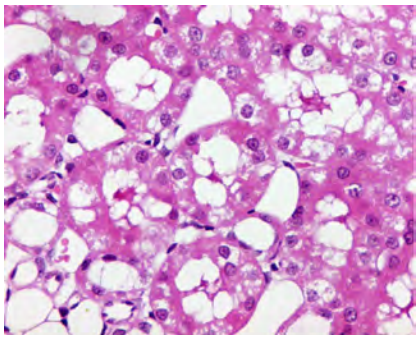
Photomicrography (4): Another section in the rat liver treated with EGF & APAP with improvement in comparison to the previous two cases, there is improved zone III necrosis around the central vein with remaining hepatocellular vacuolation in between.



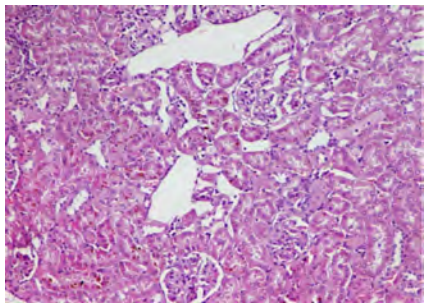
Photomicrography (5): Section in the rat kidney treated with APAP is cloudy swelling of proximal convoluted tubules. The lumen of the proximal convoluted tubules are nearly obstructed.



Photomicrography (7): Section in the rat kidney treated with APAP is vacuolar degeneration of distal tubules.



Photomicrography (6): Section in the rat kidney treated with APAP ;vacuolar degeneration in the proximal convoluted tubules.



Photomicrography (8): Section in the rat kidney treated with with EGF & APAP with improvement in comparison to the previous ,there is mild cloudy swelling of proximal convoluted tubules.

Discussion

This study reveals severe hepatotoxic and nephrotoxic effects of acetaminophen after single intrapritoneal dose. Acetaminophen (APAP) at therapeutic doses is rapidly metabolized in the liver principally through glucuronidation and sulfation, and only a small portion is oxidized by cytochrome P-450 2E1 to generate a highly reactive and cytotoxic intermediate, N-acetyl-p-benzoquinoneimine (NAPQI), which is quickly conjugated by hepatic glutathione to yield a harmless water-soluble product, mercapturic acid [13]. However, after acetaminophen overdose the capacity for glucuronidation and sulfation is exceeded and a large amount of NAPQI is formed via cytochrome P-450 2E1. After glutathione is depleted, NAPQI binds covalently to hepatic parenchymal cell proteins and DNA with resultant liver injury[10]. This search does not aim to study the mechanism of APAP induced injury. But, recent data has demonstrated that acetaminophen, a classic example of a chemical that produces dose-related centrilobular necrosis following metabolism by the cytochrome p450 system, also produces apoptosis in human hepatocytes [14].

n addition, mitochondria are targeted in APAP-induced hepatic injury. Kupffer cells (KC) become activated and release of pro-inflammatory and pro-apoptotic cytokines such as tumour necrosis factor (TNF) could contribute to paracetamol-induced liver injury by targeting mitochondria. Stimulation of TNF receptor-1 activates caspase-8 and causes mitochondrial outer membrane permeabilisation through tBid and Bax followed by mitochondrial permeability transition (MPT). Fas signaling plays a key role in this clinical setting [15].

A similar mechanism was proposed for the nephrotoxicity of acetaminophen. However, it has been proposed that the cytotoxicity of NAPQI could be also dependent on its metabolism via one electron reduction followed by re-oxidation. This redox cycle reaction can reduce molecular oxygen to form superoxide anion with a consequent formation of the oxidizing radicals, hydrogen peroxide

and hydroxyl radical [16]. Some investigators [17] reported that the generation of the reactive oxygen species appears as an early event which precedes GSH depletion and cell damage in APAP hepatotoxicity.

Therefore, strategies for preventing organ toxicity induced by therapeutic drugs, including acetaminophen, would be very meaningful in clinical practice. In this study, Epidermal growth factor (EGF) showed extreme significant protection of the kidney with moderate protection of the liver proved by both biochemical and pathological examinations. EGF is a well-known mitogen for a variety of epithelial and mesenchymally-derived cells and it is also a potent cytoprotective and reparative polypeptide growth factor for the gastrointestinal mucosa (8) and other tissues when they are exposed to injurious factors or necrogenic agents.

Signaling through epidermal growth factor (EGF) receptors (ErbB receptors; EGFRs) is important for fundamental cellular functions, such as proliferation, migration, growth, and differentiation. A number of studies have shown that both quiescent and proliferative hepatocytes maintain significant levels of the transmembrane EGF receptor. Mature hepatocytes express the highest levels of EGF-R of any normal cell type, although its functional role in liver homeostasis has remained controversial[18]. In normal tissue, EGFR exhibits a high affinity for EGF and is believed to operate primarily as a mechanism to affect the blood-to-bile clearance of its serum binding ligands [19]. Treatment of isolated murine hepatocytes with EGF, has demonstrated to induce hepatocyte resistance toward Fas-mediated apoptosis [6]. This receptor can be bound and activated by a broad family of ligands that, besides EGF, include transforming growth factor α (TGFα), heparin-binding EGF-like growth factor (HB-EGF), betacellulin (BTC), epiregulin (EPR), and amphiregulin (AR). It is known that the expression of TGFα and HB-EGF in liver tissue is markedly increased on liver injury induced by CCl_4, and is necessary for efficient hepatocyte DNA synthesis after partial

hepatectomy [20].

EGF receptors are widely distributed in the kidney, both in the glomerulus and along the basolateral membranes of the tubular epithelium. EGF is also expressed at high levels in mammalian kidneys [21]. EGF production in the adult mouse kidney has been localized to the thick ascending limb/distal convoluted tubule [22]. It has been reported that there is increased processing of prepro EGF to its soluble, active form in response to acute ischemic renal injury [23]. In addition to roles as mitogens, EGF-like growth factors may be cytoprotective for the kidney [24]. In the plethoric pathophysiology of ischemic renal injury, the generation of reactive oxygen species (ROS) by vascular, inflammatory and epithelial cells plays a critical role, whereas these cells are also the primary target of ROS toxicity[25;26]. EGF contributed to reduce the generation of ROS and lipoperoxidative metabolites[23]. Also, they suggest that EGF somehow prevented the formation or facilitated the neutralization of hydrogen peroxide, and that also prevented the over-production of inflammatory mediators (i.e., platelet-activating factor [PAF]), which may derive from free fatty acids and lysophospholipids released by PLA2 [7-27]. In other experimental systems, EGF has shown a marked antioxidant activity by stimulating the production of superoxide dismutase (SOD) [5;28].

Haussler et al. found that EGF administration decreases apoptotic cell death in the nephrogenic zone of the developing kidney cortex and in the developing medullary papilla [29]. Also, Wang et al. noticed that the absence of EGF in the culture medium of tubular epithelial cells results in apoptosis emphasizing the survival and renotrophic qualities of this polypeptide [30].

In our study, we conclude that EGF protects the kidney however; it does not fully protect the liver from acute damage induced by APAP in male rats. This is in contrast to other studies done on the heart or gastric mucosa in which EGF protects against acute stress-induced injury[8-28]. However, a lack of liver protection by exoge-

nous EGF administration was described by Caballero et al. in a model of thioacetamide-induced multiorgan failure. This suggests that EGF may protect organs against some insults but cannot protect others. The mechanisms beneath the selectivity of the protective role of EGF remain to be clarified [11].

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Author Contribution.

Shereen M. Samir designed & performed research, analyzed data and wrote the paper

Mie A. Mohamed performed the pathological part of research including analysis of the pathological data

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DOES EGF HAVE A PROTECTIVE ROLE AGAINST ACETAMINOPHEN TOXICITY IN RAT KIDNEY OR LIVER?

Shereen M. Samir MD and Mie A. Mohamed MD

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CEMENTED VERSUS UNCEMENTED HEMIARTHROPLASTY FOR DISPLACED INTRACAPSULAR FRACTURE NECK OF THE FEMUR

El-Sayed M. A. El-Morsy MD, Akram A. H. Hammad MD and Mohamed I. I. Taha M.Sc*

*Departments of Orthopedic Surgery, Faculty of Medicine, Mansoura University, Egypt and Al-Jahra Hospital-Kuwait**

Abstract

We undertook a prospective randomized controlled trial involving 66 patients with a displaced intracapsular fracture of the neck of the femur to determine whether there was any difference in outcome between treatment with a cemented Thompson hemiarhtroplasty and an uncemented Austin-Moore prosthesis. The patients were followed up post-operatively for minimum of 12 months up to 4 years. The mean age of the patients was 70.04 years (63 to 86) and 39 patients were female. The hospital stay was reduced with cemented Thompson (P=0.001). Also, The functional results including better mobility, walking ability and less residual pain were better with cemented Thompson prosthesis (P=0.009), while the surgery time and intra-operative blood loss was less with Austin-Moore prosthesis (P<0.001). No statistically significant differences are found between the two groups with regard to mortality, implant related complications, reoperations or post-operative medical complications. The use of cemented Thompson hemiarthroplasty led to less residual pain, improved return of mobility and reduced hospital stay than an uncemented Austin-Moore prosthesis.

Introduction

Femoral neck fractures are common among elderly people. These fractures can cause significant changes in people's life and many patients never return to the same activity level. Displaced intracapsular fractures of the neck of the femur in the elderly patients are commonly treated by hemiar-

throplasty [1]. There is an ongoing controversy about the type of hemiarthroplasty preferred. The typical arguments are between proponents of unipolar versus bipolar hemiarthroplasty and between cemented and cementless implants and combinations of these two groups [2].

Monobloc or unipolar prostheses are still the norm for most elderly patients. The two most common types used are the uncemented Austin-Moore prosthesis and the cemented Thompson hemiarthroplasty[3]. The cemented Thompson prosthesis is cemented into the femoral canal by using bone cement while uncemented Austin-Moore prosthesis is inserted with press-fit technique[1]. Orthopaedic surgeons are divided as to the relative merits of cemented versus uncemented prostheses in those patients. Cementing the prostheses provides more secure fixation and may result in less residual pain and better function. However, the insertion of cement complicates the operation and carries the risk of cardiovascular collapse when the cement introduced into the femur[3].

The continued use of a mixture of uncemented and cemented prostheses reflects uncertainty as to the relative advantages and disadvantages of using bone cement. We therefore undertook this randomised controlled trial comparing an uncemented Austin-Moore prosthesis with a cemented Thompson hemiarthroplasty in elderly patients with a displaced intracapsular fracture of the proximal femur, with a minimum follow-up of one year'

Materials and Methods

Patients presented at Mansoura University Hospitals and Al-Helal Hospital, Cairo with a displaced fracture of neck of femur (Garden grades 3 and 4) were included in the study. All patients were randomized for the used prosthesis either cemented Thompson or Austin-Moore with respect to number of inclusion and exclusion criteria Table1.[2,4] Patients with odd hospital number had a cemented Thompson prosthesis (group A) and those with even number had a cementless Austin-Moore prosthesis (group B).

All the patients were operated by senior surgeons or at least supervised by them. All the patients received preoperative antibiotics and thrombo-prophylaxis during their hospital stay. The surgical approach used was either posterior approach (Southern) approach or direct lateral approach (Hardinge) according to the surgeon's preferences. The surgery time, intra-operative, post-operative blood losses and the amount of blood transfusion related to the procedure was noted. The mobilization and rehabilitation programs started after drain removal and progressed gradually according to patients' tolerance and compliance. The patients were followed regularly at six week, three months, six months, 1year and every year till end of follow up. The assessment of the clinical outcome and grading of the total functional results was done by using the Harris [5] hip scoring system, while radiological assessment was done by the immediate analysis (Fig. 1,2) for postoperative x-rays and delayed analysis (Fig. 3, 4) for x-rays taken at each follow up and till the end of follow up according to Yau et. al [6], radiological evaluation of gautier et. al[7] and Parker et.al[8] and according to the type of prosthesis used. The positive changes were recorded and correlated clinically to detect whether it could be explained in terms of clinical satisfaction or dissatisfaction. Both morbidity and mortality were noted in both groups. The patients' follow- up was not regular in some cases depending on many factors according to the patients' response, rate of improvement, development of complications and their understanding of what was needed from them as well as the patients'circumstances. In some cases patients refused to attend their appointment regularly for follow up and only follow up was done for some clinical parameters through telephone interview either with the patient or with next of ken and we arranged for the follow-up x-rays to be done close to their home for review and follow-up.

Statistical analysis :

Student t-test, Chi square test, Mann-Whitney test and K-S (Kolmogorov-Smirnov) test were used for statistical measurements.

A result was considered significant if the p value = or < 0.05.

Results

The mean follow-up period of this study was 33 months. The minimum period of follow- up was 12 months, while the maximum was 48 months. The total number of the patients included in this study was 77 patients. Eleven patients were excluded for different reasons and only 66 patients had completed follow-up. Six patients were deceased before completing 12 months of follow up; the causes of death were not conclusively gathered and are not reported on with an average age of those who died was 70 years, one patient with pathological fracture of the neck of the femur was diagnosed late as the head of the femur was suspected intra-operatively and sent for pathology to be found as a bony metastases from breast carcinoma and four patients lost follow up before completing twelve months of follow up. At the end of the follow up, we have 58 out of the 66 patients still alive because eight patients died from other reasons unrelated to the procedure after they had been followed-up for one year at least.

Patient characteristics :

The study included 66 patients. Thirty-nine females and 27 males with a ratio of (1.4:1). The mean age of the patients was 70.04 years with a range from 63-86 years. Group A included twenty one females and fifteen males, while group B included twelve males and eighteen females. The majority of patients 39 were females and they were in their sixth decade of life. The right hip was affected in 25 patients and the left hip was affected in 41 patients (Table 2, 3). None of the differences between the two groups as regard patient characteristics was statistically significant.

Operative details :

The Mean delay to surgery for both groups was 9 days with a range from 1-21 days. The mean delay for group A was 9.6 days while the mean delay for group B was 8.4 days. Most of the patients (40 patients) were operated before ten days and only 13 patients were operated after fifteen days due to the presence of associated medical problems which necessi-

tated appropriate adjustment of the medical condition prior to surgery. The common problems in our series were anemia, hypertension, diabetes mellitus, chronic bronchitis, bronchial asthma, liver problems and ischemic heart disease. Forty percent of our patients had one or more of these problems. The mean hospital stay was 16.5 days for both groups with a range from 10-36 days. The mean hospital stay for group A was 15.7 days while for group B was 17.4 days. The majority of patients (43 patients; 26 in group A and 17 in group B) stayed in the hospital post-operatively less than 15 days, while only 8 patients (3 in group A and 5 in group B) stayed in the hospital more than 30 days due to less compliance to rehabilitation program and development of general or orthopaedic complications which required further management. There was significant statistical difference in the mean length of hospital stay for both groups (Table 4). There was also significant statistical differences as regard the duration of surgery and blood loss in both groups (Table 5).

Severe hypotension and inferior myocardial ischemia developed intraoperatively in one case of cemented Thompson during insertion of the bone cement. Patient was resuscitated and managed by anesthetic team and stayed in ICU for three days until he is stable and then discharged to the general orthopedic ward to complete the rehabilitation program. Fissure fracture of the calcar occurred in two cases of Austin-Moore intraoperatively. Although the fractures were wired, this didn't affect the stability of the prosthesis. Both had excellent and good results at the end of follow up with no complain. Fracture of the greater trochanter occurred in one case of cemented Thompson which was treated by tension band of the greater trochanter and this patient showed poor result which necessitated conversion to cemented total hip at 23 months postoperatively.

General complications :

Two cases had chest infection and they were managed by chest physicians in addition to chest physiotherapy, and all of them showed full recovery. GIT bleeding occurred in two cases due to

stress ulcer. Patients were resuscitated and managed in the gastroenterology unit and upper GIT endoscopy was done to investigate and control the bleeding. One patient recovered after 9 days and discharged to orthopaedic ward while the other patient showed recurrence of the bleeding due to associated varices which necessitated further endoscopy and the patient recovered after 15 days. Three cases were complicated by DVT and one case developed pulmonary embolism in group A and was resuscitated and admitted to ICU. Vena cava filter was inserted and the patient was discharged from ICU after becoming stable on anticoagulant therapy under the care of medical team to continue the rehabilitation program. No dislocation of the prosthesis had been encountered in both groups. One case of superficial bed sore in group B, which healed with mobilization and dressing with no residue. Infection was encountered in a total of 3 cases; two cases (1 in group B and 1 in group A) had superficial infection which was managed by dressing and parenteral antibiotics after culture and sensitivity. One case had deep infection (group A) which didn't respond to dressing and antibiotics and necessitated debridement and suction irrigation at 9^{th} day postoperatively, but pain and deep infection persisted which resulted in septic loosening and acetabular erosion and required conversion to cemented total hip arthroplasty by two stage revision surgery at 15th months postoperatively (Fig. 5). No statistically significant differences between both groups. (Table 6).

Mortality

Eight patients (5 patients in group A with an incidence of 13.8% and 3 patients in group B with an incidence of 10 %) died from different causes unrelated to the procedure after completing one year of follow up. No statistically significant differences between both groups. (Table 7).

Functional and radiological results :

Patients in group A showed better compliance to postoperative mobilization and rehabilitation program, thus reduced the postoperative complications and reduced the mean length of hospital

stay 15.7 days (p=0.001). The cemented Thompson prosthesis showed superior clinical results in trems of function, mobility and less residual pain (mean Harris hip score was 85,9; p=0.009) (Fig. 6). Limb length discrepancy occurred in 8 cases. No dislocation seen in this group. The prosthesis was varus in position in 3 cases. Change of position of the prosthesis occurred in one case and periprosthetic osteolysis developed in 2 cases, while acetabular erosions and heterotopic ossification both occurred in three cases. However, the number of patients who were classified as both clinically and radiologically satisfactory in this group were 31 patients. While in group B patients had less compliance to postoperative mobilization and rehabilitation program thus increased the mean length of hospital stay (17.4 days). The cementless Austin-Moore prosthesis showed inferior clinical results in terms of function, mobility and more residual pain (mean Harris hip score was 80.4). Limb length discrepancy occurred in 12 cases. No dislocation was seen in this group also. The prosthesis was varus in two patients. Two cases had change of the position of the prosthesis, marked osteolysis along the stem developed in 3 cases, while acetabular erosions and heterotopic ossification both occurred in one case. Sever disabling pain occurred in one case which is in correlation with radiological changes in the form of pivoting and subsidence needed conversion total hip arhtroplasty. However, the patients who were classified as both clinically and radiologically satisfactory in this group included 22 patients. (Table 8) .

Table 1: Inclusion and exclusion criteria for patients in the study [2,4]

Inclusion criteria were:
Displaced fractures of the neck of the femur (Garden III and Garden IV) in patients over 60 years.
Exclusion criteria were:
Fractures secondary to pathology, such as metastases in the head and neck of the femur.
Patients with Sever osteoporosis; (singh I and II were excluded which need cemented fixation).
Unsuitable medullary canal of the proximal femur which was assessed in the pre-operative x-rays of the patients.
Pre-existing sepsis.
Pre-existing disease of the ipsilateral hip as osteoarthritis of the hip.
Neurological problems and Impaired cognitive status (less than 11points according to abbreviated mental score assessment).
Associated fracture precludes routine postoperative rehabilitation.

Table 2: Demographic characterisitics of the patients.

Variable	No. or %	
Age	63-86	(mean 70.04±5.8 years)
Sex	Male	27 (40.9) %
	Female	39 (59.1) %
Side	Right	25 (37.8) %
	Left	41 (62.2) %
Prosthesis	Austin- Moore	30 (45.5) %
	Cemented Thompson	36 (54.5) %
Delay to surgery	From 1-21 days	(mean±SD 9±4.5 days)
Hospital Stay	From 10-36 days	(mean±SD 16.5±8.6 days)

Table 3 : Age groups of the patients.

Age groups	Thompson	M	F	Austin-Moore	M	F	No. (%)
60- 70	27	11	16	15	6	9	42 (63,6)
70-80	7	4	3	10	4	6	17 (25,7)
> 80	2	-	2	5	2	3	7 (10,6)
Total	36	15	21	30	12	18	66
Mean value	71.78±6.3			68.3±5.2			70.0±5.5

P=0.62

Table 4: Length of the hospital stay.

Length of hospital stay in days	No. % Thompson No =36	No. % Austin-Moore No=30	Total No. % No=66
<15 days	26(72.2)%	17(56.6)%	43(65,15)%
15-30 days	7(19.4)%	8(26.2)%	15(22,72)%
> 30 days	3(8.3)%	5(16.6)%	8(12,1)%
Total	36	30	66
Mean value	15.7±2.2	17.4± 1.9	16.5±2.06

P=0.001**

Table 5: Duration of Surgery and blood loss.

Prosthesis type	Mean Surgery time	Average blood loss			Mean amount of Blood transfusion
		Intraoperative	Postoperative	Total	
Thompson	78.3± 12.5minutes	520±75.8 ml	225±45ml	775±60ml	557±60ml
Austin-Moore	57.5±9.8 minutes	415 ±50.5ml	240±24ml	655± 37ml	550±65ml
Difference	20.8 minutes	105 ml	15ml	120ml	7ml
P	<0.001***	<0.001***	0.13	0.09	0.32

P is significant if≤ 0.05 Independent sample t-test is used

Table 6 : Complications.

Complication	Group A(%) No=36	Group B(%) No=30	Total (%) No=66	P
*General				
Cardiac	1 (2.7)%	0	1 (1.5)%	0.92
GIT.Bleeding	0	2 (6.6)%	2 (3.03)%	0.39
DVT	1 (2.7)%	2 (6.6)%	3 (4.5)%	0.87
P. Embolism	1 (2.7)%	0	1 (1.5)%	0.92
Chest Infection	1 (2.7)%	1 (3.3)%	2 (3.03) %	0.87
Bed sores	0	1 (3.3)%	1 (1.5)%	0.92
*Orthopaedic				
Fiss. Fracture of the calcar	0	2 (6.6)%	2 (3.03)%	0.39
Fracture of the Greater Trochanter	1 (2.7)%	0	1 (1.5)%	0.92
superficial				
Infection	1 (2.7)%	1 (3.3)%	2 (3.03)%	0.55
deep	1 (2.7)%	0	1 (1.5)%	0.92
Dislocation	0	0		
Failure and revision	2 (5.5)%	1 (3.3)%	3 (4.5)%	0.87
Total	9 (25) %	10 (33.3) %	19 (28.8)%	

Table 7: The mortality.

Mortality	Thompson No=36	Austin-Moore No=30	No. % No=66
12-36 months	2 (5.5%)	3(10%)	5(7,5%)
36-48 months	3(8.3%)	-	3(4,5%)
Total	5(13.8%)	3(10%)	8(12,1%)

P=0.34 P is significant if ≤ 0.05, Chi-square test is used

Table 8: Grading of total function according to H.H.S.

Grade	Cemented Thompson (%) No=36	Austin-moore (%) No=30	Total (%) No=66
Excellent	5 (13.8)%	3(10)%	8(12.1)%
Good	26 (72,2)%	19(63.3)%	45(68.2)%
Fair	4(11.1)%	6 (20)%	10(15.2)%
Poor	1(2.7)%	2(6.6)%	3(4.5)%
Total	36	30	

P=0.61 P is significant if ≤ 0.05 Chi-square test is used

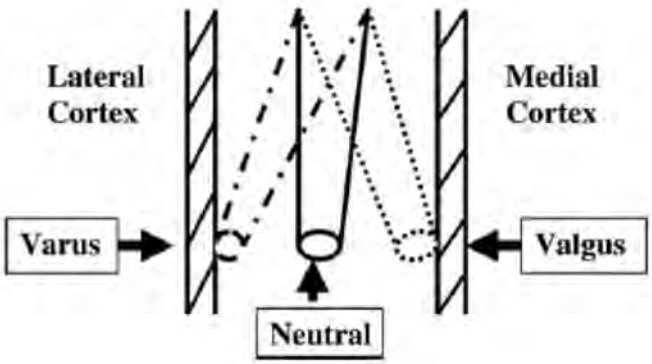

Fig. 1 The alignment of prosthesis in the early postoperative X-ray (the alignment of the prosthesis was defined by the relative position of the tip of the prosthesis to the inner cortex of shaft of femur).[6]

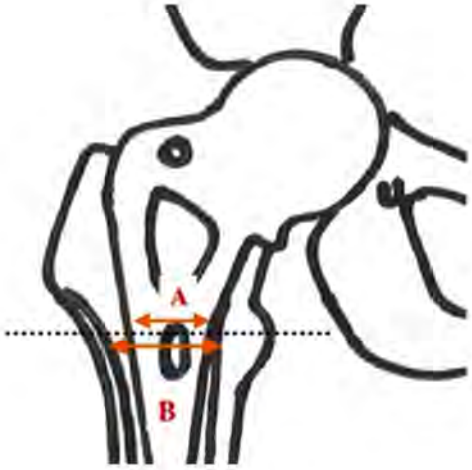

Fig. 2 Relative fill of the stem of prosthesis to the medullary canal of femur at the level of lesser trochanter on AP X-ray (A and B).[6]

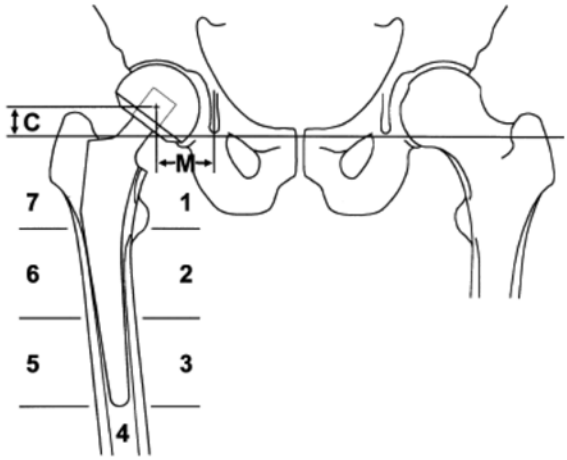

Fig. 3 Assessment of radiologic parameters: M is the distance of the teardrop to the center of rotation of the prosthetic head; C is the distance between the interteardrop line to the center of rotation of the prosthetic head. Numbers 1 to 7 represent Gruen's zone.[7]

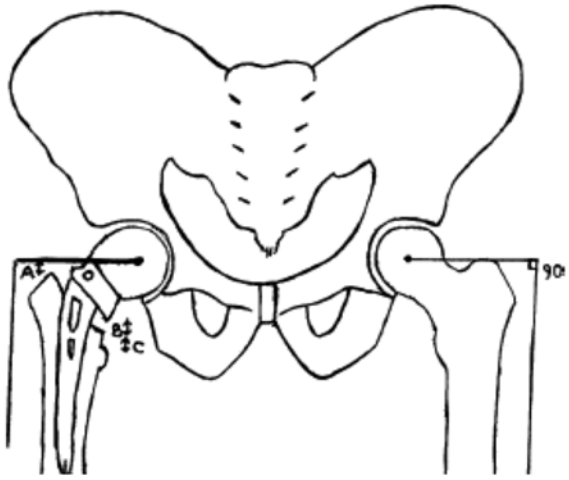

Fig. 4 Radiographic measurements made. *A* represents the leg length discrepancy; *B* the calcar seating distance; *C* the length of neck remnant.[8]

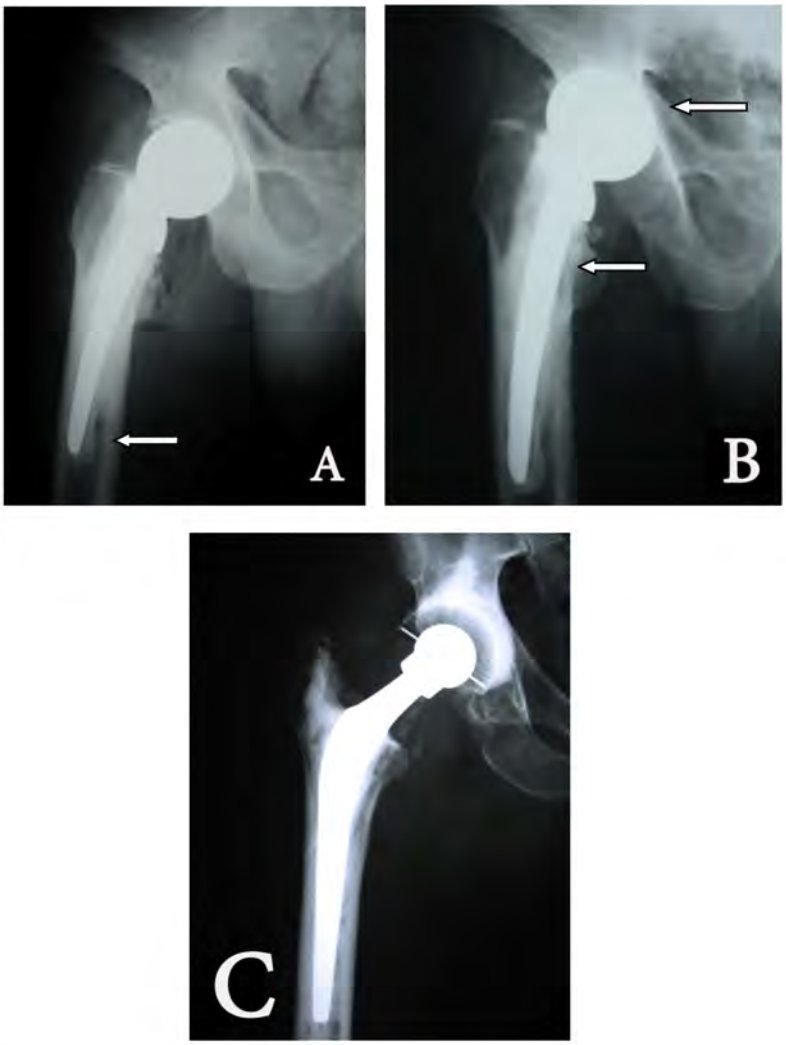

Fig. 5: (A) Immediate postoperative radiographs showing cemented Thompson prosthesis inserted via lateral approach. In male patient aged 72 years old. There is good cement mantle all around the prosthesis. However, the prosthesis is in varus position. This patient had infection which required debridement. After this Patient had painful limited range of hip motion and inability to walk. **(B)** It shows loosening due to deep infection and acetabular protrusion at 15th months postoperatively which required conversion total hip arthroplasty **(C)**

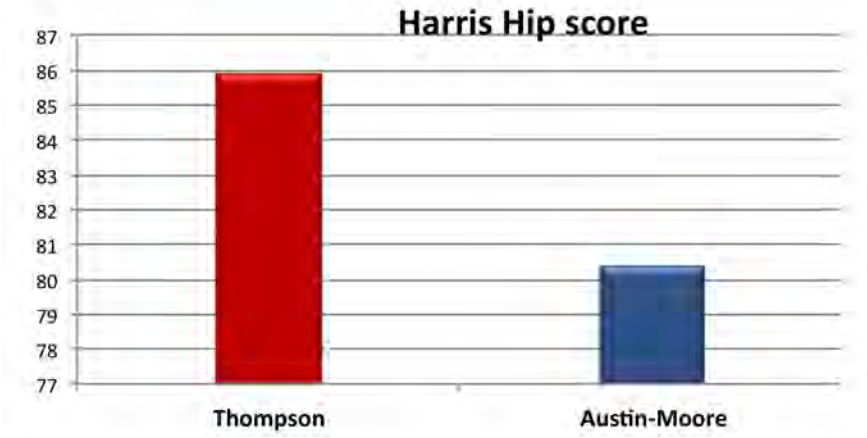

Fig. 6 show Mean harris hip score in both groups with statistical significant differences (***P=0.009** ,Mann-Whitney test is used)***

Discussion

Hip fracture is a serious injury in the elderly with a high mortality and complication rate.[9,10] The pendulum is swinging between reduction and internal fixation with various supplementary methods as osteosynthesis to total hip replacement. It is now the general consensus that reduction and internal fixation should be reserved for the younger patients in whom if needed revision surgery may be done at a later date. For the displaced femoral neck fracture in the elderly the standard surgical procedure has been hemiarthroplasty. It has been debated for years which method of fixation is the best, cementing the prosthesis or press-fit fixation without cement. There are only a handful of prospective studies comparing these two methods and in almost all of them the prostheses used are either uncemented Austin-Moore or cemented Thompson.[2,4]

In Egypt, the technically demanding and advancing procedures of unipolar modular, bipolar and total hip replacement will lack universal application for a long time to come and the hemireplacement procedures by using Austin-Moore (uncemented) and Thompson (cemented) will need to

have continued application to fill the lacuna produced by deficient resources and finances. In this context we undertook the present study to evaluate the outcome and patient satisfaction of monoblock unipolar hemiarthroplasty in displaced fracture neck of the femur in the elderly using Austin-Moore (uncemented) versus Thompson (cemented) prosthesis keeping in view the living condition of an average Egyptian.

The common problems in this series were anaemia, hypertension, diabetes mellitus, chronic bronchitis, bronchial asthma, liver problems and ischaemic heart disease. Forty percent of our patients had one or more of these problems. These problems were reported in similar studies[11,12,13] except for liver problem which was common in this series and neurological problems which were common in western series and were excluded from this study. Liver problem was a problem which is not commonly found in western literature as it is common in the Egyptian community. This is because of the increased number of the patients with viral hepatitis and bilharzial liver cirrhosis in Egypt. There is another important difference, the patients with nervous system disorder and mental problems are not found in this series as they were excluded, whereas they were common in western series. They were excluded in this series because they most probably would assume different prognosis and are difficult to assess and compare their results.

It can be noticed that in this series that we had more delay to surgery but less total hospital stay than other series[2,3,12] and this could be probably due to good patient preparation before surgery. Although the difference in hospital stay between the two groups in this series is the least, it is statistically significant; (p value = 0.001) and it still can be clearly seen that patient in cemented group reported less hospital stay. This could be explained that patients in cemented group can tolerate ambulation and showed better rehabilitation than cementless group. Early ambulation and comparatively less hospital stay following hemiarthroplasty have also been reported

in other series[2,3,12]. This is an advantageous factor in relation to economy of hospital beds and favours financial condition of the patients.

In this study, the mean difference in surgery time between both groups was 20.8 minutes. The duration of surgery depends on many factors; experience of surgeon and assistants, the method of anaesthesia used, the development of any intraoperative complications and issues related to the technique like the method of cementing in case of cemented Thompson. The mean duration of surgery in this study for both groups was average in relation to other series[3,12]. However, the mean difference in surgery time between group A and group B is slightly longer and statistically significant; (p value < 0.001). This could be explained by the method of cementing used by less experienced surgeons and the time consumed by the surgeons to remove the excess of bone cement and to make sure that the cement is not retained in the acetabulum. Also, the intraoperative blood loss was less in cementless group and there was statistically significant difference; (p value < 0.001).

Previously published randomized trials comparing cemented and uncemented hemiarthroplsties for patients with a fracture neck of femur have been identified and summarized in the cohrane review on this subject. It showed that in most series there was no statistical significant differences in mortality and morbidity between the two groups except for increased incidence of pneumonia in cemented group and also increased incidence of conversion arthroplasty in cementless group because of disabling thigh pain.[14] In this series also, there is no significant difference as regard morbidity and mortality. We had no intra-operative deaths in this series. We also had no hospital mortality. The possible explanation for this could be the younger age of our patients in relation to western series, proper management of the associated medical problems preoperatively, use of antibiotics routinely and early mobilization.

The causes for revision arthro-

plasty include disabling pain, infection, dislocation and periprosthetic fractures. In this series the incidence of revision arthroplasty was 4.5%.We had three cases; one in group B and two in group A. The first case in group A had deep infection which ended by disabling pain and septic loosening while the second case had peiprosthetic fracture in the greater trochanter and ended by pain, acetabular erosion and subsidence. In group B only one case had disabling thigh pain and loosening. Although most of series[14] in the literature showed tendency to more revision arthroplasties in cementless group, we had less cases in cementless group in this series. One possible cause is that operations in this series either done by senior surgeons or at least supervised by them which decreased technical errors leading to implant failure. Another thing that elderly population in Egypt are less likely to undergo revision arthroplasty, even if significant symptoms of residual pain exists.

The functional outcome was for the cemented Thompson group better than that of the cementless Austin Moore group as regard mean Harris hip score (p value = 0.009) and regarding the excellent and the good results it was better by 12.8%. However, the difference between the two groups as regard the total functional results was not statistically significant(P=0.39)

In summary, the cemented Thompson hemiarthroplasty led to less pain in the hip, improved return of mobility and a reduced hospital stay compared to an uncemented Austin-Moore prosthesis. Also, there was no increase in complications or mortality related to the use of cement. However, the Austin-Moore prosthesis had less operative time and intraoperative blood loss compared to cemented Thompson prosthesis. No significant statistical differences were found as regard morbidity and mortality in both groups and as regard the total functional results. We suggest that when a hemiarthroplasty is used for a fracture of the hip it should be cemented in place. However, there is still a reserved place for Austin-Moore prosthesis especially in the frail elderly people with low cardiopulmonary reserve and

when time factor is an important concern.

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REPRINT

BENHA MEDICAL JOURNAL

CEMENTED VERSUS UNCEMENTED HEMIARTHROPLASTY FOR DISPLACED INTRACAPSULAR FRACTURE NECK OF THE FEMUR

El-Sayed M. A. El-Morsy MD, Akram A. H. Hammad MD and Mohamed I. I. Taha M.Sc

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RELIABILITY OF GLYCEMIC CONTROL IN HEAD TRAUMA PATIENTS : A CORRELATION OF TWO GLYCEMIC RANGES WITH THE OUTCOME

Mona Gad M. El-Ebiedy Msc*, Amgad A. Zaghlol MD, Amr M. Yasseen MD, Mahmoud M. Othman MD and Mohamed A. A. Sultan MD

Departments of Anesthesia and Surgical ICU, Faculty of Medicine, Mansoura University, Egypt.*

Abstract

Introduction: *Tight glycemic control is currently used widely in critically ill patients. However , it is accompanied with side effects especially in patients with traumatic brain injury .*

Aim of the work (Objective): *This study was designed to find out the reasonable and suitable range of blood glucose level in head trauma patients. We assumed that intermediate control of blood glucose (110-140 mg/dl) will be a reasonable option to protect the brain injured patients from the possible hazards of hypoglycemic episodes of tight glycemic control.*

Patients & Methods: *This study was carried out on patients with severe traumatic brain injury.They were randomly divided into 2 major groups via sealed envelope assignment: GroupT (tight glycemic group) with random blood sugar between 80-110 mg/dl (129 patients) and groupI (intermediate glycemic group) with random blood sugar between 110-140 mg/dl (124 patients). Glasgow coma score (GCS), Glasgow outcome score (GOS), incidence of hypoglycemic episodes and number of patients who developed one or more episodes of hypoglycemia were considered as the primary end point of the study. Incidence of infection, vital organ dysfunction development that needed support, patients needed mechanical ventilation for more than 48 hours, ICU stay and 28-day mortality were considered as secondary end point of the study.*

Results: *Intermediate glycemic control with random blood sugar*

between 110-140 mg/dl recorded lower incidence of hypoglycemic episodes, days of ventilation and period of stay in ICU compared with tight glycemic control with lower range between 80-110 mg/dl. Their was no significant difference regards infection and the need for vital organ support between both groups, the intermediate range recorded significant improvement in GOS with lower 28 day mortality.

Conclusion: *we concluded that tight glycemic control with random blood sugar ranging between 80-110 mg/dl did not offer any advantage over intermediate glycemic control with random blood sugar ranging between110-140 mg/dl in patients with severe traumatic brain injury. More over, tight glycemic control recorded higher mortality with higher incidence of hypoglycemia. We found that intermediate glycemic control appeared to be safer avoiding hazards of hypoglycemia with better neurological outcome.*

Key Words: *traumatic brain injury; tight, intermediate glycemic control*

Introduction

Stress related hyperglycaemia, previously considered to be a protective physiological response to meet the increased demands of tissue injury, has been recently a challenge in critically ill medical and surgical patients [1].

Intensive Insulin Therapy (IIT) is defined as the maintenance of normoglycemia to keep the blood glucose concentration at 80-110 mg/dl by titration of intravenous insulin [2]. Intensive Insulin Therapy plays an important role in protecting both the central and peripheral nervous system in critically ill patients with traumatic brain injury and it demonstrated significantly fewer seizures as well as lower intracranial pressures [3].

However, using IIT to lower blood glucose to normal values in TBI requires careful monitoring of blood glucose, as the classic neurological symptoms of hypoglycemia can be offset by sedation or the underlying impairment of mental status [4]. The safety of IIT has been questioned [5].

The physiological response to hypoglycemia itself can be im-

paired as reviewed recently. Another concern is that even asymptomatic episodes of hypoglycemia can blunt the physiological response of the sympathoadrenal system, thereby leading to a vicious circle in which the patient is at risk of recurrent hypoglycemia and therefore neurological injury [6].

In traumatic brain injury intensive insulin therapy was associated with increased incidence of microdialysis markers of cellular distress [7]. Recent data suggest targeting safe effective blood glucose control with intermediate range of 140-180 mg/dl. This range will decrease hypoglycemia and workload while still reducing the adverse of severe hyperglycemia [8].

Nevertheless, as demonstrated in critically ill patients, blood glucose levels higher than 180 mg/dl can no longer be considered as an acceptable target for insulin therapy. However, the issue of the safest range below this level is still unresolved and has not been specifically addressed in prospective clinical trials to date [9].

Intermediate glycemic control with random blood sugar between 110-140 mg/dl may be a safer alternative to tight glycemic control with random blood sugar between 80-110 mg/dl in patients with traumatic brain injury.

Patients & Methods

This prospective study was carried out on patients with traumatic brain injury admitted to Surgical Intensive Care Unit of Mansoura Emergency Hospital from April 2009 to end March 2011. Local ethical approval and informed consent was taken from the next of kin.

This study included head trauma patients above 18 years old, expected to be in ICU for >5 days, Glasgow Coma score (GCS) of ≤ 8 and with no history of major organ dysfunction.

Patients with pre-injury diabetes \ mellitus, on corticosteroid therapy, associated major extracranial injuries or associated systemic organ dysfunction were excluded from the study.

The patients included in the

study were followed up and randomly classified by closed envelops method according to target blood glucose level (BGL) into 2 groups:

Tight glycemic control group (GroupT) with target BGL 80-110 mg/dl ?(129 patients) and intermediat glycemic control group (GroupI) with target BGL 110-140 mg/dl? (124 patients).

- Insulin infusion: 50 iu regular human insulin (humulin R 100iu/ml, Lilly Egypt) in 50 ml 0.9% normal saline was prepared and given by syringe pump. Insulin infusion rate was adjusted according to Algorithm 1-4 in table 1 (10) and continued during the first 5 days .

Moving from algorithm to another algorithm:

We started using algorithm 1 then we moved up or down according to patient's response.

- Moving up: means an algorithm failure (BG outside the goal range, and the BG did not change by at least 60 mg/dl within 1 hour).

- Moving down: when BG was <70 mg/dl after 2 consecutive readings.

Patient monitoring: capillary BG was checked every 1 hr until within the goal range for 4 hrs, then BG measurement were decreased to every 2 hrs for 4 hrs. When the target range was reached measurement was every 4 hrs. The average of readings of RBS was calculated daily for the first 5 days.

Nutritional support: All patients received glucose free fluids for the first 24 hours. Then enteral feeding via nasogastric tube was started gradually according to our ICU protocol:

First day: started at 8 am, 50 ml/hour for the 1st 4 hours then 75 ml/hour for the next 4 hours then 100 ml/hour for the next hours till 12 midnight then rest till 8 am. (total volume 1300 ml/day).

Second day: started with 75ml/hour for 4 hours then 100 ml/hour till 12 midnight and rest

till 8 am. (total volume 1500 ml/ day).

Third day: started with 75ml/ hour for 4 hours then 125 ml/ hour till 12 midnight and rest till 8 am. (total volume 1800 ml/day)

Fourth day and on?

we gave 150 ml/hour started at 8 am till 12 midnight and rest till 8 am.

(total volume 2400 ml/day)

- We added Ringer's solution by intravenous route to complete the daily fluids to 2500-3000 ml/ day according to the body weight.

Treatment of hypoglycemia (BG<60 mg/dl): insulin infusion was discontinued and we gave dextrose 25% intravenous (100ml) then BG was rechecked every 20 minutes and we repeated dextrose 25% if BG was still <60 mg/dl. Insulin infusion was restarted if BG is >70 mg/dl for 2 consecutive readings and we started with lower algorithm.

Table (1):

Algorithm l		Algorithm 2		Algorithm 3		Algorithm 4	
BG	U/hr	BG	U/hr	BG	U/hr	BG	U/hr
<70	Off	<70	Off	<70	off	<70	Off
70- 109	0.2	70-109	0.5	70- 109	1	70- 109	1.5
110-119	0.5	110-119	1	110-119	2	110-119	3
120-149	1	120-149	1.5	120-149	3	120-149	5
150-179	1.5	150-179	2	150-179	4	150-179	7
180-209	2	180-209	3	180-209	5	180-209	9
210-239	2	210-239	4	210-239	6	210-239	12
240-269	3	240-269	5	240-269	8	240-269	16
270-299	3	270-299	6	270-299	10	270-299	20
300-329	4	300-329	7	300-329	12	300-329	24
330-359	4	330-359	8	330-359	14	330-359	28
>360	6	>360	12	>360	16	>360	32

Nurse Training: before the start of the study, the ICU nurses received intensive training course for two weeks with tight glycemic control in a trial to improve the nurse competence and correct management.

- After 5 days of the study, glycemic control was shifted from intravenous infusion to subcutanous insulin 6 hourly (or 4 hourly in resistant cases) according to our ICU protocol:

• RBS >130-150 mg/dl we

gave 4 units.

- RBS 150-200 mg/dl we gave 8 units.
- RBS 250-300 mg/dl we gave 12 units.
- RBS 300-350 mg/dl we gave 16 units.
- RBS>350 mg|dl we gave 20 units.

The main outcome measures in this study were:

GCS, GOS, incidence of hypoglycemic episodes and number of patients who developed one or more episodes of hypoglycemia were considered as the primary end point of the study. Incidence of infection (hospital acquired pneumonia ,urinary tract infection and venous catheter related infection), vital organ dysfunction development that needed support, patients needed mechanical ventilation for more than 48 hours, ICU stay* and 28-day mortality were considered as secondary end point of the study.

* patients were discharged from ICU when they no longer needed vital organ support and received at least 2/3rds of their caloric intake by normal enteral route.

Statistical Analysis

The statistical analysis of data was done by using SPSS program (Chicago, IL, version 16, USA). The data were displayed as mean ± SD for quantitative data, median (interquartile range) for scores and episodes of hypoglycemia and number (percentage) for qualitative data.

K-S (Kolmogorov-Smirnov) test was used to test the normality of distribution. For parametric data, paired and unpaired t-test was used for intra and intergroup differences respectively. Mann Whitney test was used for non-parametric data. Chi square test was used analysis of number (percentage) of qualitative data.

Mean group differences and their 95% confidence intervals were calculated to determine which of the specific variables differed between groups. If the 95% confidence intervals include 0, it indicates no significant difference between groups. P value < 0.05 was considered significant.

Power analysis was performed by G *power, version 3.0.4 pro-

gram (two tailed). The sample size was based on estimated enrollment of head trauma patients managed with two different regimens of insulin therapy over two years period. The Glasgow outcome score was used as a primary variant. Post hoc power analysis was done at an ?- error of 0.05 and 0.5 effect size convention that yield study power of 0.97.

Kaplan Meier survival graph was plotted to study 28-mortality between the studied groups by using the log-rank test.

Results

The two studied groups were comparable in age, , body mass index(BMI) and sex (Table2).

Although there was no significant difference in random blood sugar at admission, it was significantly higher (p=0.0001) in the first five days in group I compared with group T. There was significant decrease in the first five days compared with the admission value for each group(table 3).

There was a significant difference (p=0.0001) in number of patients with hypoglycemia in group I compared with in group T.(19 versus 58 patients). As regards hypoglycemic episodes also there was significant decrease (p=0.001) in group I. compared with group T.(Table 4).

Glasgow coma score(GCS) showed no significant difference between the two groups neither at admission nor at the first three days but it was significantly higher in 4th (P=0.04) and 5th (P=0.005) day in group I compared with group T and also in comparison with the admission value in the same group. Glasgow outcome score(GOS) improved significantly (P=0.0001) in group I compared with group T (Table 5).

The need for vital organ support showed no significant difference between both groups. In group I 24 patients (19.3%) versus 30 patients (23.2%) in group T needed vasopressor support, 6 patients (4.8%) developed renal impairment in group I versus 8 patients (6.2%) in group T, 52 patients (41.9%) had respiratory support in group I versus 59 (45.7%) in group T. Days of venti-

lation were significantly less (p=0.0001) in group I (6.8±3.5) in comparison with group T (11±3.1) (Table 6).

Out of 129 studied patients in group T, 40 patients(31%) had infection compared with 34 patients (27.4%) out of 124 studied patients in group I with no statistical significance between both groups [8 patients in group T developed bactremia versus 6 patients in group I, 25 patients in group T developed pneumonia versus 18 patients in group I , 4 patients in group T developed urinary tract infection versus 5 patients in group I and 3 patients in group T developed catheter related infection versus 4 patients in group I] (Table 7).

Days of ICU stay were significantly less (p=0.0001) in group I (9.4±3.1) compared with group T. (12.1±2.3).

In the two studied groups, regarding 28 day hospital mortality although there was no significant difference between both groups, group I recorded lower mortality as 35 patients (28.2%) died out of 124 patients in group I versus 44 patients (34.1%) out of 129 patients in group T [ICU mortality was 33 patients (26.6%) in group I versus 41patients (31.7%) in group T and ward mortality was 2 patients (1.6%) in group I versus 3 patients in group T] (Table 8).

Kaplan Meier survival graph which was plotted to study 28-mortality between the two studied groups showed better survival in group I. compared with group T. (Figure1).

Table 2. Demographic data of patients with severe traumatic brain injury in the two studied groups .

	Group $_T$ (n=129)	Group $_I$ (n=124)	Difference (95%CI)	P
Age (yr)	34.66±7.96	33.65±8.99	1.01(-2.1, 2.2)	0.987
BMI (kg/m^2)	32.8±7.9	32.6±8.4	0.2(-1.7,2.2)	0.822
Male/ Female (n,%)	84(65.1%) 45(34.9%)	88(71%) 36(29%)	4%(11.4% - 12.4%) 5.9%(6.2% - 17.6%)	0.319

Group $_T$: Patients with tight glycemic control (80-110 $mg.dl^{-1}$) .
Group $_I$: Patients with intermediate glycemic control (110-140 $mg.dl^{-1}$).
Data are in mean ± SD,except sex is in number(percent).

Table 3. Random blood sugar (RBS) ($mg.dl^{-1}$) in patients with severe traumatic brain injury in the two studied groups at admission and in the first five days.

	Group $_T$ (n=129)	Group $_I$ (n=124)	Mean difference (95%CI)	P
RBS admission	204.7±39.8	205.5±42.6	0.8(-9.4,11.1)	0.876
1st day	92.3±18.3*#	120.9±23.1*#	28.6(23.4,33.7)	<0.0001
2nd day	93.4±18.4*#	121±20*#	27.6(22.8,32.3)	<0.0001
3rd day	91.2±19*#	123.2±22.4*#	32(27.1,36.8)	<0.0001
4th day	94.1±18.9*#	122.6±20.7*#	28.5(23.6,33.4)	<0.0001
5th day	92.4±19.1*#	126.4±21.8*#	34(28.9,39.1)	<0.0001

Group $_T$:Patients with tight glycemic control (80-110 $mg.dl^{-1}$).
Group $_I$: Patients with intermediate glycemic control (110-140 $mg.dl^{-1}$).
Data are in mean ± SD, * Significant increase in comparison with group T, # Significant decrease in comparison with the admission value.

Table 4. Incidence of hypoglycemia in patients with severe traumatic brain injury in the two studied groups.

	Group $_T$ (n=129)	Group $_I$ (n=124)	Difference (95%CI)	P
Number of patients with hypoglycemia	58(45%)	19(15.3%)*	29%(18% to 40.2%)	<0.0001
Hypoglycemic episodes (per patient)	3(2-4)	2(1-2)*		0.001

Group $_T$: Patients with tight glycemic control (80-110 $mg.dl^{-1}$).
Group $_I$: Patients with intermediate glycemic control (110-140 $mg.dl^{-1}$).
Hypoglycemic episodes is in median (interquartile range) , number of patients with hypoglycemia is in number(percent),* Significant decrease in comparison with group $_T$.

Table 5. Glasgow coma scale (GCS) at admission and in the first five days and Glasgow outcome score (GOS) in patients with severe traumatic brain injury in the two studied groups.

	Group $_T$ (n=129)	Group $_I$ (n=124)	P
GCS admission	7(6-8)	7(6-8)	0.188
1st day	8(6-9)	8(6-9)	0.278
2nd day	8(6-9)	8(7-9)	0.231
3rd day	8(7-9)	8(7-10)	0.341
4th day	8(6-10)	9(7-11)*#	0.04
5th day	9(6-10)	9(8-11)*#	0.005
GOS	3(2-4)	4(3-5)*	<0.0001

Group $_T$: Patients with tight glycemic control (80-110 $mg.dl^{-1}$).
Group $_I$: Patients with intermediate glycemic control (110-140 $mg.dl^{-1}$).
Data are in median (interquartile range),* Significant increase in comparison with group $_T$ # Significant increase in comparison with the admission value.

Table 6. Vital organ support and duration of ventilation(days) in patients with severe traumatic brain injury in the two studied groups .

	Group $_T$ (n=129)	Group $_I$ (n=124)	Difference (95%CI)	P
Patients received vasopressor treatment	**30(23.2%)**	**24(19.3%)**	**3.9%(6.8% -14.4%)**	**0.443**
Patients needed dialysis	**8(6.2%)**	**6(4.8%)**	**1.4%(5.3% - 8.1%)**	**0.631**
Patients needed mechanical ventilation	**59(45.7%)**	**52(41.9%)**	**3.8%(8.9% -16.3%)**	**0.734**
Duration of mechanical ventilation(days)	**11±3.1**	**6.8±3.5***	**4.2(2.9,5.4)**	**<0.0001**

Group $_T$: Patients with tight glycemic control(80-110 mg.dl^{-1}).
Group $_I$: Patients with intermediate glycemic control(110-140 mg.dl^{-1}).
Vital organ support is in number(percent), duration of ventilation(days) is in mean±SD. * Significant decrease in comparison with group $_T$.

Table 7. Incidence of infection in patients with severe traumatic brain injury in the two studied groups.

	Group $_T$. (n=129)	Group $_I$ (n=124)	Difference (95%CI)	P
Total patient number(%)	40(31%)	34(27.4%)	3.6%(8.1% - 15.1%)	0.531
Bactremia	8(6.2%)	7(5.6%)	0.6%(6.3% - 7.4%)	0.937
Pneumonia	25(19.3%)	18(14.5%)	4.9%(5.1% - 14.6%)	0.312
Urinary tract infection	4(3.1%)	5(4%)	0.9%(5.2% - 6.5%)	0.952
Catheter related infection	3(2.3%)	4(3.2%)	1%(4.4% - 6.5%)	0.957

Group $_T$: Patients with tight glycemic control (80-110 mg.dl^{-1}).
Group $_I$: Patients with intermediate glycemic control (110-140 mg.dl^{-1}).
Data is in number (percent).

Table 8. ICU stay(days) and 28 day mortality in patients with severe traumatic brain injury in the two studied groups .

	Group $_T$ (n=129)	Group $_I$ (n=124)	Difference (95%CI)	P value
ICU stay(days)	12.1±2.3	9.4±3.1*	2.68(1.9-3.3)	<0.0001
Hospital 28 day mortality				
ICU mortality	41(31.7%)	33(26.6%)	7.5%(4.3% - 19%)	0.444
Ward mortality	3(2.3%)	2(1.6%)	0.7%(4% - 6.1%)	0.964

Group $_T$: Patients with tight glycemic control (80-110 mg.dl^{-1}).
Group $_I$: Patients with intermediate glycemic control (110-140 mg.dl^{-1}).
ICU stay(days) is in mean ± SD, 28 day mortality is in number(percent),P significant ≤ 0.05, *
Significant decrease in comparison with group $_T$.

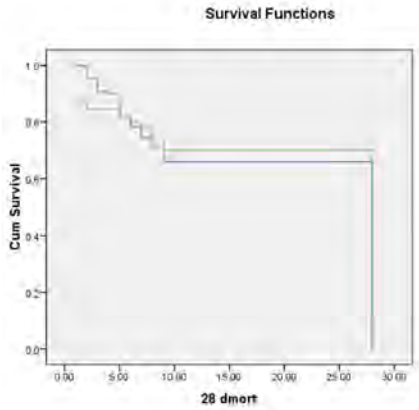

Figure 1. Kaplan Meier survival graph. showed better survival in Group I than Group T.

Disscusion

In the current study , intermediate glycemic control with random blood sugar between 110-140 mg/dl was shown to be safer than tight glycemic control with lower range between (80-110 mg/dl) in patients with severe traumatic brain injury as the former was associated with significant decrease in incidence of hypoglycemic episodes, days of ventilation and period of stay in ICU. In spite of that their was no significant difference regarding infection and the need for vital organ support between both groups. Patients with intermediate glycemic control had significant improvement in Glasgow outcome score with lower 28 day mortality that denoted more favorable neurologic outcome.

Ten years ago, introducing tight glycemic control was like a bomb going off in critical care medicine. Mortality and morbidity were markedly reduced in a group of critically ill whose blood glucose was held between 80-110 mg/dl, compared with conventional treatment group(1). Intensive insulin therapy seemed to be the magic key to a favorable outcome in severely ill neurologic patients. Other studies concerning blood glucose control appeared fast worldwide and it almost felt like a crime not to target tight glycemic control in critically ill patients[11].

The two landmark studies in this field were done by Van den Berghe et al.[1,2]. The first study included 1548 surgical critically ill

patients. They demonstrated that a protocol of intensive insulin therapy, targeting blood glucose levels between 80-110 mg/dl, resulted in decreased intensive care unit (ICU) mortality from 8.0 to 4.6% in these patients compared with a protocol targeting blood glucose levels between 120-180 mg/dl. The second study included 2748 surgical and medical patients, they used the same insulin protocols as in the first study. They reported a lower mortality also with intensive insulin therapy. These findings stands in contrast to our results that showed higher 28 day mortality in the tight glycemic group than the intermediate glycemic group. However unfortunately in spite of that both studies included great number of patients, none of these studies has specifically addressed patients with TBI. Even neurologic groups were not classified neither by GCS nor by injury severity.

In concordance with our study Meier et al.,[12] reported higher mortality in the intensive glycemic control group in a retrospective study that compared the incidence of hypoglycemia and other adverse consequences when intensive glycemic control (goal 70-110 mg/dl) was used versus intermediate glycemic control (goal 90-140 mg/dl). Our study also showed worsened neurologic outcome in tight glycemic control compared with intermediate glycemic control. This finding comes in agreement with the study of Schlenk et al., (13) that treated blood glucose levels above 140mg/dl with intensive insulin therapy. They reported that a major concern with intensive insulin therapy for neurocritically ill patients is the danger of brain tissue hypoglycemia.

Later on Odd et al., [14] differentiated between tight, intermediate and high blood glucose ranges in critically ill neurological patients. They found that tight blood glucose control did not seem to be part of the treatment for critically ill neurologic patients as low cerebral extracellular glucose and brain energy crisis were significantly increased in the tight range and led to unfavorable outcomes in critically ill neurologic patients. These findings are not new as Vespa et al.,[7] found that in traumatic brain injury, intensive insulin

therapy was associated with increased incidence of microdialysis markers of cellular distress that led to poor outcome.

Both studies of Billota et al., and Raimundo et al., [15,16] differed with our study as they postulated that there were no difference in neurological outcome among patients with TBI treated with intensive insulin therapy compared with conventional glucose mangment. However we do believe that both studies had some limitations. The first study included 78 patients and only 69% of patients were randomized to intensive insulin therapy achieved target glucose level. The small number of patients and the inability to achieve target glucose level may have reduced the power of the study to detect significant difference. Also they studied only one type of TBI (SAH) so their results can not be generalized to all types of TBI. Similarly the second study included 48 patient that was a small number also to show a significant difference and they failed to target glycemic control as they assumed.

Tight glycemic control in our study did not show benefits over intermediate control regarding incidence of infection, need for vital organ support although the latter decreased ICU stay and days off ventilation significantly in comparison with tight glycemic control. In a recent study done by Abdulaziz et al.,[17] that included 188 patients(108 undergone tight glycemic control and 80 undergone conventional insulin therapy) they found that there were no significant difference neither in incidence of infection , renal replacement therapy, ICU stay nor days of ventilation between both groups .More over Meier et al.,(12) reported a trend towards increased rate of bacteremia and urinary tract infections in intensive glycemic group.

Our study showed significant increase in hypoglycemia in 45% in tight glycemic group compared with 15.3% in intermediate glycemic group. Many studies agreed with us as regards this finding but with different percentages as it was for example 18.5% vs.1.3% in the study of Abdulaziz et al (17), 18.7% vs. 4.3% in the study of

Donald et al., (18). We believe that these studies had lower percentages than we did due to the different definition of hypoglycemia. Since they defined hypoglycemia <40 mg/dl. in their studies while we defined it<60mg/dl in ours.

As mentioned above we do believe that because of the risks associated with both hyperglycemia and hypoglycemia regarding patients with TBI and their deleterious effects on neurological outcome, glycemic control as early as possible has its positive impact on improvement of neurologic outcome. But to what extent? From our study it is clear that tight glycemic control is not as safe as more intermediate ranges for glycemic control.

Our study had a number of limitations. First, we did not study specific type of head trauma. Second, we did not study the effect of glycemic control on cerebral microdialysis markers that had important relation with neurological outcome but we had not the facilities to did that. Third, the follow up of patients to evaluate neurological outcome was done for short period as we recorded Glasgow outcome score at 28 days. Our evaluation for these patients did not extend beyond this period that if we assume that we followed up those patients for longer period, this have made this evaluation more valuable but that was due to some difficulty to communicate with those patients and their families for longer periods.

No one can deny that severe hyperglycemia should be avoided in these patients but there is still debate about what is the optimal blood glucose level target in these patients? and what about the recent recommendations concerning safe and effective blood glucose control ? The saying "just to do intensive insulin therapy" and "every thing will go right" has not become strong any more for critically ill neurologic patients. So we recommend further more researches with more different ranges of glycemic control for these critically ill neurologic patients out of tight glycemic control to find out the "optimal" range of glycemic control regarding this group of patients . Also we hope that these studies will be carried on specific types of

head trauma and also on moderate and mild traumatic brain injury as we focused in our study on patients with severe traumatic group with GCS≤8. We do believe that further researches should study the effect of glycemic control on cerebral microdialysis markers as this will make the results of these researches more valuable and accurate.

Finally we concluded that, the tight glycemic control with random blood sugar ranging between 80-110 mg/dl did not offer any advantage over intermediate glycemic control with random blood sugar ranging between110-140 mg/dl in patients with severe traumatic brain injury. More over, tight glycemic control recorded higher mortality with higher incidence of hypoglycemia. We found that intermediate glycemic control as we assumed appeared to be safer avoiding hazards of hypoglycemia with better neurological outcome.

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RELIABILITY OF GLYCEMIC CONTROL IN HEAD TRAUMA PATIENTS: A CORRELATION OF TWO GLYCEMIC RANGES WITH THE OUTCOME

Mona Gad M. El-Ebiedy Msc, Amgad A. Zaghlol MD, Amr M. Yasseen MD, Mahmoud M. Othman MD and Mohamed A. A. Sultan MD

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ENDOSCOPE-ASSISTED MICROSURGERY OF CEREBELLOPONTINE ANGLE LESIONS PRELIMINARY EXPERIENCE

Mostafa M. Nabeeh MD, Nabil M. Ali MD, Abdelwahab M. Ibrahim MD, Ashraf A. Ezz Eldin MD and Mohamed A. Kassem MD

Department of neurosurgery. Faculty of Medicine, Mansoura University., Egypt.

Abstract

Introduction: *Due to limitations to view angles by the operating microscope Endoscope Used as instruments to add Stronger magnification, better visualization, additional illumination during microsurgical procedures. Surgery of CPA encountered hidden angles and difficult pathologies.*

Material and methods: *A 0° or 30° rigid neuroendoscope was introduced into the CPA during the intracranial procedure of 11 cases; Trigeminal nerve compression 2 cases, Epidermoid tumor 5 cases, Vestibular schwanoma 2 trigeminal schwanoma 1 case, Craniopharyngioma 1 case early in surgery and after completion of microsurgical work. Results, observations, difficulties and complication noticed.*

Results: *6 out of 9 tumoral patients achieved total removal while near total in another 2 patients and subtotal only in craniopharyngioma patient. In two cases of trigeminal neuralgia endoscope gives better evaluation of conflict than microscope and detect incomplete decompression in one case.*

Conclusion: *Using neuroendoscopy during microsurgical procedures for CPA lesions is safe procedure adding better visualization of lesion anatomical relations, excellent to look at corners of CPA specially anterior to brain stem, internal auditory meatus and Mickle's cave. Not adding much morbidity and reasonable operative time.*

Keywords: *Endoscopy; epidermoid cyst; cerebellopontine angle.*

Introduction

Most current clinical applications of neuroendoscopy concern the ventricular system and transsphenoidal route. Its use in the basal cisterns either alone or as assistance for microsurgery is still a matter of controversy. Endoscope Used as instruments to add stronger magnification, better visualization and additional illumination during microsurgical procedures.[11]

The limitations to view angles by the operating microscope contrasts with the ability for broad panoramic surveys and different angles of view when endoscope is used. The endoscope is particularly well suited to application at the cerebellopontine angle (CPA), where neurovascular structures are often obscured by the protrusion of the petrous portion of the temporal bone, and some lesions like vestibular schwannomas (VSs) that include portions within the internal auditory canal (IAC), are not always completely exposed microscopically.[13]

Endoscope provide the advantages of Reduceing the approach-related trauma, Reduces the necessity for intraoperative retraction of nervous or vascular intracranial structures and Provide access to hidden angles of operative field.[8]

Hidden structures in CPA includes the internal auditory meatus, the ventral surface of the brain stem, the ventral aspect of root entry zones of cranial nerves, anterior contents of the foramen magnum and lesions extended anterior to Meckle's cave. Some lesions like epidermoids grow along the subarachnoid spaces around delicate neurovascular structures and often extend into the middle cranial fossa.[10]

Difficulties in using endoscopes during microneurosurgery includes; bimanual surgical procedures cannot be achieved when holding a scope with one hand, No information immediately around the endoscope is available, Clouding of vision by blood, Insufficient development of endoscopic equipment.[9,14]

Material and Methods

A 0° or 30° rigid neuroendo-

scope was introduced into the CPA during the intracranial procedure of 11 cases; Trigeminal nerve compression in 2 cases, Epidermoid tumor in 5 cases, Vestibular schwanoma in 2cases, trigeminal schwanoma in 1 case and Craniopharyngioma in 1 case.

Under microscopic control, an endoscope was inserted parallel to the posterior surface of the petrous bone The CPA was explored by using neuroendoscop. Neurovascular anatomy, as well as the relationship between tumour and surrounding structures, was evaluated first. The offending compressing vessel was identified in the cases of trigeminal neuralgia by 0 then 30-degree endoscope. This is followed by ordinary microsurgical procedure using microscope for dissection and excision of tumor allowing working bimanually. At the end of the operation, the IAC or ventral surface of brainstem and the other subarachnoid cisterns of CPA were inspected again using the 0° and 30° rigid neuroendoscope. If residual tumour was found, neuroendoscopic assisted tumour dissection was performed using angled endoscope for resection of tumor parts at hidden angles to microscope.

Results

Clinical manifestation :

We managed 11 cases of CPA lesions 8 females and 3 males The patients' average age was 39.2 years (range, 3-65 yr). The right side was affected in 4 patients, and the left side was affected in 7 patients. The onset of symptoms occurred 11.4 years (range, 2-28 yr) before the initial operative procedure. Initial complaints included TN in 2 patients (18.2%) and HFS in 1 patient (9.1%), headache in 9 patients (81.2%), deterioration of vision and hormonal disturbance in only one craniopharyngioma case. By examination: cranial nerve deficite identified in 10 patients neurological deficite in 7 cases includes deafness, facial palsy and hemiparesis.

Neuroradiological evaluation :

Preoperative neuroradiological studies included computed tomographic (CT), T1- and T2-weighted magnetic resonance imaging (MRI) scanning in all patients, the tu-

mors largest diameter averaged 4.1 cm ranged from 3-6cm. In 2 cases of vestibular schwanomas there is widening of internal auditory meatus on CT bone window with significant intra meatal tumor appeared in post contrast CT and MRI. T1-weighted MRI showed epidermoids to be hypointense, and on T2-weighted MRI, they appeared hyperintense in relation to brain tissue and slightly hyperintense to isointense with regard to cerebrospinal fluid (Figure 2). Normal imaging in trigeminal neuralgia, in case of craniopharyngioma lesion had solid suprasellar part with large cystic component extended posterior to fill the left CPA and exerting significant brain stem compression calcification was evident in both CT and MRI.

Surgery :

We managed 5 epidermoid 1 craniopharyngioma 2 neurovascular compressions 2 vestibular schwanoma one non vestibular schwanoma. All patients underwent tumor removal via the retrosigmoid approach. Microsurgical rules applied like ordinary procedures operative time averaged 212 minutes ranged from 180 to 250 minutes, endoscopic time averaged 29.5 minutes ranged (10 - 45m), time of microsurgery averaged 110.45m and ranged (45-170m).

Total removal was accomplished in 6 patients (66.6%) near total in 2 (22.2) subtotal only in craniopharyngioma patient.

In 2 cases of trigeminal neuralgia Intraoperative observations regarding the locations of nerve-vessel conflicts were examined by endoscope using 0 then 30 degree lenses which not observed clearly by operating microscope. In both cases the conflict involved the nerve root entry zone anterior in location in one the pole of loop was medially embedded in the nerve and the other there were complex vascular relationship of multiple vessles around nerve and the offending vessel was medial and raped the nerve anteriorly. After that microscope used for dissection and introduction of Teflon barrier, followed by endoscpic exploration revealed inapproprioate position in both due to incomplete dissection of the nerve

which completed and corrected under endoscopic vision.

In the 5 epidermoid cysts the lesion is quite small in two leaving space in CPA allowing initial introduction of endoscope for assessment of relation of posterior fossa content to tumor specially anteriory located lead to expectation of posterior inferior cerebellar artery PICA loop in one case and relationship of cranial nerves to capsule in both patient. In the other 3 patients adhesions and size of lesions omit this initial step. After completion of removal using bimanual manipulation under microscope introduction of angled endoscopic lens 30, 70 degree revealed residual in all patients anterior to brain stem in 4 and in Micckle's cave in 1 patients. 4 residuals removed under endoscopic guidance. In one patient small part couldn't be reached by available instruments.

Neither exploration nor excision of the residual part needed retraction to cerebellum, brain stem or cranial nerves (Figure 1).

In two cases of vestibular schwanomas intial exploration by endoscope gives better orientation of cranial nerves especially 10th nerve which found stretched over tumor anteriorly in both cases. Endoscopy allowed improved identification of tumor and adjacent neurovascular relationships in all cases without the need for significant retraction of the cerebellum or brain stem. after complete excision endoscope angled lens revealed meatal residual in both cases which removed under endoscopic vision.

In the case of craniopharyngima and after excision of the CPA part the extension to middle fossa was not feasible by microscope so the dissection done under endoscopic guidance, at bed of the field dissection ended by lashes of viens which is stretched, due to large lesion and disturbance of anatomy we thought it stretched over cystic part of lesion and after introduction of endscope which cleared it as the 3rd ventricle so we corrected our orientation and avoid unnecessary dissection and liability of injury of basal veins and vital structure in floor of 3rd ventricle.

No endoscope related complicatin encountered except in only one case in which introduction of endoscope lead to injury of bridging vein and subsequent bleeding which controled easily.

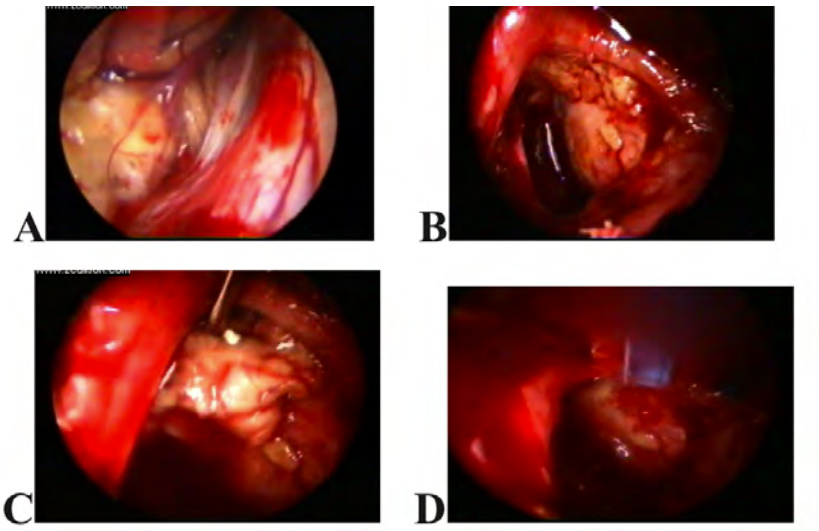

Fig. (1) : Pictures taken through 30^{O}endoscope A shows the exploration of tumor to surrounding vessles and cranial nerves B residual appears under 5^{th} nerve and not apperared by microscopic view C during removal of residual under endoscopic view D assure complete excision.

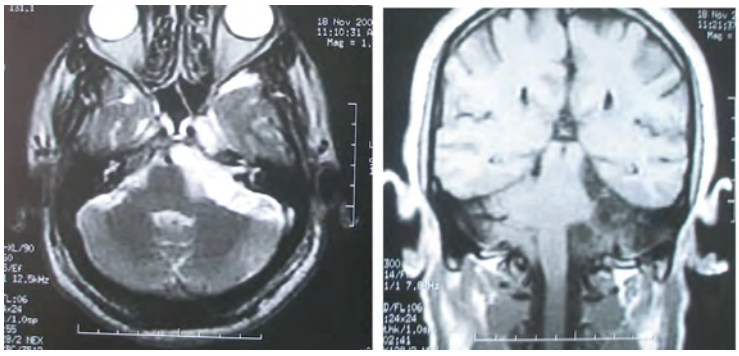

Fig. (2) : MRI of the same patient in Figure 1 A T2 axial view show extension of epidermoid anterior to brain stem. B T1 coronal cut show extension up to supratentorial compartment and down to foramen magnum.

Discussion

Our 5 epidermoid cases presented by headache mainly associated with atypical facial pain , hemiparesis, hydrocphalus, tinnitus and vertigo, hemifacial spasm each in one case. Although small and none randomized sample clinical presentation showed headache as main symptomatology. Meta-analysis of 263 cases disclosed that hearing difficulty was the most common symptom, accounting for 37.6%, followed by TN, 29.7%; dizziness or vertigo, 19.4%; facial palsy, 19.4%; headache, 17.9%; and diplopia, 16.7%. Hemi facial spasm was found in only 4.9% of patients in these reports.(6)

Our result support the same results of De Divitiis etal that epidermoid tumors located in the cerebellopontine angle (CPA) are challenging lesions because they grow along the subarachnoid spaces around delicate neurovascular structures and often extend into the middle cranial fossa.(1)

As all 5 cases has extensions out the microscopic view of CPA extending to anterior to brain stem, middle fossa or supratentorial. Same conclusion reported by Ebner etal who managed 25 consecutively patients with an epidermoid cyst in the posterior fossa. Surgeries were performed both with an operating microscope and endoscopic equipment including 7 (28%) recuurent patients. In 5 cases the endoscope was used as an adjunctive tool for inspection/ endoscope-assisted removal of remnants. The effective time of use of the endoscope was limited to the end stage of the procedure, but was very effective. And stated that the combined use of microscope and endoscope offers relevant advantages in demanding anatomic situations.(2)

We evaluated the fesability and outcome of using endoscope during surgery of CPA lesions we achieved complete resection in 6 out of 9 and near total in 2 with a total percentage of 88.8% overall the other craniopharyngioma case the subtotal resection was related to the specific pathology of the lesion and its anatomical relation as it is extended from suprasellar region posterioly the effectiveness of endoscopic assisted microscopic

surgery in improving outcome support many reports which concluded that application of endoscope assisting in retrosigmoid approach can be helpful for totally resection of vestibular schwannoma in inner acoustic meatus. Application of the endoscope assisting in retrosigmoid approach is helpful to provide the precise information of CPA anatomic structure and decrease the incidence rate of complication.[7,15]

They concluded that the application of endoscope during microsurgical removal of Vestibular schwanoma is a safe procedure that does not lead to heat-related or mechanical neural or vascular injuries.[4]

And concluded that the endoscope-assisted microsurgical technique enables safe removal even when tumor parts are not visible in a straight line. Tumor extensions into adjacent cranial compartments can be removed with the same approach without retracting neurovascular structures or enlarging the craniotomy.[12]

Jarrahy observations on the relevance of endoscopy in defining trigeminal nerve conflicts have shown that of the fifty-one total nerve-vessel conflicts identified in their series, fourteen (27%) could only be detected endoscopically. These were in areas that were inaccessible to adequate microscopic visualization. Furthermore, they have specifically analyzed the impact of the endoscopic survey upon the actual decompression performed. In 24% of cases, an endoscopic survey of the decompression revealed areas where the nerve-vessel conflicts were not completely resolved.[5] in our 2 cases of trigeminal neuralgia we found same results as endoscope was superior to microscop in identification of conflicts and in assurance of complete decompression. Same results was supported by a study comprises 42 cases of trigeminal neuralgia that underwent operation with endoscopic-assisted microvascular decompression. El-Garem etal performed decompression by means of a minimally invasive retrosigmoid approach without a cerebellar retractor.[3]

Morita et al. concluded that the

endoscope can be applied safely in routine microsurgery with specific equipment and has proven useful in 1 of 10 patients. To perform more effective procedures using endoscopes, we need to develop specially designed instruments usable through a narrow corridor and in an angled field. And we faced the same problem of angled instruments and found it is very difficult to use straight instrument while viewing through angled lens. So special different angled instrument have to be designed for such procedure[9].

We can conclude the usefulness of endoscope in small tumours of the CPA in which the diagnosis is unclear, Intraoperative inspection to determine the cause of trigeminal neuralgia or other neurovascular compression, Resection of small acoustic neuromas within the IAC or into the pontine cistern, Removal of residual acoustic neuroma within IAC, or following hidden residual of epidermoid, selective cranial nerve dissections, neurovascular decompression of any of the cranial nerves

Conclusion

Using neuroendoscopy during microsurgical procedures for CPA lesions is safe procedure adding better visualization of lesion anatomical relations, excellent to look at corners of CPA specially anterior to brain stem, internal auditory meatus and Mickle's cave. Not adding much morbidity and reasonable operative time.

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VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION AND MICROVESSEL DENSITY IN LIVER BIOPSIES FROM CHRONIC HEPATITIS C PATIENTS WITH DIFFERENT STAGES OF HEPATIC FIBROSIS

Wagdi F. Elkashef M.Sc, Khaled R. Zalata MD, Mohamed F. Mohamed MD, Nagwa M. Helal MD and Nadia A. Nada MD

Departments of

Mansoura Faculty of Medicine, Mansoura University, Egypt

Abstract

Chronic HCV infection is the leading cause of liver disease in Egypt. HCV infection is associated with progressive fibrosis and cirrhosis. Angiogenesis was found to be associated with cirrhosis and development of HCV. ***Aims:*** *To evaluate the VEGF expression and MVD as markers of angiogenesis in different stages of hepatic fibrosis.* ***Methods:*** *Liver biopsy from 100 patients with chronic HCV was enrolled in this research (25 for each stage of hepatic fibrosis). Pathological assessment was done using METAVIR scoring system. Immunohistochemical staining for CD34 and VEGF was performed.* ***Results:*** *(VEGF) expression was detected in (100%) of the cases. It shows highly significant correlation with the degree of hepatic fibrosis and the degree of necro-inflammatory injury. (MVD) showed highly significant correlation with stage of fibrosis and the degree of necro-inflammatory injury. Also micro-vessel density (MVD) shows significant correlation with VEGF expression.* ***Conclusions:*** *Progression of fibrosis is associated with increased angiogenic activity in the liver. This activity involves increased expression of VEGF by the hepatocytes and increased micro-vessel formation in the expanded portal areas and fibrous bands.*

Introduction

Hepatitis C infection is considered the leading cause of chronic liver disease in Egypt[1]. Chronic hepatitis C virus (HCV) infection typically is characterized by slowly progressive hepatic fibrosis and eventually development of cirrhosis [2].

Chronic activation of the wound healing response is the major driving force for progressive accumulation of extracellular matrix components, finally leading to liver cirrhosis and hepatic failure [3]. The proposal that angiogenesis may significantly contribute to fibrogenesis and disease progression relies first on the fact that vascular remodeling, irrespective of etiology, is a common finding in human cirrhotic livers [4]. Several mediators of the inflammatory response may stimulate other cells (for example hepatocytes) in the surrounding microenvironment to express vascular endothelial growth factor (VEGF) and other pro-angiogenic factors as well as to sustain angiogenesis [5].

VEGF is known to play an important role not only in the angiogenesis and prognosis of different human tumors, but also in physiological and nonmalignant pathological conditions including liver cirrhosis [6] and the expression of VEGF is reported in the hepatocellular carcinoma (HCC) and surrounding liver tissue by several authors [6-7].

Micro-vessel density (MVD) is the measure of the number of vessels per high-power (microscope) field and, as such, reflects the number of capillaries and intercapillary distance [8]. Quantification of MVD is thought to constitute a surrogate marker for the efficacy of antiangiogenic agents as well as a means by which to assess which patients are good candidates for antiangiogenic therapy prior to treatment [9]. Micro-vessel density was reported to be increased in HCC and surrounding cirrhotic liver tissue [6].

Angiogenesis was well investigated in relation to the cirrhosis, its role in development of HCC and the progression of HCC, however, limited investigation about the role of angiogenesis in the pro-

gression of HCV associated hepatic fibrosis and its status in different stages of hepatic fibrosis. In the current study, we evaluated the relationship between (MVD and VEGF) as markers of fibrosis and the progression of hepatic fibrosis in cases of chronic HCV infection.

Patients and Methods

In this retrospective study the data of patients with chronic HCV in The Association of Hepatic Patients Care (AHPC), in Mansoura city were reviewed for the personal and clinical data. Patients proved to be PCR positive were included in this study.

One hundred liver biopsies were retrieved (25 for each stage of hepatic fibrosis) as paraffin blocks and cut at 3-5 um. Slides stained with Hematoxylin and Eosin stain were prepared. Immunohistochemical reaction was performed using anti human CD34 (Clone QBEnd/10, Thermo Fisher Scientific, Fremont, CA, USA) and VEGF (Clone SP28, Thermo Fisher Scientific, Fremont, CA, USA) antibodies on paraffin sections with enzyme-conjugated polymer technique (EnVision + System, Dako, Glostrup, Denmark).

Both necro-inflammatory activity and fibrosis stage assessment was done using METAVIR scoring system according to Bedossa and Poynard [10].

Immunohistochemical scoring of VEGF:

Vascular endothelial growth factor was expressed as cytoplasmic brown color of hepatocytes and endothelial cells lining blood vessels and counting the sum of intensity (scored as 0: None; 1: mild; 2: moderate; and 3: strong) and extent (scored as 0: <5%; 1: 5-33%, 2: 33-66% and 3: 66-100%) with power of magnification (x 400) according to Takahashi et al [11].

Determination of microvascular density (MVD):

MVD of was evaluated according to the method described by Weinder et al[8] Brown-stained endothelial cell or endothelial cell cluster, which was clearly separate from adjacent microvessels and other connective tissue elements, was considered a single,

countable blood vessel. Screening of the cores was first performed at a low power (40 X) to identify areas of the highest MVD. Counting was performed in the three highest MVD areas at high power (400 X). The mean value of the counted three fields was considered as the MVD of an individual case.

Statistical analysis:

The statistical analysis of data done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 16.

Results

Most of the patients were males 73 (73%) while the females were 27 (27%). There is significant difference in distribution with male predominance. The age of the patients ranges from 20 years to 57years with a mean age (40.29).

(VEGF) expression was detected in all cases (Figures: 1-2). It shows highly significant correlation with the degree of necro-inflammatory activity determined by METAVIR score (P value: 0.002) (Graph 1). VEGF expression shows highly significant correlation with the degree of hepatic fibrosis measured by METAVIR score (P value : 0.000) (Graph 2).

(MVD) (Figures: 3-4) was 9.67±2.54/0.16mm2 for the F1 stage of fibrosis, 18.17±5.99/ 016mm2 for F2 stage of fibrosis, 17.68±5.8/0.16mm2 for F3 stage of fibrosis and 34.5±10.15/ 016mm2 for F4 stage of fibrosis. (MVD) shows highly significant correlation with the degree of necro-inflammatory activity measured by METAVIR scores (P value: 0.000) (Graph 3). It also shows highly significant correlation with the stage of hepatic fibrosis measured by METAVIR scores (P value: 0.000) (Graph 4). Also microvessel density (MVD) shows significant correlation with the VEGF expression (P value : 0.005) (Graph 5).

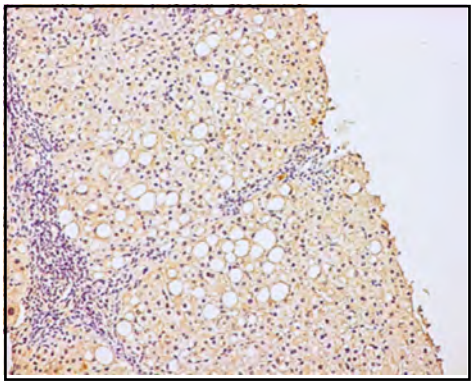


Fig (1): Moderate diffuse cytoplasmic reaction of hepatocytes with marked bridging fibrosis (VEGF immunohistochemical staining x 100).

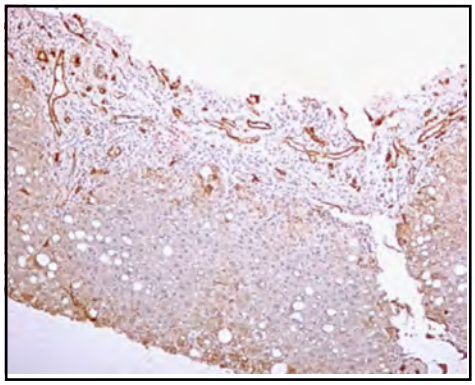


Fig (3) : Micro-vessel density in expanded portal area (CD34 immunohistochemical staining x 100).

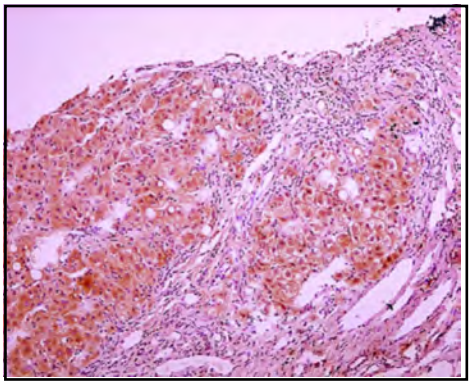


Fig (2): Strong diffuse cytoplasmic reaction of hepatocytes with marked bridging fibrosis (VEGF immunohistochemical staining x 100).

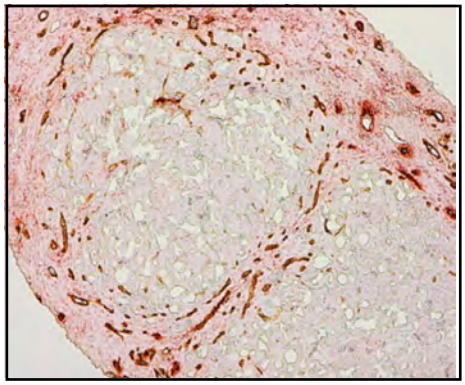
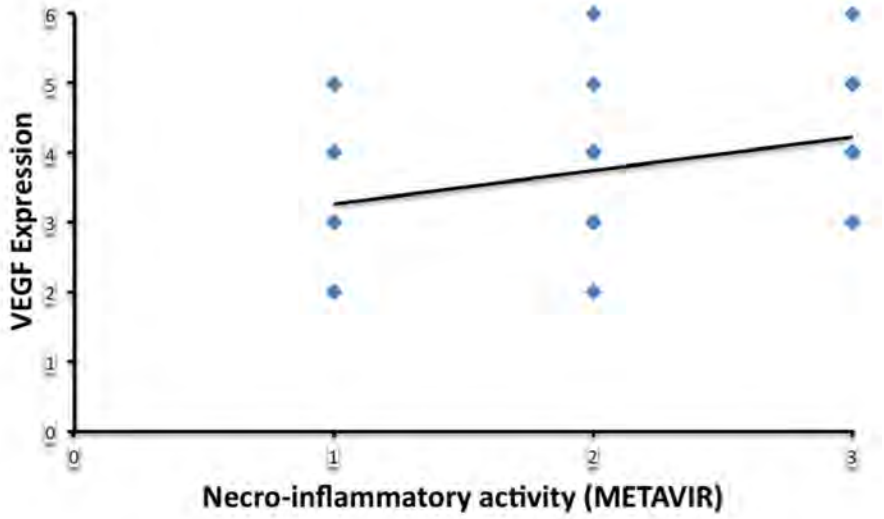
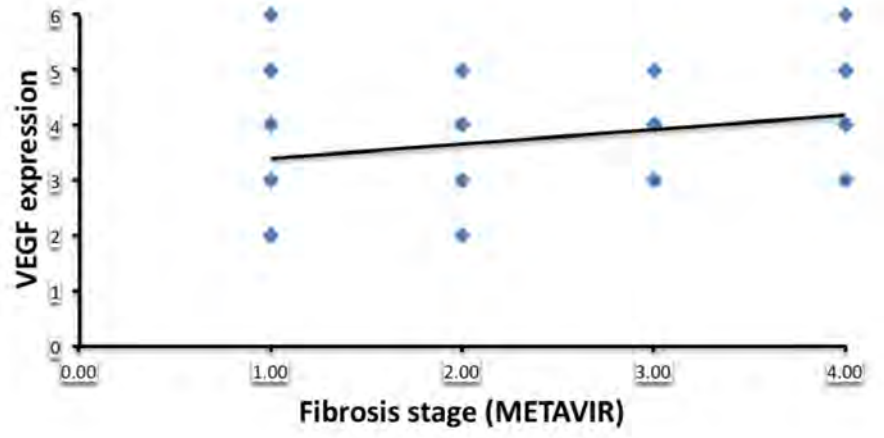


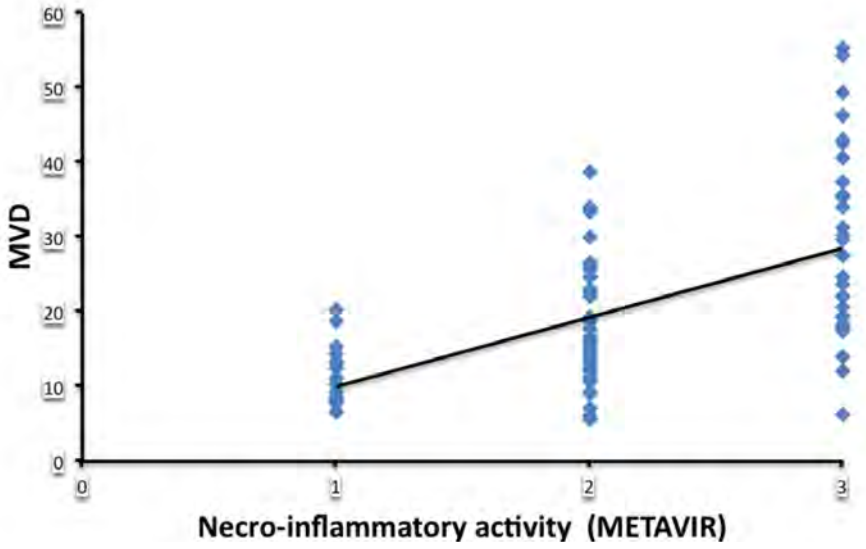
Fig (4) : Micro-vessel density in cirrhotic liver (CD34 immunohistochemical staining x 100).



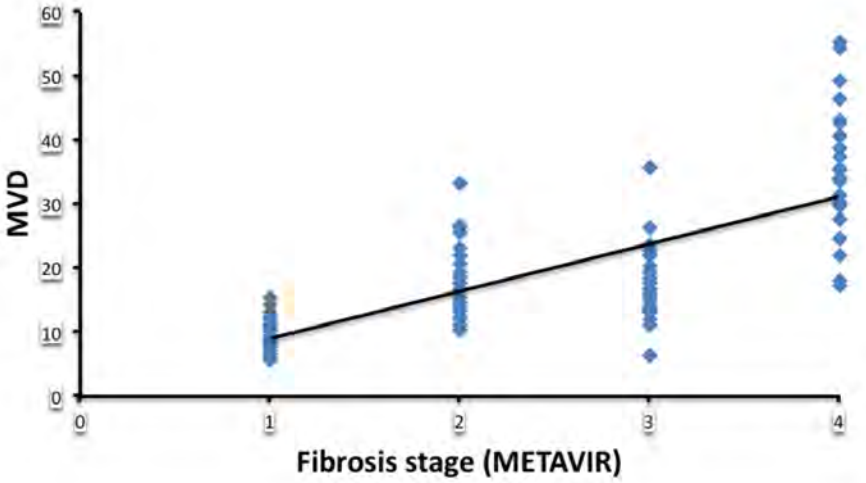
Graph 1: Significant positive correlation between VEGF expression and the necro-inflammatory activity (METAVIR score).



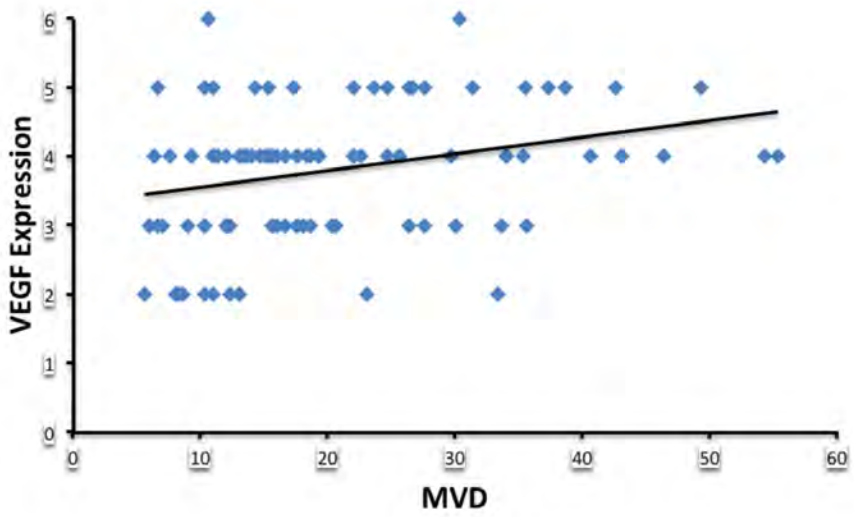
Graph 2: Significant positive correlation between VEGF expression and the stage of fibrosis.



Graph 3: Significant positive correlation between micro-vessel density (MVD) and the necro-inflammatory activity (METAVIR score).



Graph 4: Significant positive correlation between VEGF expression and the stage of fibrosis.



Graph 5: Significant positive correlation between micro-vessel density (MVD) and VEGF expression.

Discussion

The results shows that VEGF expression was detected in all our cases. It shows highly significant correlation with the degree of hepatic fibrosis determined by METAVIR score (P value: 0.002). VEGF expression shows highly significant correlation with the degree of necro-inflammatory injury (P value: 0.000). Recent study reported that HCV infection has the ability to trigger the production of VEGF proteins [12]. Similar results are reported also by [13], they found that VEGF expression is a common response to liver injury and progression to cirrhosis. VEGF expression was reported in different models of experimental hepatic fibrosis and it was more evident at the earliest stages of fibrosis development [14]. Higher VEGF expression in cirrhotic liver tissue than adjacent hepatocellular carcinoma tissue was reported by [7]. Also in a recent study on HCV infected patients, VEGF expression was commonly found in early stages of fibrosis and increased significantly during fibrogenesis and carcinogenesis [15]. No significant correlation was found between VEGF expression

and capillarization of sinusoidal wall endothelial cells.

In present study Micro-vessel density (MVD) shows highly significant correlation with stage of fibrosis (P value: 0.000). It also shows highly significant correlation with the degree of necro-inflammatory activity (P value: 0.000). Increased MVD during chronic hepatitis due to HCV infection was first reported by [16], then [6] reported increased MVD in the cirrhotic livers adjacent to hepatocellular carcinoma compared with non cirrhotic ones. These results were found also by [7]. Increased MVD in cirrhotic patient was found to be associated with the development of hepatocellular carcinoma [17]. Also micro-vessel density (MVD) shows significant correlation with the VEGF expression (P value: 0.005). This result comes in agreement with [13] who reported that the liver density of microvessels was correlated to the abundance of VEGF in fibrotic and cirrhotic livers.

Increased expression of VEGF and increased MVD with the progression of hepatic fibrosis from mild expansion of portal tracts (F1) to established cirrhosis (F4) supports the conception of wound healing theory of hepatic fibrosis and indicate that the process of angiogenesis is go hand in hand with fibrosis progression in chronic HCV infection.

Interesting therapeutic agents are used as an inhibitor of angiogenesis in treatment of hepatic fibrosis and cirrhosis. These drug was proved to have a potent anti-tumor and antiangiogenic agent in clinical trials for treatment of cancer. Its antiangiogenic efficacy is attributable to inhibition of vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR), both of which are essential for angiogenesis development [18].

These therapeutic trials are based on the evidence that the development of fibrosis and cirrhosis especially in cases of HCV infection is associated with significant angiogenesis evidenced by increase microvessel density and its correlation with the stage of hepatic fibrosis. This increase in

MVD is may be regulated by increased VEGF expression during the development of hepatic fibrosis which is correlated with increased MVD.

Conclusions

Progression of fibrosis is associated with increased angiogenic activity in the liver. This activity involves increased expression of VEGF by the hepatocytes and increased micro-vessel formation in the expanded portal areas and fibrous bands. No significant difference was observed between stages F2 and F3 regarding the MVD and this may indicat significant difference between early and advanced fibrosis. More research efforts are needed to explore the molecular aspects of angiogenesis in relation to hepatic fibrosis and to study its relation to the progression of fibrosis. Regarding the fast progression of anti-angiogenic drugs, investigation of the role of anti-angiogenic therapy in treatment of hepatic fibrosis is indicated.

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DETATCHED PODOCYTES IN THE BOWMAN'S SPACE AS A PARAMETER OF ACTIVITY INDEX IN LUPUS NEPHRITIS

Nahed A. Soliman M.Sc, Ashraf M. Abd-Elbast MD, Fatma E. Moustafa Ph.D, Asmaa M. Gadoo Ph.D, and Ebrahim M. El-Shwaf Ph.D

Departments of Pathology, Mansoura Faculty of Medicine, Mansoura University, Egypt

Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a generalized dysregulation and hyperactivity of immune system with production of autoantibodies to a variety of nuclear and non-nuclear antigens. Nephritis is a frequent and potentially serious component of systemic lupus erythematosus [2,3].

The glomerular morphological patterns of immune complex-mediated injury in lupus nephritis are related to the site of accumulation of immunoglobulins, their antigen specificity, the capacity to bind and activate complement and other serine proteases, and their ability to evoke a cellular inflammatory response [4,13].

Podocyte loss seems to be an important component of disease progression in glomerular diseases [6].

Close correlation between the degree of glomerular damage and the reduction of podocyte number per glomerulus was observed by Kim et al. [6]. More severe form of podocyte injury may occur, leading to podocyte detachment and death. This event initiates an injury cascade that results in the segmental scar characteristic of FSGS [1,9].

Skoberne et al., [11] suggested that, it seems reasonable to look for podocytes in the urine as a possible marker of disease activity or adverse prognosis.

However, no attempts to count the number of detatched podocytes in Bowman's space and score it as an activity parameter in different glomerular diseases.

This study was preformed on 37 female patients with the clinical diagnosis of lupus nephritis that were recruited consecutively from Mansoura University children Hospital and indicated for biopsy to determine the activity index and chronicity index of lupus nephritis. The main age at presentation was (14 ± 2.4). Renal biopsies were received in 10% neutral buffered formalin and processed for paraffin embedding and subsequent histopathological examination under the light microscope. Paraffin blocks are cut at 2-3 µm in thickness and 15 slides were obtained with serial numbers.

In this study, counting of detatched podocyte in Bowman's space of the mostly affected glomerulus and its relation to activity index or chronicity index in lupus nephritis was done.

The nature of detatched podocytes was confirmed by chemical stain colloidal iron stain and immunohistochemistry with monoclonal antipodocalyxin antibodies (Catalog number: AF1556) (R&D) by immunoperoxidase chromagen labeled using tissue staining kit (R&D system catalog CTS008) (enzymatic protocol 1(R&D system) to ensure the detatched cells were podocytes not monocytes or other cells. Counting and scoring of detatched podocytes in the mostly affected glomerulus according to the number were performed.

The statistical analysis methods that were used are descriptive statistics median range, compare means test (One Way Anova test), cross tabs (chi square test), bilinear correlation test, linear regression (multiple stepwise test).

This study reveals significant association and correlation of detatched podocyte score with activity index in lupus nephritis.

So, we recommended that, detatched podocytes in the Bowman's space should be considered as a parameter of activity added to those already known parameters of activity.

Introduction

The main podocyte surface antigen podocalyxin, a highly electronegative sialoglycoprotein, prevents the podocyte foot processes from collapsing due to its high negative charge. Podocyte damage in glomerular disease is supposed

to be accompanied by podocyte detachment [5].

Pavenstadt et al.,[9]; Petermann and Floege [10] said that, the loss of nephron most likely starts with the injury of podocytes. Three different patterns of changes initiated by podocyte injury may be distinguished including degenerative change ending in sclerosis, inflammatory change leading to crescent formation, and dedifferentiation from nonproliferative form to proliferative form as in collapsing nephropathy.

The degenerative Changes and the development of "Classic" FSGS comprise foot process effacement and apically microvillus transformation, cell hypertrophy, cell body attenuation, pseudocyst formation, and detachment from the GBM [7, 12, 10].

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a generalized dysregulation and hyperactivity of immune system with production of autoantibodies to a variety of nuclear and non-nuclear antigens. Nephritis is a frequent and potentially serious component of systemic lupus erythematosus [2;3].

The glomerular patterns of immune complex-mediated injury in lupus nephritis are related to the site of accumulation of immunoglobulins, their antigen specificity, and the capacity to bind and activate complement, and their ability to evoke a cellular inflammatory response [4;13]. These patterns of injury can be divided into three groups:

In the mesangial pattern, mesangial hypercellularity and matrix accumulation result from mesangial immune complex accumulation [13].

The endothelial pattern, (an exudative component) characterized by leukocyte accumulation, endothelial cell injury, and endocapillary proliferation. This pattern is often associated with capillary wall destruction, mild to marked immune complex deposition, and varying degrees of mesangial proliferation and crescent formation [19].

In the epithelial pattern, antibodies and complement inflict cytotoxic injury on the podocyte resulting in a nonexudative, nonproliferative capillary wall lesion [13].

D'Agati, [3] described activity and chronicity indices as a histologic grading system of lupus nephritis. These provide useful information about response to therapy and long-term renal survival. Each of the histologic features (proliferation, neutrophilic infiltration, cellular crescent, necrosis, hyaline lesion, interstitial inflammation) is graded on a scale of 0, 1, 2, or 3 (absent, mild <25%, moderate 25-50% and severe >50% respectively). Both fibrinoid necrosis and cellular crescents are multiplied by a factor of 2 because of their prognostic importance, so the maximum activity index is 24 and the maximum chronicity index is [10].

Patient and Methods

This study was preformed on 37 female patients with the clinical diagnosis of lupus nephritis that were recruited consecutively from Mansoura University children Hospital and indicated for biopsy to determine the activity index and chronicity index of lupus nephritis. The main age at presentation was (14 ± 2.4). Renal biopsies were received in 10% neutral buffered formalin and processed for paraffin embedding and subsequent histopathological examination under the light microscope. Paraffin blocks are cut at 2-3 µm in thickness and 15 slides were obtained with serial numbers. Sections are stained by the following stains, Hematoxylin and eosin (slides1, 5, and 10), Periodic Acid-Schiff (slides 2,6, and 11), Masson Trichrome (slides 3,7, and 12), Periodic Acid Silver Methanamin (slides 4, 8, and 13). The minimum number of glomeruli to consider the biopsy as adequate was 5-10 glomeruli per section [8].

The nature of detatched podocytes was confirmed by chemical stain colloidal iron stain and immunohistochemistry with monoclonal antipodocalyxin antibodies (Catalog number: AF1556) by immunoperoxidase chromagen labeled using tissue staining kit (R&D system catalog CTS008)

(enzymatic protocol 1(R&D system) to ensure the detatched cells were podocytes not monocytes or other cells. Counting and scoring of detatched podocytes in the mostly affected glomerulus according to the number were performed.

The activity indix was detected in each case. Counting and scoring of detatched podocytes in the mostly affected glomerulus according to the number were performed.

In this study, the importance of detatched podocyte as a marker of activity or chronicity was assessed using thorough statistical workup in the following steps:

First counting and scoring detatched podocytes in each class of lupus nephritis using descriptive statistics (median-range).

Second Comparing the means of activity index in different scores of detatched podocytes using compare means tests (One Way Anova test).

Third Correlation between detatched podocytes and either activity or chronicity index using Bivarient correlation test.

Lastly detecting the best predictors of activity index using regression test (linear multiple stepwise method).

Results

The commonest class of lupus nephritis in this study was class 4 (51.4%) 19 cases. Then class 3 (21.6%) 8 cases. The least class was class 2 (2.7%) one case.

Highest grade of detatched podocytes was present in lupus class 4 (median score 2(3-5 detatched podocytes per glomerulus)). No detatched podocytes were present in lupus class 1, 2.

Table (1) and figures (1,2) reveal significant difference in the means of the activity index in cases of lupus nephritis in different pattern of proliferation (p=0.000), the presence or absence of hyaline lesion (p=0.00), the presence or absence of cellular crescent (p=0.01), the presence or absence of necrosis (p=0.00), in different scores of detatched podocytes

(p=0.00) and different grades of neutrophilic infiltration (p=0.00).

From table (2) and figure (3) in this study, there were significant association between scores of detatched podocytes and the pattern of sclerosis (p=0.01), there were non significant association between scores of detatched podocytes and the presence of fibrous crescent (p=0.209).

Table (3) reveals significant high positive linear correlation between detatched podocytes and activity index but not with chronicity index.

Table (4) shows the best predictor(s) of activity index in cases of lupus nephriitis were cellular crescent, proliferation, necrosis, and neutrophilic infiltration but not detatched podocytes..

Table (1) : The activity index and detatched podocytes in lupus nephritis

Activity		N	Activity index Mean±SD	One-Way Anova
Detatched podocytes	N	13	0.84±1.5	0.000
	<3pod/glm	5	5.4±2.5	
	3-5pod/glm	10	7.8±3.1	
	>5pod/glm	9	8.7±5.2	

Significant (P≤ 0.05)

Table (2) : Association () detatched podocytes and fibrous crescent.

		Detatched podocytes					χ^2 test
		Absent	**<3**	**3-5**	**>5**		
		N %	**N %**	**N %**	**N %**	**Total %**	
Fibrous crescent	Absent	28 96.6%	27 90%	14 77.8%	13 92.9%	82 90.1%	0.209
	Present	1 63.4%	3 10%	4 22.2%	1 7.1%	9 9.9%	
	Total	29 100%	30 100%	18 100%	14 100%	91 100%	

Significant ($P \leq 0.05$) using χ^2 test

Table (3) : Correlation () activity, chronicity indices, detatched podocyte.

		Detatched podocytes
Activity index N=37	Pearson Correlation	.712**
	Sig. (2-tailed)	.000
Chronicity index N=37	Pearson Correlation	0.190
	Sig. (2-tailed)	0.26

Table (4) : The best predictors 0f activity index.

Predictor variable	**B**	**P**	**OR (C.I.)**
Cellular crescent	0.602	0.000	0.378(0.312-0.893)
Proliferation	0.431	0.000	0.404(0.255-0.286)
Necrosis	0.369	0.02	0.229(0.063-0.675)
Neutrophil	0.161	0.025	0.200(0.022-0.300)
Constant	-0.048	0.692	-0.295-0.199)(

Model F test=41.19, p=0.000

This model predicts 82% of the activity index (predictors)

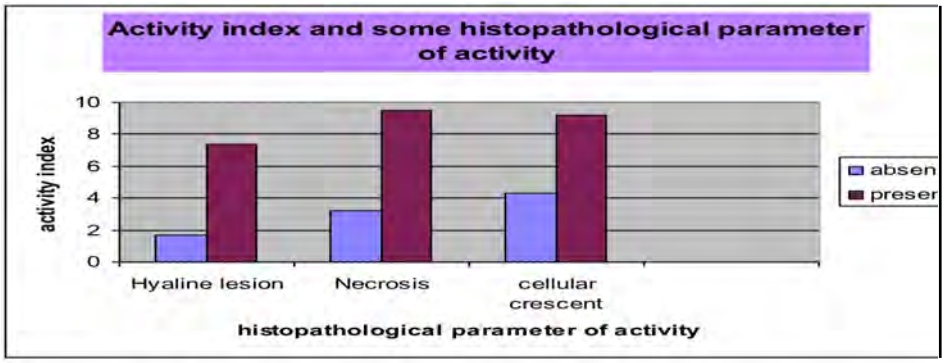

Figure (1) : Activity index and some histopathological parameters of activity.

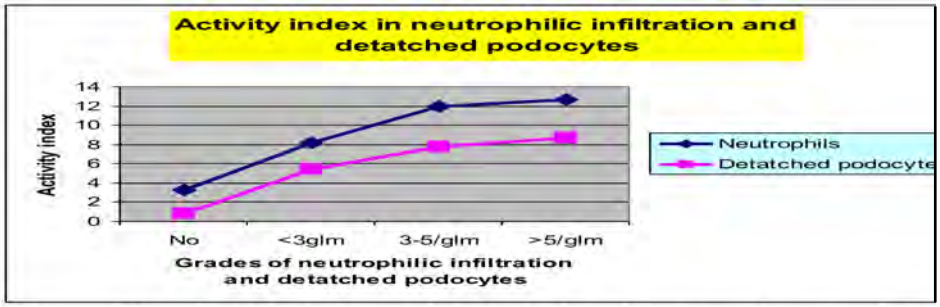

Figure (2) : Activity index and detatched podocytes and neutrophilic infiltration.

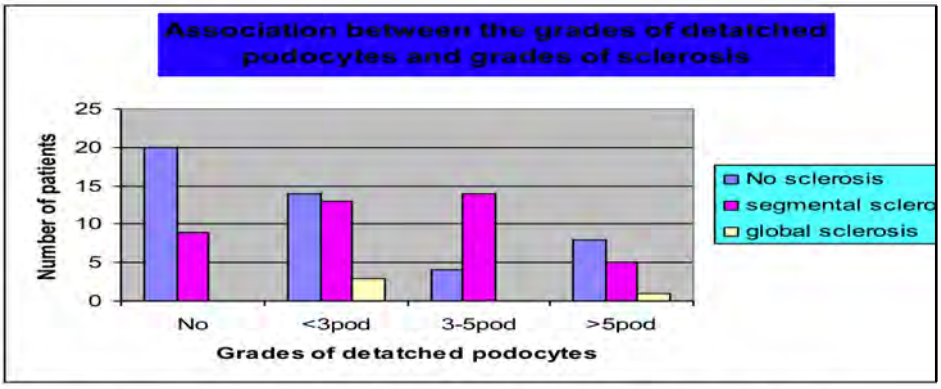

Figure (3) : Association between detatched podocytes and grades of sclerosis.

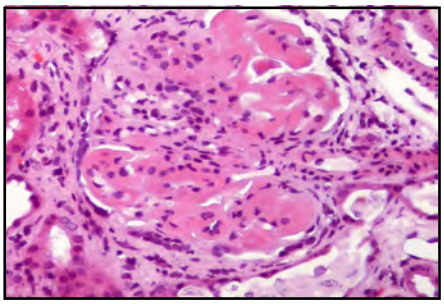

Fig (19): LN class IV AI: 6/24, CI: 3/12 (200 H&E): proliferation, wire loop.

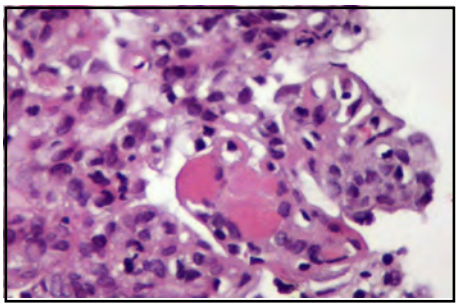

Fig (20) : LN class IVa AI: 8/24, CI: 1/12, prolifertaion, hyaline thrombi, neutr. inf (H&E 400).

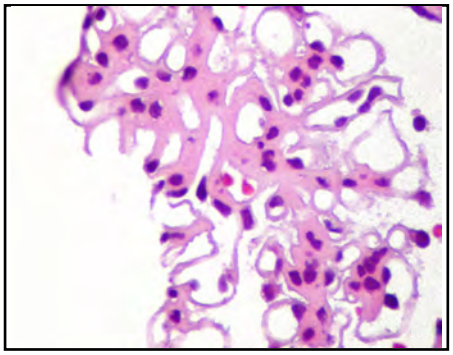

Fig (14): lupus nephritis class V, wire loop, detatched podocytes (H&E 400).

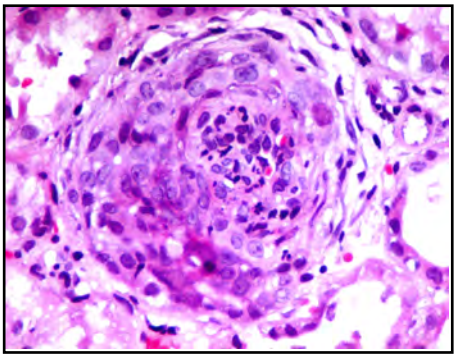

Fig (4) : LN, class IVa AI: 18/24, CI: 2/12, neutrophilic infiltration, cellular crescent (H&E 200).

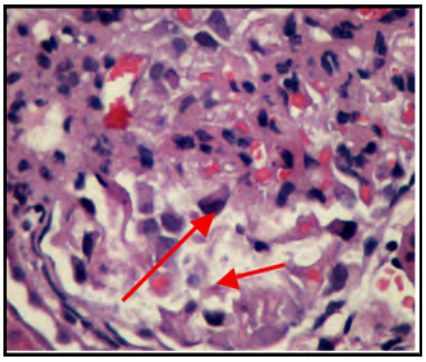

Photo (11): lupus nephritis class IV-V activity index 5/24, chronicity index = 0/12 detatched podocytes. (H & E 400).

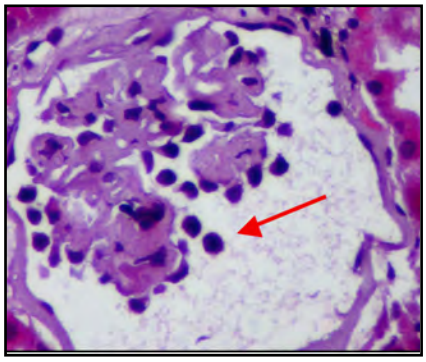

Photo (18): LN class V with detatched podocytes (H&E 400).

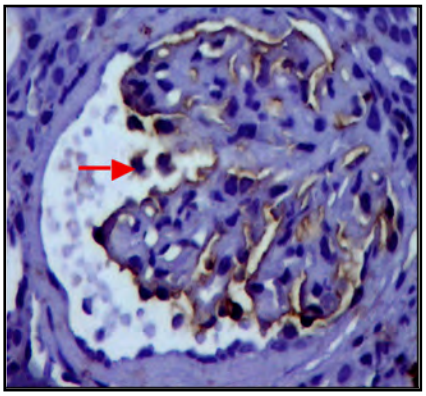

Photo (19) : LN class V with detatched podocytes (IHC antiPXN 200).

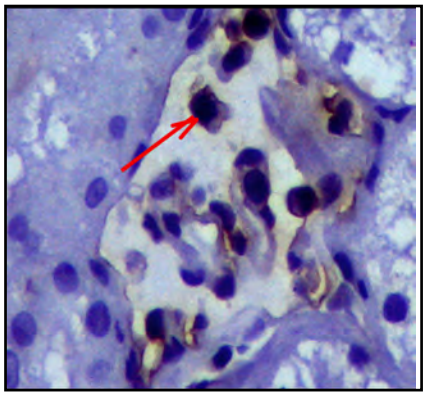

Photo (20): LN class V with detatched podocytes (IHC antiPXN (400).

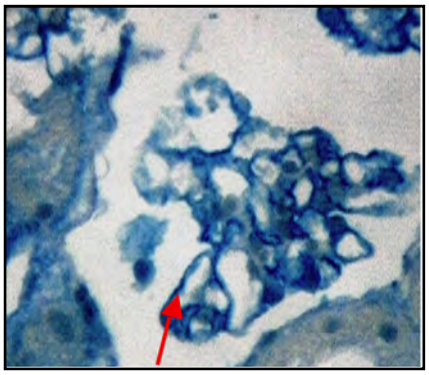

Photo (61): Detatched podocytes in sclerotic glomerulus in LN (colloidal iron stain 400).

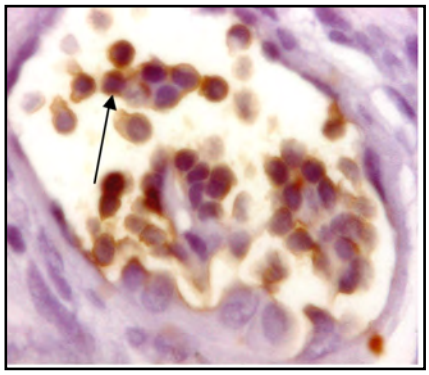

Photo (15): lupus nephritis class IV SB, sclerosis, detatched podocytes (H&E 400).

Discussion

The importance of counting detatched podocyte can be explained by the close correlation between the degree of glomerular damage and the reduction of podocyte number per glomerulus that was observed by [6,1,11].

For this reason, we look for detatched podocytes in the Bowman's space as a marker of activity in all cases.

We observed significant difference in the means of the activity index (p=0.000) in cases of lupus nephritis in different scores of detatched podocytes. The highest activities were found in the presence of more than five podocytes per glomerulus.

The importance of each parameter in determining the activity index in this study shows little difference from that reported in D'Agati, [3] as shown in table (4) because not all the cases of the study were active lupus. D'Agati, [3] reported that the most predictors of activity include cellular crescent and necrosis. So they should be multiplied by a factor of

2 because of their prognostic importance.

There were significant association between scores of detatched podocytes and the pattern of sclerosis (p=0.01), Highest degree of detatched podocytes were associated with segmental sclerosis These result can be explained as detached podocytes initiates an injury cascade that results in the segmental scar characteristic of FSGS (1).

From all of the above results we can say detatched podocytes is a marker of disease activity in lupus nephritis and it is the first step of a cascade of injury ending in progression to sclerosis but not represent a marker of chronicity index.

Conclusion

From this study, we concluded that, detatched podocytes can be one of the parameters of activity index but not of the chronicity index in lupus nephritis.

Scoring of detatched podocytes in the mostly affected glomerulus as 1=<3, 2=3-5, 3=>5 podocytes in the mostly affected glomerulus. Counting podocyte can be done using routine H&E stain. Colloidal iorn stains or immunohistochemistry by anti PCX can be used as confirmatory stain to be sure of podocyte. Colloidal iron stain is a very simple and cheap method in this purpose and can be also a background cytoplasmic stain with other nuclear stain.

Since the detatched podocytes were not important as cellular crescent or necrosis because it is not included in the best predictor of activity index by regression analysis so we did not recommend multiplying it by factor 2 as cellular crescent and necrosis. So the maximum summation of activity index in lupus nephritis should be 27 not 24.

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BENHA MEDICAL JOURNAL

DETATCHED PODOCYTES IN THE BOWMAN'S SPACE AS A PARAMETER OF ACTIVITY INDEX IN LUPUS NEPHRITIS

Nahed A. Soliman MD, Ashraf M. Abd-Elbast MD, Fatma E. Moustafa MD and Ebrahim M. El-Shwaf MD

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UNIFOCAL DIFFERENTIATED THYROID CANCER SMALLER THAN 1 CM ARE BETTER MANAGED BY TOTAL THYROIDECTOMY

Mohamed A. F. Hegazy MD, Waleed Elnahas MD, Mohamed T. Hafez MD and Khaled M Abdel Wahab MD

Surgical Oncology Department, Oncology Center,
Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Abstract

Background: *The optimal extent of surgery for differentiated thyroid cancers (DTC) is controversial. The aim of this study is to assess the frequency and potential predictive factors of residual malignancy in the lobe contralateral to the main tumor. The secondary aim was to assess the safety of completion thyroidectomy*

Methods: *All total thyroidectomies performed at our institution between 2004-2010 were reviewed identifying 185 patients (70 cases underwent completion thyroidectomy and 115 cases underwent initial total thyroidectomy). The predictive value of sex, age, tumor size, histology, multifocality, perithyroid extension , and lymph node involvement was analyzed*

Results: *We report a high rate of multifocality (51%) in the whole cohort of cases and of contralateral disease (35%). There were no differences in multifocality rates for sex, age, pathology types and tumor size. PTC cases < 1cm have a similar contralateral disease to cases > 1 cm (45% versus 59% respectively). Moreover, there was no significant correlation between ipsilateral multifocality and contralateral disease in our cases. In our series, the complication rates for both completion thyroidectomy and total thyroidectomy were comparable.*

Conclusion: *Absence of significant predictive factors that could suggest a residual disease justifies total thyroidectomy as a primary treatment for cases of DTC. Hemithyroidectomy for management of cases of DTC smaller than 1 cm with absence of multifocal disease in the ipsilateral lobe should be questioned.*

Key words: *differentiated thyroid cancer- total thyroidectomy- completion thyroidectomy- multifocal carcinomas.*

Introduction

Although controversy exists regarding the extent of surgery in patients with differentiated thyroid cancer of follicular origin which includes papillary, follicular, and Hurthle cell malignancies, there is general agreement that a total thyroidectomy is indicated in patients considered to be high risk [1,2]. Approximately 90% of all thyroid cancers are well differentiated and of follicular origin and approximately 80% of these tumors are staged as low risk [3].

Thyroid lobectomy has been considered as adequate therapy for a papillary thyroid carcinoma (PTC) in a low-risk patient that is less than 1.0 cm (papillary microcarcinoma, mPTC), is confined to the thyroid gland, does not have insular or tall cell features and does not demonstrate angioinvasion or metastasis. A completion thyroidectomy would, therefore, not be indicated in this group of patients. Patients with this PMC have a death rate of 0.1% and a recurrence rate of 5% [4]. For follicular thyroid carcinoma, the absence of multicentric disease is an argument against bilateral thyroidectomy for management of primary tumors. However, other authors recommend complete removal of all thyroid tissue in all case of differentiated thyroid cancers (DTC) at least to facilitate subsequent radioactive iodine therapy[5]

Among the main considerations for the limited surgery recommendations is the estimated low rate of tumor multifocality in the thyroid gland and more specifically in the contralateral lobe. Several studies established a clear association between tumor multifocality and local recurrence, regional recurrence, and lymph node involvement, and distant metastases. Hence the need for a more aggressive approach [6-8]. The incidence of multifocality is understudied and ranges between 18% and 87% with some of the studies dated more than 4 decades ago[7-11].

Although the debate is still going, once the diagnosis of DTC is made in one lobe many physicians think that a completion thyroidectomy should be considered to achieve total surgical ablation of thyroid [12,13]. Completion thyroidectomy not only deals with resid-

ual carcinoma in the opposite lobe, but also facilitates I131 whole-body scanning, allowing for the diagnosis and treatment of unrecognized metastatic carcinoma [13]. However, the morbidity after completion thyroidectomy is reported to be many times more than the primary procedure [14], only a few studies reporting comparable morbidity for these two procedures [15]. As a protocol at our centre, we recommend patients for completion thyroidectomy if they are referred to us after subtotal thyroidectomy or the diagnosis of thyroid cancer is confirmed after hemithyroidectomy.

The primary objective of this study is to assess the frequency of malignancy in the residual thyroid tissue removed after completion thyroidectomy. Furthermore we analyzed a number of clinical parameters as potentially predictive factors that may anticipate the presence of malignancy in the residual thyroid tissue. The secondary objective was to review our institution's experience with completion thyroidectomy in the management of DTC and to compare the results of recurrent laryngeal nerve (RLN) injury, and hypocalcaemia against a cohort of patients treated by primary total thyroidectomy.

Patients and Methods

Patients:

We reviewed the medical records of 343 patients who underwent total thyroidectomy in our institution in the period between 2004 and 2010. After exclusion of 132 cases that underwent treatment for benign diseases and 26 cases that lost follow up, our cohort included 185 patients with DTC. Seventy cases underwent completion thyroidectomy. Among those cases, 56 patients were referred to our center from other hospitals. Fourteen cases underwent initial surgery at our clinic for benign goiter or adenoma which was diagnosed as DTC at postoperative biopsy. Preoperative investigations included estimation of serum thyroxin (T4) and thyroid stimulating hormone (TSH), calcium, neck US, radioactive thyroid scan and indirect laryngoscopy. The TNM classification system was used for staging. FNAC was performed to all cases in our institute preoperatively. For cases re-

ferred to our center from other hospitals, the following data were collected: size of the initial tumor, location, radiologic status of the residual thyroid tissue and cervical LNs and postoperative pathology. Slides of the initial surgery were revised with special emphasis on multifocality.

Surgical technique :

For total thyroidectomy, the capsular dissection method was used. The external branch of the superior laryngeal nerve was saved by individual ligation of vessels in the upper pole. An attempt was made to follow the course and preserve both recurrent laryngeal nerves and four parathyroids. However, we took care not to dissect the parathyroids out of their fatty envelopes. The blood supply to the parathyroids was preserved by ligating the individual branches of the inferior thyroid artery on the capsule of the thyroid. When there was inadvertent injury to the parathyroid glands or their blood supply, they were auto transplanted in the sternocleidomastoid muscle on the same side.

Completion thyroidectomy was done within 7 days of the initial operation or after 6 weeks. When re-opening the operative site, particularly if this is performed in the early postoperative period, the thyroid bed should be thoroughly rinsed with saline before dissection for removal of clots or debris. All cases were performed via the lateral approach. The carotid sheath is retraced laterally, the omohyoid muscle is transected, and the lateral thyroid is approached. Harmonic Scalpel has been used routinely in our center since 2006 and is effective in reducing operative bleeding, operating time and facilitating meticulous dissection. When re-operating on the ipsilateral thyroid gland, the recurrent laryngeal nerve is often difficult to identify and dissect as it is buried in scar tissue. Palpation of the cricothyroid junction and the inferior cornu of the thyroid cartilage was helpful to identify the nerve just anterior to this point and it can then be traced inferiorly. Any structure that is suggestive of being a parathyroid gland should be considered as such until proven otherwise.

Central lymphadenectomy (removing level VI pretracheal and paratracheal LNs) was routinely

performed. Dealing with lateral cervical LNs (level II-V) depended upon the preoperative clinical and radiologic assessment. When preoperative examination indicated positive LNs, functional neck dissection (removing nodal levels, II, III, IV, and V with preservation of the internal jugular vein, sternocleidomastoid muscle and spinal accessory nerve) was performed. For those who showed negative results in preoperative imaging, lateral neck nodes were explored at surgery and any palpable LN was sent for frozen section examination. Cases that showed positive frozen underwent lateral neck dissection.

Postoperative I131 whole body scans were done to all patients after 4-6 weeks to detect residual thyroid tissue and to exclude functional metastases. Ablative doses (80-100 mCi) of I131 were given, if the uptake in the thyroid bed was greater than 1.5%. Subsequently, all patients were given hormone suppressive therapy (Eltroxin 200-300 mcg per day) and were closely monitored by FT3, FT4 and TSH aiming to keep TSH level below 0.1μIU/ml. Follow-up was done every 6 months by I131 whole body scan, neck ultrasound, and non-contrast CT chest.

Pathologic evaluation:

The histopathology reports (including reports of the initial surgery that were revised in our center) were analyzed for the following information.

Tumor subtype was noted as papillary carcinoma, papillary carcinoma-follicular variant, follicular carcinoma, or Hurthle cell carcinoma.

Tumor size with the largest tumor considered the primary tumor.

Focalityuuuuuuu: Patients were divided into 2 groups: single lesion and multiple lesions. The second group was then further subdivided to 3 subgroups: lesions located only at the same lobe as the main tumor (ipsilateral), lesions located at the other lobe (contralateral), and lesions located in both lobes (bilateral). The number and size of all tumors were also registered.

Perithyroid tumor extension: defined as positive or negative.

Node status: graded as positive or negative and number of nodes involved.

The following factors were analyzed as potentially predictive factors for presence of malignancy in the residual thyroid tissue and/or cervical LNs: age older than 40 years, gender, tumor pathologic type, size of the primary tumor, evidence of perithyroid tumor extension, lymph node status, and ipsilateral multifocality.

Procedure related complications:

The complications with completion thyroidectomy were compared with the primary total thyroidectomy. We gathered data regarding wound infection, hematoma, seroma, RLN injury (temporary or permanent), and hypoparathyroidism (temporary or permanent). Temporary RLN injury was defined as a new onset vocal cord paralysis, identified in the postoperative period that was noted to recover completely at subsequent visits. Injuries were deemed permanent if paralysis persisted past 12 months from operation. Permanent hypoparathyroidism was diagnosed as either an unrecordable or abnormally low serum parathormone level, with a persistent dependence on vitamin D and calcium replacement at 6 months postoperatively. Temporary hypoparathyroidism was defined as hypocalcemia in the immediate postoperative period requiring treatment with calcium, vitamin D, or both, which was eventually withdrawn.

Statistical analysis:

Statistical analysis was done using the two tailed Fisher's exact test with the use of statistical software SPSS version 11.5 (SPSS, Inc, Chicago, IL) and a P value <0.05 was considered to represent statistical significance for all comparisons.

Results

The final cohort of patients in the current study included 185 patients with WDTC, 70 cases underwent completion thyroidectomy, and 115 cases underwent initial total thyroidectomy. In the completion group there were 52

women and 18 men, with a mean age of 42.8 years. In the initial total thyroidectomy group there were 86 women and 29 men with a mean age of 46.2 years (Table 1). The difference between both groups regarding the age and gender was statistically insignificant (P=0.4 and P = 0.67 respectively).

Completion Thyroidectomy Group :

Most cases (n =52, 70%) in the completion group were referred from other hospitals. Preoperative FNAC was performed to 26 cases (all 18 cases that were operated initially in our center and 8 cases referred from other hospitals). FNAC demonstrated follicular lesion in 5 cases, papillary carcinoma in 1 case, colloid goiter in 16 patients, suspicious smear in 2, and non representative in 2 cases. The primary operation in these cases were hemithyroidectomy (n=45), subtotal thyroidectomy (n=18), or subtotal lobectomy (n=7). The neck nodes were assessed in only 11 cases; 8 cases underwent unilateral modified radical neck dissection and 3 cases underwent lymph node sampling. Nodes were positive in all 8 cases of neck dissection and in 1 case of LN sampling. Final pathology reports in these patients revealed papillary carcinoma in 43 cases, follicular variant of papillary carcinoma in 12 cases, follicular carcinoma in 11 cases and Hurthle cell carcinoma in 4 cases. The median tumor size was 2.3 cm (range 1.2-6.7 cm). Tumors were single in 53 cases (76%) and multifocal in 17 cases (24%). Multifocal tumors were present in 10/52 cases who underwent hemithyroidectomy or lobectomy (ipsilateral), and in 7/18 cases who underwent subtotal thyroidectomy (3 bilateral, 1 ipsilateral, and 3 contralateral).

Completion thyroidectomy was performed within a median of 3.1 month (range 12 days- 5 months). The previously resected lobe had to be explored in 22 patients because of the presence of radiologic evidence of residual thyroid tissue. Residual tumor was found in 27 specimens after completion thyroidectomy; 14 contralateral, 3 ipsilateral, and 10 bilateral. The median tumor size was 0.7 cm (range: 0.2-2.1 cm). Cervical lymph node status was negative

clinically and radiologically in 57 cases and positive in 13 cases. Intraoperatively, nodal metastasis was suspected in 9 patients (of the 57 radiologically node negative cases), and confirmed by frozen section examination in 6 cases. Modified radical neck dissection was performed in 19 patients, bilateral in 5 and unilateral in 14 cases. Final node positive cases after both surgeries were 28. Overall, on pathologic examination of specimens obtained from both the initial surgery and completion surgery, a total of 40 multifocal cases was obtained (13 ipsilateral, 10 bilateral, and 17 contralateral). Multifocality was significantly correlated only with LN stratus. Multifocal tumors were present in 79% of node +ve cases (22/28) and in 43% of node -ve cases (P= 0.03)

The contralateral lobe showed residual tumor tissue in 27 patients (39%). None of the predictive factors analyzed for contralateral disease reached a statistical significance.

Complications of the second surgery were as follows: 15 patients had transient hypocalcaemia that needed oral calcium supplement for less than a month. , 1 patients had permanent hypoparathyroidism that needed long term treatment with oral and IV calcium. Temporary RLN palsy was found in 2 cases, and only 1 case had permanent RLN injury. This case underwent ST as the primary operation outside our institution (Table 2).

Primary total thyroidectomy group:

115 cases underwent initial total thyroidectomy. Preoperative FNAC was performed to all cases. It demonstrated follicular lesion in 21 cases, papillary carcinoma in 49, suspicious smear in 19 cases, colloid goiter in 16 and non representative in 10 cases. Final pathology was papillary carcinoma in 72 cases, follicular variant of papillary carcinoma in 25 cases, follicular cancer in 15 cases and Hurthle cell carcinoma in 3 cases. The mean tumor size was 2.6 (range: 0.7-6.3 cm). Single lesion was identified in 60 patients as opposed to 55 patients with multifocal disease. Patients with multifocal disease were further classified into ipsilateral lesions only

(n= 17), contralateral only (n=13), or bilateral (n= 25). The neck nodes were assessed in 56 cases; 43 cases underwent unilateral modified radical neck dissection and 8 cases underwent bilateral MRND, and 5 cases underwent lymph node sampling. Cervical lymph node metastasis was present in 52 cases. Multifocality was present in 69% of node positive cases (n=36) and in 30% of node negative cases (n=19) with a significant P value (P=0.01).

The overall morbidity after the 115 primary total thyroidectomy included temporary hypocalcaemia in 24 cases (21%), permanent hypocalcaemia in 2 cases (2%), temporary hypoparathyroidism in 3 cases (3%), seroma in 5 cases and 1 case developed wound infection that was managed by antibiotics. Comparison of complications of both groups is illustrated in table (2).

Final histopathologhic report of the entire cohort of patients (n=185) revealed 115 cases with papillary carcinoma, 37 with follicular variant of papillary carcinoma, 26 with follicular carcinoma, and 7 with Hurthle cell carcinoma. The overall rate of multifocality is 51% (n=95). Multifocal cases were ipsilateral (16%, n=30), contralateral (16%, n=30), or bilateral (19%, n=35). Multifocality was only significantly affected by LN positivity (P= 0.02). The rate of contralateral positivity was not significantly correlated with any of the predictive factors assessed including tumor multifocality. Contralateral lobe was positive for malignancy in 65 cases (37%); 30 cases were +ve despite the absence of focality in the ipsilateral lobe, and 35 +ve in presence of multifocality in the ipsilateral lobe (P=0.3) (Table 3).

Follicular carcinoma cases occurred more in males (19/26) and in older age groups (20 cases were > 40 years). The tumor size in follicular carcinoma cases tend to be larger [mean 2.3 cm (range: 1.4-5.6 cm) versus a mean of 1.9 cm in papillary cancer cases (range: 0.2-6.7 cm)]. Furthermore, multifocality was present in 9 cases (35%) of follicular carcinoma (7 +ve contralateral lobe) that was once considered a unifocal disease. Most of these tumors were

found to be PMCs with 68% measuring <1cm. Hurthle cell carcinoma was present in 7 cases, 2 cases showed ipsilateral multifocality.

Papillary carcinoma group (including follicular variant of papillary carcinoma) included 152 cases. The rate of multifocality in PTC cases was 51% (77/152). Patients were analyzed in 2 groups; those with tumors ≥ 1cm (n=105, 69%) and those with tumors < 1 cm (n= 47, 31%). No significant difference existed between both groups in age or sex. Multifocality was present in 62 cases (59%) with tumors ≥ 1cm (44 cases showed +ve contralateral lobe) and in 21 cases (45%) with tumors < 1 cm (13 cases showed +ve contralateral lobe) (P=0.3) (Table 4).

Table (1): Clinicopathologic data of completion and total thyroidectomy groups.

	Completion thyroidectomy Group (n=70)	Total thyroidectomy Group (n=115)	Total (n=185)
Age			
< 40 years	32	53	85
>40 years	38	62	100
Sex			
Males	18	29	47
Females	52	86	138
FNAC			
Follicular lesion	5	21	26
Papillary carc	1	49	50
Colloid nodulre	16	16	32
Suspicious	2	19	21
Non respresentative	2	10	12
Lymph Nodes			
Status			
+ve	28	52	80
-ve	42	63	105
Operation			
Mod Neck Dissection	27	51	78
Berry picking	3	5	8
Final Pathology			
Papillary Ca	43	72	115
Follicular variant Papillary ca	12	25	36
Follicular ca	11	15	26
Hurthjle cell ca	4	3	7
Mean tumor size	2.2 cm (0.2-6.7cm)	2.6 cm (0.7- 6.3 cm)	
Perithyroid tumor extension			
+ve	14	22	36
-ve	56	93	149
Multifocal tumors			
Ipsilateral	13	17	30
Contralateral	17	13	30
Bilateral	10	25	35
Comtralateral lobe			
Positive	27	38	65
Negative	43	77	120

Table (2): Complications of completion thyroidectomy and initial thyroidectomy groups.

Complication	Completion thyroidectomy (n=70)	Initial Total thyroidectomy (n=115)
Temporary hypoparathyroidism	15 (21%)	24 (21%)
Permanent hypoparathropidism	1 (1%)	2 (2%)
Temporary RLN palsy	2 (3%)	3 (3%)
Permanent RLN palsy	1 (1%)	0
Hemorrhage	1 (1%)	0
Wound infection	2 (3%)	1 (1%)
Seroma	4 (6%)	5 (4%)

Table (3): Predictive factors of residual malignancy correlated with ipsilateral multifocality in the whole cohort of patients (n= 185).

	Contralateral lobe –ve (n= 120)		Contralateral lobe +ve (n=65)		P value (for contralateral disease)
	Ipsilateral multifocality -ve (n=90)	Ipsilateral multifocality +ve (n=30)	Ipsilateral multifocality -ve (n=30)	Ipsilateral multifocality +ve (n=35)	
Age					
< 40 years (85)	46 (54%)	13 (15%)	12 (14%)	14 (16%)	0.61
> 40 years (100)	44 (44%)	17 (17%)	18 (18%)	21 (21%)	
Gender					
Males (47)	26 (55%)	6 (13%)	8 (17%)	7 (15%)	0.36
Females (138)	64 (46%)	24 (18%)	22 (16%)	28 (20%)	
Perithyroid extension					
-ve (149)	74 (50%)	25 (17%)	23 (15%)	27 (18%)	0.29
+ve (36)	16 (44%)	5 (14%)	7 (19%)	8 (23%)	
LN status					
-ve LN (105)	66 (63%)	8 (8%)	14 (13%)	17 (16%)	0.09
+ve LN (80)	24 (30%)	22 (28%)	16 (20%)	18 (22%)	
Pathology					
Papillary carcinoma (152)	69 (45%)	25(16%)	27(18%)	31 (20%)	0.12
Follicular carcinoma (26)	16 (62%)	3(12%)	3 (12%)	4 (15%)	
Hurthle cell carcinoma (7)	5 (71%)	2 (29%)	0	0	

Table (4): Multifocality in papillary thyroid carcinoma cases (n= 152) according to tumor size.

	Single lesion	Multiple lesions			P for contralateral disease
		Ipsilateral	Contralateral	Bilateral	
<1cm (47)	26 (55%)	8 (17%)	6 (13%)	7 (15%)	0.33
>1cm (105)	43 (41%)	18 (17%)	20 (19%)	24 (23%)	

Discussion

The preoperative diagnosis of DTC can be defined with FNA if the tumor is greater than 1cm in size, total thyroidectomy in the treatment of choice for the treatment [16]. The controversy exists for the treatment of tumors less than 1cm in diameter and where the diagnosis is made after hemi- or partial thyroidectomy for thyroid nodule. Options include completion thyroidectomy or I131 ablation. However the later approach is associated with many disadvantages, including multiple doses of I131 for successful ablation, difficulty in ablating large thyroid residue. In addition, high doses of radioactive iodine can result in pulmonary fibrosis, temporary bone marrow suppression and leukemia [17]. Therefore surgical resection remains the best way to remove the remnant thyroid tissue.

Ideally, if one could identify with high accuracy the extent of the disease and be able to excise all malignant tissue leaving only normal thyroid tissue, the issue might be resolved. However, this goal is not attainable with the current diagnostic techniques. In view of that limitation, interesting questions that may arise would be "How frequent is residual malignancy in patients who had partial thyroid surgery?" and "Could one predict its presence in any given patient?" [18].

The incidence of separate malignant lesion at completion thyroidectomy following the initial thyroid lobectomy ranged in the literature from 31 to 77% and lymph node metastases ranged from 17 to 40% [18-20]. Although studied for several decades the exact incidence and ramifications of multifocal disease, and more specifically contralateral disease, is not well established for 2 reasons. First, some patients undergo unilateral lobectomy only, so that no information is available on the contralateral lobe. Second, there is some inconsistency regarding the terminology. Multifocal disease, defined as more than 1 cancerous lesion, can be located in the ipsilateral lobe only and should not be mistaken for contralateral disease. Published data, some of which is 4 decades

old, cite multifocality to occur between 18% and 87%[7-11]. However, the rate of contralateral disease is estimated as 13% to 56% only[21,22]. In a more recent study, Pitt et al identified a rate of 29% for contralateral disease in a cohort of 228 patients [11]. In our single-institution study in a cohort of 185 patients with DTC, we report a total rate of 51% multifocality and 35% contralateral disease. Our data failed to find any influence on multifocality or on the rate of contralateral disease by age, sex, pathology subtype or tumor size.

Papillary thyroid cancer is known to be multifocal in about 30-80% [20,23], thus initial total thyroidectomy remains an effective and safe treatment method to reduce the surgical risk of the patients and facilitate to perform radioactive iodine therapy. Follicular thyroid cancer tends to be less multifocal than papillary thyroid cancer does but they are much more aggressive and, if they are widely invasive or extrathyroidal disease is found, completion thyroidectomy should also be performed [24].

The reported rates of the incidence of contralateral PTC discovered in completion thyroidectomy or total thyroidectomy specimens ranges from 13% to 56% [21,25]. Regarding mPTC, 28% of patients have been reported to have PTC in the contralateral lobe on histologic review [21,26]. Our series is consistent with these previous reports. Thirty six percent (n= 57/152) of all patients and 42% (n= 44/105) of patients with primary tumors >1 cm had contralateral PTC. Interestingly, patients with primary tumors < 1cm had a similar rate of contralateral disease (28%). These data suggest that the rate of contralateral PTC is independent of primary tumor size. In a review of 150 patients who underwent completion thyroidectomy, Grigsby et al also found that the size of the primary tumor did not predict the presence of contralateral disease [27].

Although primary tumor size does not correlate with PTC in the contralateral lobe, multiple factors have been shown to predict contralateral disease. Researchers have attempted to detect such connections to better identify

which patients would benefit from completion thyroidectomy when PTC is discovered incidentally after lobectomy. In completion thyroidectomy specimens, positive lymph node metastases at the initial surgery and a longer time interval between lobectomy and completion thyroidectomy have been associated with PTC in remaining lobe [28]. In addition, infiltration of the thyroid capsule, "tall-cell" variant, and the presence of a tumor capsule have been linked to bilateral PTC. Another variable shown to be predictive of PTC in the contralateral lobe is ipsilateral multifocal disease [24].

Tumor multifocality has been reported previously to correlate with the presence of the disease in the contralateral lobe [7] and with a higher risk of tumor recurrence [29]. In our study, we report a multifocality rate of 49%; ipsilateral only in 16%, contralateral only in 16% and bilateral in 17%. There was no significant correlation between ipsilateral multifocality and +ve contralateral lobe. Contralateral disease occurred in 30 cases in absence of ipsilateral multifocality versus 35 in presence of ipsilateral multifocality.

The absence of significant correlation between ipsilateral multifocality and contralateral disease could be explained by two reasons. First: multifocal disease has been considered for years an intraglandular spread of the primary tumor and hence regarded as more aggressive disease with increased risks of locoregional recurrence, as well as lymph node and distant metastases [6-8]. Recent advances in molecular genetics allowed investigators to challenge the assumption of intraglandular spread. In the last 5 years a few studies explored the genetic origin of mPTC. Shattuck et al [30] published their data in 2005 proving that tumor foci in patients with mPTC arose in 5 of 10 patients as independent tumors. Independent clonal origin was also observed by Park et al[31] in 2006 and Giannini et al[32] in 2007. This finding strengthens the argument of performing total thyroidectomy or completion thyroidectomy for tumors <1cm. Similarly, our findings in patients with follicular carcinoma also support this

observation. Follicular carcinoma is considered a unifocal tumor that spreads to the bloodstream as opposed to the intraglandular route. Nevertheless, in 35% of our follicular carcinoma patients multifocal disease was identified. However, these were found to be mPTCs with 68% measuring <1cm. These are all obviously incidental independent clones, unassociated with the original, main follicular carcinoma.

Second: pathology guidelines for thyroid specimen examination recommend that only representative sections of the entire gland should be examined. At our institution, pathologists follow these recommendations. Mazeh et al [33], suggested that entire gland examination rather than representative seconds offers a superior representation and better diagnosis of multifocality (64% versus 54%, P=0.16) and more specifically bilateral disease (60% vs 37%, P= 0.04).

Despite the high frequency of malignancy on second surgical exploration, an important question would be the significance of this finding. Alzahrani et al [18] in his study stated that the median tumor size on second surgery was 0.8 cm, and only a minority of cases showed evidence of perithyroidal extension (7 cases) or soft tissue invasion (2 cases). Some investigators argue that such small residual tumors have little bearing on the patient's outcome.[34] Others have reported higher recurrence rates and higher chances of pulmonary metastases [35] and other distant metastases. The policy in our institution calls for completion thyroidectomy in most cases of DTC with significant residual thyroid tissue. Because of this policy and because of absence of control group in which completion thyroidectomy was not done, we cannot draw a conclusion on the long tern impact of completion thyroidectomy in patients with DTC.

One of the most feared complications of repeated thyroid surgery is RLN injury. Beahrs and Vandertoll [36] found a 17% incidence of vocal cord paralysis in 548 secondary thyroidectomies. At the time of reoperation, the surgeon usually does not

know if 1 or several parathyroid glands have been unintentionally removed with the thyroid. Furthermore, even if they are left in place, it is not possible to predict the functional value of the remaining parathyroid glands that may have been devascularized during the previous surgery. As technique and experience have improved, this incidence of complications has gradually decreased. We report a low complication rate with cases of completion thyroidectomy that was comparable to those of initial total thyroidectomy. Our data cope with Chao et al. [12] who reported a 2.6% incidence of transient RLN palsy in completion thyroidectomy. Mishra et al. [37] reported that the incidence of transient RLN palsy was 4% and there was no permanent RLN palsy.

Conclusion

In this study, we report a high rate of 51%% multifocality and 35%% of contralateral disease. We also show that the rate of contralateral thyroid cancer of patients with tumors > 1cm are similar compared with those having tumors < 1 cm. Moreover we failed to find a significant correlation between the factors commonly used for thyroid cancer staging including ipsilateral lobe multifocality and the occurrence of contralateral cancer. Therefore consideration for TT or CT should be made in cases of DTC regardless the tumor size or ipsilateral multifocality. Completion thyroidectomy is a safe procedure with low complication rates that are comparable to initial TT.

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UNIFOCAL DIFFERENTIATED THYROID CANCER SMALLER THAN 1 CM ARE BETTER MANAGED BY TOTAL THYROIDECTOMY

Mohamed A. F. Hegazy MD, Waleed Elnahas MD, Mohamed T. Hafez MD and Khaled M Abdel Wahab MD

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IMMUNOHISTOCHEMICAL EXPRESSION OF CYCLIN D1, KI67/MIB, AND PROGESTERONE RECEPTORS IN MENINGIOMAS

Abdelhadi M. Shebl M.Sc, Dina A. El-Tantawy MD, Maha M. Ameen MD, Wageha M. Kandil Ph.D and Nadia E. Basiony Ph.D

Department of Pathology, Faculty of Medicine, Mansoura University, Mansoura, 35516, EGYPT.

Abstract

Objective: Recurrence after apparently complete resection is one of the most relevant problems of meningioma treatment. The mechanisms of which are still unclear. Purpose of the study: The aim of this study is to correlate immunohistochemical expression of progesterone receptors, cyclin D1, and KI67/MIB-1 with meningioma recurrence. Methods: 153 cases of completely resected meningiomas have been reviewed. Immunohistological studies include Ki-67 LI, progesterone receptors, and cyclin D1. All these factors have been correlated with the recurrence. Results: The recurrence rate was 7.5%, 56.25%, 33.3% for WHO grade I, grade II, and grade III tumours respectively. The tumor recurrence was not significantly correlated with PR and cyclin D1 status. However, Ki-67 LI was significantly correlated with the recurrence (P<0.001). Non-recurrent cases had a mean KI67 LI of 3.47 ± 4.21% (range, 0-25), while that of recurrent cases was 16.75 ± 7.61% (range, 6-40). Conclusions: WHO grade and proliferative index (KI67 LI) are statistically significant to predict recurrence, while PR and cyclin D1 status are not.

Keywords: *Meningioma, PR, KI67, Cyclin D1, recurrence.*

Introduction

Meningiomas are neoplasms derived from meningothelial cells [1]. They normally line the inner aspect of the arachnoid membrane, and fill the cores of the arachnoid villi that project into the lumens of dural veins and venous sinuses. Arachnoid cells are also identified outside

the neuraxis and give rise to meningiomas in extracranial locations [2].

Meningiomas are the second most common central nervous system neoplasm in adults and account for 15-20% of all primary brain tumors [3]. Ninety percent of meningiomas are benign [4]. Atypical meningiomas account for 4.7%-7.2% of all meningiomas [5], while malignant meningiomas account for 1-3% [6,7].

Meningiomas are one of the most recurring tumours which affect the central nervous system [8]. Between 7 and 32% of benign meningiomas recur after total resection and between 19 and 50% after subtotal removal [9]. Grade II meningiomas exhibit a higher risk of recurrence (29% to 40%). Recurrence rate of anaplastic meningiomas is reported to be 50% to 78% [10]. Recurrence after apparently complete resection is one of the most relevant problems of meningioma treatment [11].

Routine histological examination fails to identify the subset of grade I meningiomas that behave aggressively. Therefore, other methods than using morphologic characteristics alone were sought to predict biological behavior of meningiomas [12].

Expression of progesterone receptors (RP) had an inverse correlation with recurrence [13], however other studies showed no significant correlation between the PR status and recurrence [14-16].

Most studies reported higher KI67/MIB-1 labeling indices in recurrent meningiomas than in non-recurrent [16,17].

Hsu and colleagues suggested that only a combination of three factors, WHO grade, proliferative index, and PR status, should be used to predict meningioma recurrence [18].

In this study, we evaluated the immunohistochemical expression of PR, KI67/MIB-1, and cyclin D1 and correlated them with recurrence.

Materials & Methods

- **Samples**

In this study, 153 meningioma

cases were examined. These have been obtained from the surgical pathology files at the pathology department and other pathology laboratories in Mansoura, Egypt between 2000 and 2007. Paraffin wax embedded sections were stained with haematoxylin and eosin. Meningiomas were categorized into subtypes according to the new WHO classification 2007. As the extent of resection is the most important factor in predicting recurrence, incompletely resected tumours were excluded. So testing for the validity of predictive recurrence markers would be of value.

- **Array Construction**

Hematoxylin and eosin (H&E) tissue sections of formalin-fixed paraffin-embedded tissue blocks were used as a guide to select the regions for sampling. Tissue microarray (TMA) was assembled manually by personal trial. First, a hole in the recipient TMA block was made, then a cylindrical 1.0 mm core sample from the donor tissue block was obtained and deposited onto TMA block at a distance of 1mm between each core. Four cores were punched from each donor block to minimize the number of cases which cannot be evaluated due to tissue loss.

- **Immunohistochemistry**

Immunohistochemical staining was performed on 4 μm thick, formalin fixed, paraffin embedded tissue sections that were mounted on coated slides.

DAKO kit (Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse, Produktionsvej 42, DK-2600, Glostrup, Denemark) was used. The horseradish peroxidase and diaminobenzedene hydrochloride (DAB) are the enzyme and chromogen employed.

Briefly, the sections were deparaffinized, followed by incubation in xylene and hydration in a series of decreasing concentration of ethanol. After that, heat-induced epitope retrieval was done using pressure cooker and EDTA buffer (PH 9). The sections were washed in PBS buffer, and immersed in peroxidase-blocking solution of DAKO to inhibit endogenous peroxidase activity. The slides were incubated with primary antibodies, monoclonal mouse antihuman progesterone receptor (Clone

PgR636, IR06861 (12 ml), ready to use (Link), Dako, North America, Inc.), monoclonal rabbit antihuman Ki67 (Clone SP6, RM-9106-R7 (7.0 ml), ready to use, Neomarkers, Fremont, CA, USA), and monoclonal rabbit antihuman cyclin D1 (Clone SP4, RM-9104-R7 (7.0ml), ready to use, Neomarkers, Fremont, CA, USA). The immune reaction was detected by incubating for 30 min at room temperature with labelled polymer (Labelled Polymer-HRP), from DAKO kit. The peroxidase activity was detected with diaminobenzidine as a chromogen, and the sections were then counterstained with hematoxylin, dehydrate, coversliped, and mounted with DPX mounting media.

• Immunohistochemical analysis

1. Cyclin D1

A distinct brown nuclear stain was considered as positive. The percentage of immunostained cells was determined. The percentages of cyclin D1 immunopositive tumor cells were counted in 5 microscopic fields per tumor sample in area that showed the highest density of these cells. In each field, 100 tumor cell nuclei were evaluated and the mean for every 5 fields were calculated manually. A cutoff value of <5% immunopositive cells was considered negative, and ≥5% immunopositive cells was considered positive. The positive samples were scored according to the frequency of cyclin D1 immunopositive cells as 5% - 25%, 26% - 50%, 51% - 75%, and >75%. Samples from patients with <50% cyclin D1-positive tumor cells were considered low expressors, whereas those with >50% cyclin D1-positive tumor cells were considered high expressors [19,20].

2. Progesterone receptors

The slides were examined for positively stained tumour cell nuclei. The receptor status was determined by a semiquantitative scoring scale with respect to staining intensity (graded as: 0, absent; 1, weak; 2, moderate; and 3, strong) and percentage of positive tumour cells (0, indicating the absence of positive nuclei; 1, the presence of a few positive tumour nuclei <10%; 2, an estimated 10–50% positive nuclei; 3, 51-80% positive tumour nuclei; and 4, >80% positive tumour nuclei). As

recommended for breast cancer, and verified with meningioma tissue, an immunoreactive score (IRS) was calculated for each tumour by multiplying the staining intensity by the indicator for positive tumour cells, producing an IRS range from 0 to 12. Tumours with an IRS of 2 or more were considered as receptor positive [14].

3. Ki-67/MIB-1 proliferation index

The Ki-67/MIB-1 Labeling Index (LI) can be calculated as a percentage of nuclear positive cells by two methods: (i) Highest Labeling Index (HLI): 1000 tumor cells located in the area of highest MIB-1 labeling were counted and the percentage of positive-staining tumor cell nuclei is evaluated; and (ii) Random Labeling Index (RLI): 2000 tumor cells in randomly selected fields were counted [21]. In this study, we used the Highest Labeling Index (HLI).

- **Statistical analysis**

Statistical analysis of data was performed by chi-squared test and Student's t-Test. A P value less than 0.05 was considered to be significant. Mean (average) value and standard deviation (SD) were calculated.

Results

The mean age was 47.58 years (range, 6-80; SD, 13.92). Females (73.9%) were affected more than males (26.1%) with F/M ratio: 2.8/1.

The majority of cases (87.6%) were WHO grade I. Grade II meningiomas account for 10.4% and grade III tumours represent 2%.

Most of studied cases (90.2%) were located intra-cranially, 9.15% were spinal, and only one case (0.65%) was ectopic (in the middle ear). The spinal to intracranial meningioma ratio is approximately 1:9.9. Parietal region was the commonest intra-cranial site affected (13.07%) followed by frontal one (12.43%).

Transitional and meningothelial meningiomas were the commonest subtypes. About 40.52% of the cases were transitional while 27.45% were meningothelial.

About 13% of studied cases re-

curred. The recurrence rate was 7.5%, 56.25%, 33.3% for WHO grade I, grade II, and grade III tumours respectively (table 1).

There was significantly lower PR expression in WHO grade II (37.5%) and III (0%) meningiomas compared with WHO grade I (58.9%) meningiomas (P= 0.04). Statistically, there was no significant association between PR status and tumour recurrence (P = 0.59) (table 2).

Cyclin D1 reactivity was significantly elevated in meningioma grades II (18.7% are high expressors) and III (66.7% are high expressors) in comparison with grade I (1.7% are high expressors) (p<0.001). The association between cyclin D1 status and recurrence is not statistically significant (Chi-2 p = 0.20) (table 3).

Mean ki67 LI were 3.58±4.04% (range, 0-16), 14.75±7.42 (range, 1-25), 26.67±12.58 (range, 15-40) for grade I, grade II, and grade III meningiomas respectively. Differences between three grades was statistically significant (P<0.001). Non-recurrent cases had a mean KI67 LI of 3.47±4.21% (range, 0-25), while that of recurrent cases was 16.75±7.61% (range, 6-40). The difference was significant (P <0.001) (table 4).

Table 1. Relationship between tumour recurrence and WHO grade

	Recurrence				**P value**
	Yes		**No**		
	No.	%	No.	%	
WHO grade					
Grade I (134)	10	7.5%	124	92.5%	
Grade II (16)	9	56.25%	7	43.75%	<0.001
Grade III (3)	1	33.3%	2	66.7%	

Table 2. Relationship between PR, gender, age, WHO grade, and tumour recurrence.

	PR expression				P value
	Negative		Positive		
	No.	%	No.	%	
Gender					0.02
Females (113)	44	38.9%	69	61.1%	
Males (40)	24	60%	16	40%	
Age, mean ± SD (range), y	47.29 ± 15.26 (6–72)		47.81 ± 12.84 (18–80)		<0.001
WHO grade					
Grade I (134)	55	41.1%	79	58.9%	0.04
Grade II (16)	10	62.5%	6	37.5%	
Grade III (3)	3	100%	0	0%	
Recurrence					
Yes (20)	10	50%	10	50%	0.59
No (133)	58	43.6%	75	56.4%	

Table 3. Relationship between Cyclin D1, gender, age, WHO grade, and tumour recurrence.

	Cyclin D1 expression						P value
	Negative		<50% (low expressors)		>50% (high expressors		
	No.	%	No.	%	No.	%	
Gender							0.35
Females (113)	50	44.2%	60	53.1%	3	2.7%	
Males (40)	15	37.5%	22	55%	3	7.5%	
Age, mean ± SD (range), y	46.68 ± 15.57 (6–75)		48.09 ± 12.77 (15–80)		50.5 ± 11.11 (32–64)		<0.001
WHO grade							
Grade I (134)	60	44.8%	73	54.5%	1	1.7%	<0.001
Grade II (16)	4	25%	9	56.3	3	18.7%	
Grade III (3)	1	33.3%	0	0%	2	66.7%	
Recurrence							0.20
Yes (20)	6	30%	12	60%	2	10%	
No (133)	59	44.4%	70	52.6%	4	3%	

Table 4. Relationship between KI67 LI, gender, WHO grade, and tumour recurrence

	KI67/MIB-1 LI		P value
	Range	Mean + SD	
Gender			<0.001
Females (113)	0-40	4.51 ± 6	
Males (40)	0-25	7.15 ± 7.61	
WHO grade			<0.001
Grade I (134)	0-16	3.58 ± 4.04	
Grade II (16)	1-25	14.75 ± 7.42	
Grade III (3)	15-40	26.67 ± 12.58	
Recurrence			<0.001
Yes (20)	6-40	16.75 ± 7.61	
No (133)	0-25	3.47 ± 4.21	

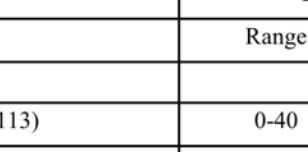

Fig. (1): Transitional meningioma (H&E x 40), TMA.

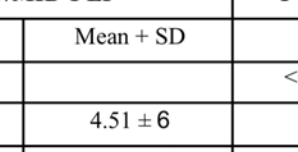

Fig. (2): Positive PR (H&E x 40), TMA.

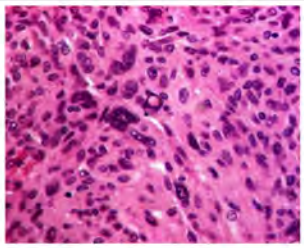

Fig. (3): Pleomorphism, nuclear pseudoinclusion (H&E x 100).

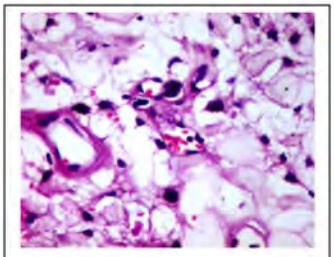

Fig. (4): Microcystic meningioma (H&E x 400).

Discussion

Meningiomas are usually benign, slowly growing localized tumors. However, they can cause significant morbidity and, in rare cases, mortality. Even benign meningiomas have morbidity and mortality risks in addition to gaining anaplastic characteristics [22].

The mean age of our cases was 47.58 years (range, 6-80; SD, 13.92) and this is slightly different from that reported by Shayanfar et al. The mean age of their studied cases was 54 years (range, 22-82 years) [11].

Our data showed that females were affected more than males (F/M ratio: 2.8/1). The results reported by Akyildiz et al. (female:male ratio of approximately 2.3:1) [12] and by Uzüm and Ataoglu (female:male ratio of approximately 2.2:1)[22] are nearly consistent with our finding.

In the literature, intracranial meningiomas account for 91% - 94.5% of all meningiomas[9,12]. Spinal meningiomas account for approximately 12-15% of all meningiomas [23]. The spinal to intracranial meningioma ratio is ranging in the literature from 1:2.5 to 1:16 [24]. Primary extracranial meningiomas involving the ear and temporal bone comprise <1% of all meningiomas [25]. It is noticed from our study that (90.2%) are located intra-cranially, (9.15%) are spinal, and only one case (0.65%) is ectopic (in the middle ear). The spinal to intracranial meningioma ratio, according to our study, is approximately 1:9.9. Our data differs little from those reported.

Meningothelial, fibrous, and transitional meningiomas are the most common grade I meningiomas [26]. According to Uzüm and Ataoglu study, meningothelial meningiomas was the commonest subtype [22], however, transitional meningioma was the commonest subtype in our study.

Ninety percent of meningiomas are benign [4]. Atypical meningiomas account for 4.7%-7.2% of all meningiomas [5], while malignant meningiomas account for 1-3% [6-7]. There is minor difference between our results (87.6% of our cases were WHO grade I,

10.4% WHO grade II, and 2% WHO grade III) and those mentioned in the literature.

It has been reported that WHO grade II and III tumors had significantly lower PR values than benign meningioma (grade I) [11,14,27]. Our data confirmed the presence of significantly higher PR values in benign meningiomas compared with WHO grade II or III tumours.

It has been observed that the deregulation and alteration of genes involved in the control of G1/S phase transition of the cell cycle play an important role in malignant transformation of several neoplasms. In particular, cyclin D1 amplification/overexpression occurs in a variety of tumor systems contributing to the early stages of carcinogenesis. Cyclin D1 is frequently reported to be overexpressed in higher grade tumors. Significant correlation of cyclin D1 overexpression in higher malignancy of tumors has been viewed in breast cancer, colorectal cancer, parathyroid adenoma, prostate cancer, melanoma and lymphoma [28].

Alama et al. suggested that all grade II/III meningiomas showed increased expression of cyclin D1. However, this association appeared of borderline statistical significance ($p = 0.07$) [29]. Our results confirmed the reported data, however, our data was statisticaly significant ($p < 0.001$).

Ki-67/MIB-1 appears to be the best and is widely accepted as a biomarker for meningioma prognosis [30]. Due to limitations of routine histological examination of meningioma tissue in predicting tumor behavior, Ki67/MIB1 immunostaining has been introduced for its potential to improve the information given by the grading system [17]. As a general rule, the Ki-67 proliferation index correlates well with WHO tumor grade for meningioma. However, considerable variation in Ki 67 immunolabelling of meningiomas has been reported. This is due to differences in interpretation methodology among individual laboratories. Therefore, there is no standard cut-off level for the Ki-67 proliferation index. In addition, there are no conclusive

results to show that Ki-67 is an independent prognostic marker for meningioma [9,12,30,31,32,33].

Our results showed that Ki-67 labeling index was significantly highest in meningiomas of WHO grade III and lowest in WHO grade I tumors. This result matched with those mentioned in literature. However, the values of Ki-67 labeling indices of our result (3.58 ± 4.04 % among grade I tumors, 14.75 ± 7.42 in grade II and 26.67 ± 12.58 in grade III meningiomas) were different from those mentioned in literature. For example, the values reported by Roser et al.[9] were (3.54% ± 4.97). in WHO grade I tumours, 11.9 ± 8.25) in WHO grade II and, 18.2 ± 9.53) in WHO grade III) and those reported by Akyildiz et al. [12] were (mean Ki67 LI values for grade I, II, and III were 2.78%, 7.23%, and 23.78% respectively). The difference in these values may be due to variation in methodology used.

Concerning relapse, 17 (14.2%) patients reported by Metellus et al. showed recurrence [13]. Data mentioned in our results, 20 (13%) patients showed recurrence, are nearly similar.

Expression of progesterone receptors (RP) had an inverse correlation with recurrence [13], however other studies showed no significant correlation between the PR status and recurrence [14-16]. Our results confirmed the absence of significant association between the PR status and recurrence.

Between 7 and 32% of benign meningiomas recur after total resection [14]. Grade II meningiomas exhibit a higher risk of recurrence (29% to 40%). Recurrence rate of anaplastic meningiomas is reported to be 50% to 78% [10]. However, in our study, the recurrence rate for WHO grade I meningiomas is 7.5%, for WHO grade II 56.25% and for WHO grade III 33.3%. The difference between our results and those mentioned in literature may be due to few cases in our study.

On the basis of the available data and from the literature, it is conceivable that the subset of meningioma patients with increased cyclin D1 expression might be at higher risk of recurrence [29]. In

the present study, 10% of recurrent cases were high cyclin D1 expressors while, only 3% of non-recurrent cases were high cyclin D1 expressors. This agree with the data reported, however, the association between cyclin D1 status and recurrence, in the current study, is not significant (P = 0.20).

Most studies reported higher KI67/MIB-1 labeling indices in recurrent meningiomas than in non-recurrent [16,17]. Our results are consistent with those mentioned in literature.

Hsu and colleagues suggested that only a combination of three factors, WHO grade, proliferative index, and PR status, should be used to predict meningioma recurrence [18]. However, in our study, we found that only WHO grade and proliferative index (KI67 LI) are statistically significant to predict recurrence.

Conclusion

Relapse was not significantly correlated with PR or cyclin D1 status, however, the Ki67 LI clearly demonstrate a statistically significant different mean KI67 LI. between recurrent and non-recurrent cases. Since the mean KI67 L.I. in recurrent cases is higher than in non-recurrent, strict follow-up after resection in those cases is recommended.

However, no clear-cut value of KI67 LI to distinguish meningiomas that have tendency for recurrence from those without recurrence tendency. So, we advise various laboratories to apply the same technique to reach a clear cut-off value and make it universal. We also recommend usage of other biological markers in researches to find a standard marker for recurrence.

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IMMUNOHISTOCHEMICAL EXPRESSION OF CYCLIN D1, KI67/MIB, AND PROGESTERONE RECEPTORS IN MENINGIOMAS

Abdelhadi M. Shebl M.Sc, Dina A. El-Tantawy MD, Maha M. Ameen MD, Wageha M. Kandil Ph.D and Nadia E. Basiony Ph.D

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DIFFERENTIATION OF HUMAN UMBILICAL CORD BLOOD MESENCHYMAL STEM CELLS INTO PANCREATIC ISLETS LIKE CELLS : IN VITRO STUDY

Adel A. Elhawary MD, Fathy A. El-Ghany MD, Mohamed Abdo MD. and Mohamed H. Elsherbiny M.Sc

Department of

Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Abstract

Background: *Diabetes mellitus (DM) is a chronic disease with great social and economic impact. Transplantation of pancreatic islet cells and stem cells differentiation into insulin producing cells have become the subject of interest over the past two decades.*

Material and Methods: *Human umbilical cord blood-derived mesenchymal stem cells (HUCB-MSCs) were isolated, characterized and induced to differentiate into islet-like cell clusters using a combination of high-glucose, fetal bovine serum (FBS), pencillin, and exendin-4.*

Results: *Islets like cell clusters appeared about 21days after mesenchymal stem cells (MSCs) differentiation; expressed cytoplasmic insulin positive cells and could synthesize and secrete functional islet proteins at the end of the experiment (21 days). Flowcytometric analysis of insulin positive cells, showed that (1.1%) of undifferentiated cells were positive compared to 40% of differentiated cells. The mean content of total cellular insulin in undifferentiated cells was (0.75 ng/mg protein) while that of the differentiated cells was (14.28 ng/mg protein).*

Conclusion: *UCB-MSCs have the ability to differentiate into islet-like cells in vitro and may be a potential new source for islet transplantation for treatment of diabetes mellitus.*

Introduction

Diabetes mellitus (DM) is a chronic disease with great social and economic impact. It affects nearly 285 million people worldwide [7].

In spite of recent advances in diabetes care, chronic hyperglycemia persists despite all the available means of insulin therapy, causing serious long-term complications in most patients. Pancreas or islet transplantation are the only methods available to date which are able to establish long-term normoglycemia or near-normoglycemia [13].

Transplantation of pancreatic islet cells as a potential cure for DM has become the subject of interest over the past two decades. However, islet transplantation has been hampered by immune rejection and recurrent attacks against islets by the underlying autoimmunity [10]. These factors motivate efforts to develop renewable sources of islet-replacement tissue.

The development of a simple, reliable procedure to obtain autologous stem cells having the ability of self-renewal and differentiation into insulin-producing cells, either in vivo or in vitro, would provide a potentially unlimited source of islet cells for transplantation and alleviate the major limitations of availability and allogeneic rejection. Therefore, the utilization of stem cells is becoming the most promising therapy for diabetes mellitus [9].

Stem cells are capable of self renewal and giving rise to more committed progenitor cells. There are embryonic stem cells and adult stem cells. Embryonic stem cells have ethical problems that impede their application into the clinic. Postnatal stem cells offer fewer concerns in terms of moral issues [12].

As an adult stem cell, recent studies have shown that mesenchymal stem cells (MSCs) have the ability to differentiate into several neuroectodermal, endothelial, mesenchymal, and endodermal cell types, and the ability to differentiate into insulin-expressing cells has also been demonstrated. The multipotential of these cells as well as their high ex vivo expansive potential makes these cells an attractive therapeutic tool for diabetes [14].

But until now, a key question has remained: Do human umbili-

cal cord blood derived mesenchymal stem cells (UCB-MSCs) have the plasticity to differentiate into insulin-producing cells in vitro? Therefore, this study aimed to evaluate the hypothesis of differentiation capacity of (UCB-MSCs) into islets like cells in order to provide support for continuing efforts aimed at using adult stem cells as a steady and renewable source of insulin-producing cells for transplantation in patients with DM.

Material and Methods

Collection of the umbilical cord blood (UCB) :

Umbilical cord blood cells from full-term deliveries were obtained from Obstetric and Gynecology departments, Mansoura and Cairo Universities. The source of each collection was identified by name. Informed written consents were taken from the parents. Blood was obtained from the removed umbilical cord by needle aspiration of exposed, engorged vessels on it. In all samples, collection was made into a sterile syringe containing 2 ml heparin and transferred immediately to be separated and cultured.

Separation and culture of MSCs from UCB:

To isolate mononuclear cells (MNCs), each UCB unit was diluted 1:1 with phosphate buffered saline (PBS) and carefully loaded onto Ficoll Hypaque solution (1.077 g/mL; Sigma-Aldrich Co, St. Louis, Mo). After density gradient centrifugation at 1800rpm for 16 min at room temperature, MNCs were removed from the interphase and washed twice with PBS and resuspended in low glucose Dulbecco Modified Eagle Medium (L-DMEM, 5.5 mmol/L glucose; Invitrogen Corporation, Grand Island, NY) supplemented with 30% fetal bovine serum (Invitrogen). After counting, cell suspension was seeded in uncoated T25 culture flasks (Orange Scientific, Belgium) at a concentration of $1x10^6$ cells/ml. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO^2, and the medium was changed 8 days later. When fibroblast-like cells at the base of the flask reached confluence, they were harvested with 0.25% trypsin-ethylenediamine tetraacetic acid (EDTA; SigmaAldrich) and passaged at 1:3 dilution as pas-

sage 1. Then the medium was half-changed with L-DMEM containing 20% fetal bovine serum (FBS) every other day to make 30% FBS concentration decrease to 20%.

In vitro differentiation cultures:

The cells (UCB-MSCs with 80% confluence) were cultured (37°C, 5% CO2) for one week in a basic medium composed of 4 mml high glucose (25 mmol/L) Dulbecco's modified Eagle's medium (HG-DMEM); 200 µ fetal bovine serum (Biochrom AG, Germany); 10 nmol/L exendin-4 (Sigma, Aldrich); 100 µ amphotrecin B (Biochrom AG, Germany); 200µ penicillin and streptomycin (Invitrogen).

In vitro assessment of differentiated cultures:

• Test for viability of cells by using Trypan blue exclusion staining teqnique.

• Analysis of insulin expression by flowcytometry:

Differentiated UCB-MSCs were tested for differentiation by quantitating cells expressing cytoplasmic insulin. This can be done through flow cytometric analysis using Anti-human/bovine/mouse Insulin-Allophycocyanin (APC) Monoclonal Antibody purchased from R&D Systems, Inc. Measurement of positive insulin rate was done by FACSCalibur (PowerMacintosh, 7600/132, USA). Isotype-matched antibodies served as controls for autofluorescence.

• **Insulin detection assay:**

To estimate total insulin levels, the total cell protein content was tested using the BCA Protein Assay Kit (Beyotime). Measurement of secreted insulin was performed with chemiluminescence immunoassay system ADVIA centaur (Bayer, Tarrytown, NY). Undifferentiated MSCs were used as a control group.

Results

I. Assessment of cell viability:

After collection of the UCB, the mononuclear cells were isolated by using Ficoll, the viability of the cells was confirmed by trypan blue exclusion. The live cells were not stained and the dead cells were stained blue (Figure 1).

II- Morphological changes of expanded undifferentiated UCB-MSCs

Examination of the primary culture of UCB-MSCs (day one) by inverted microscope revealed the presence of rounded overcrowded cells. These cells were variable in size and shape (Figure 2).

The onset of fibroblast-like cell colony formation could be observed approximately during 7 to 14 days after first seeding (Figures 3, 4&5).

II- Morphological changes of differentiated UCB-MSCs

By day 14, induction of in vitro UCB-MSCs into insulin producing cells was done by culturing the cells into high glucose media supplemented with exendin-4. Clusters of islet like cells were observed by inverted miscroscpe on day 21(Figures 6, 7&8).

II- Phenotype characteristics of the undifferentiated UCB-MSCs

Flow cytometric analysis of insulin positive cells showed that (1.1%) of undifferentiated cells were positive (Figure 9) compared to 40% of differentiated cells (Figure 10).

The mean content of total insulin in undifferentiated cells showed no significant release of insulin in the presence or absence of glucose challenge (0.75 ng/mg protein), while after differentiation, the differentiated cells showed significant increase in insulin cellular content (14.28 ng/mg protein) ($P < 0.05$) (Figure 11).

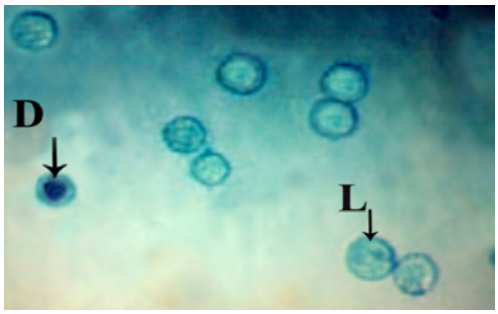

Fig. (1): A photomicrograph of mononuclear cells after seperation. L, live cells (non-stained); D, dead cells (stained). (Trypan blue x 1000).

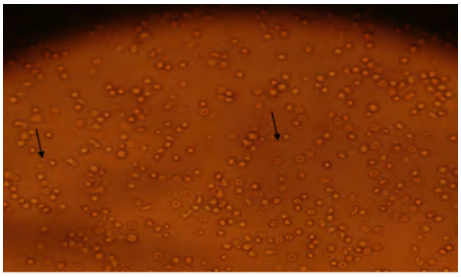

Fig. (2) : Inverted microscopic picture of a primary culture of UCB-MSCs on day one of isolation and culture, showing cultured cells crowded and variable in size and shape. Most of the cultured cells appear rounded (arrows) (X200).

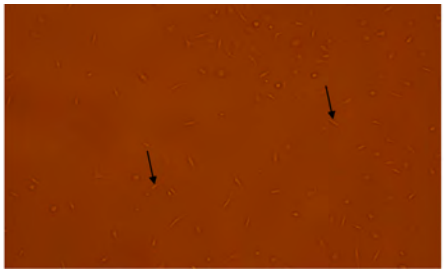

Fig. (3) : Inverted microscopic picture of a primary culture of UCB-MSCs on day seven of isolation and culture and after removal of the supernatant and changing the media showing adherent cells with long processes (arrows) (X100).

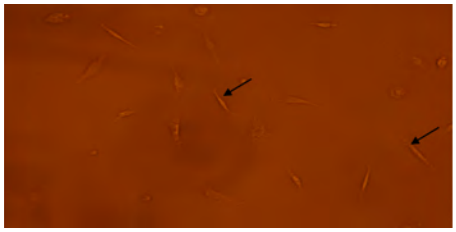

Fig. (4) : Inverted microscopic picture of a primary culture of UCB-MSCs on day seven of isolation and culture and after removal of the supernatant and changing the media showing adherent cells with long processes (arrows) (X200).

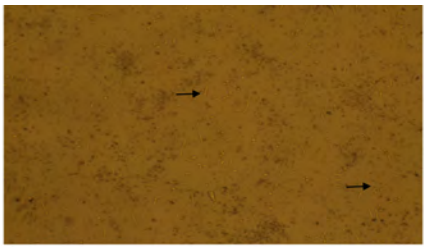

Fig. (5) : Inverted microscopic picture of a primary culture of UCB-MSCs on day 14 reaching 80% confluent and after removal of the supernatant and changing the media showing the appearance of confluent adherent cells consisting of dense population of spindle-shaped fibroblast-like cells with long processes (arrows) (X100).

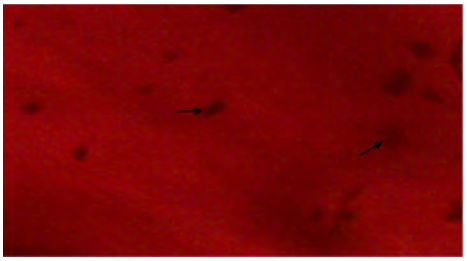

Fig. (6) : Inverted microscopic picture of the differentiated UCB derived mesenchymal stem cells showing islet like clusters (arrows) (X100).

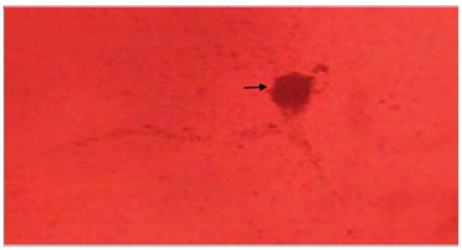

Fig. (7) : Inverted microscopic picture of the differentiated UCB derived mesenchymal stem cells showing islet like clusters (arrow) (X200).

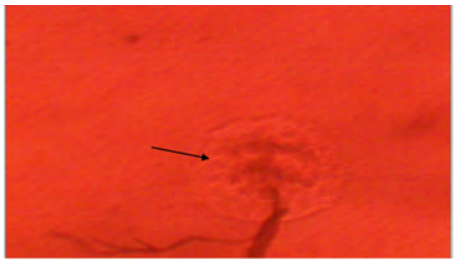

Fig. (8) : Inverted microscopic picture of the differentiated UCB derived mesenchymal stem cells showing islet like clusters (arrow) (X400).

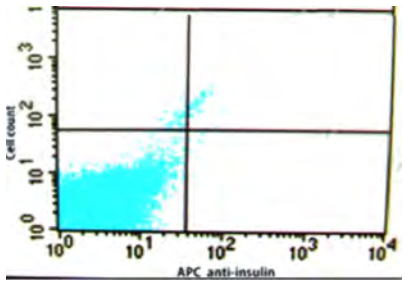

Fig. (9) : Flow cytometric analysis of undifferentiated UCB-MSCs showed that insulin positive rate was 1.1% as shown in right upper and lower quadrants.

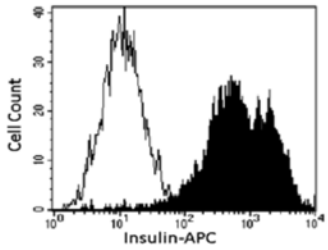

Fig. (10) : Flow cytometric analysis of differentiated UCB-MSCs showed that insulin positive rate was 40%.

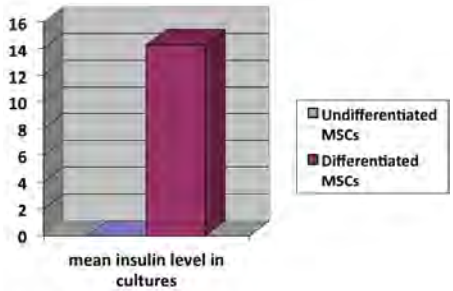

Fig. (11) : Mean insulin content in undifferentiated and differentiated cells.

Discussion

Recent studies have demonstrated the feasibility of generating insulin-producing cells obtained from progenitor cells of various cellular sources (OH et al., 2004). Despite their promising potential, it also proved difficult to obtain enough autologous adult stem cells from these organs. Some obstacles, such as the immune rejection and autoimmunity against newly formed β-cells derived from pancreatic stem cells, still remain. To overcome these limitations, we explored the possibility of using HUCBMSCs as a source for cellular differentiation into insulin-producing cell clusters under de novo culture conditions. HUCBMSCs are easily obtained compared with embryonic and other stem cells.

In the present work, high glucose DMEM media were used. Glucose is a growth factor for β-cells. It promotes β-cell replication in vitro and in vivo at a 20-30 mmol/L concentration. It induces in vitro differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells at a 23 mmol/L concentration and increases insulin content in cell lines derived from embryonic stem cells at a 5 mmol/L concentration. Moreover, glucose could have a dual role. In the proliferation phase, the high glucose content may support the extra-energy needed for cell division. In the differentiation stage, it could modulate specific gene programs linked to glucose sensing and insulin secretion [4&6].

In the present study, exendin-4 was used in culture in a dose 10 nmol/L. Exendin-4 is a glucagon-like peptide-1 (GLP-1) receptor agonist which stimulates both β-cell replication and neogenesis from ductal progenitor cells, and decrease β-cell apoptosis [5&7].

According to Jahr and Bretzel, (2003)[8], Oh et al., (2004)[11], Chen et al., (2004)[3], Tang et al., (2004)[13] and Doyle and Egan, (2007)[5] high concentrations of fetal bovine serum lead to occurrence of intense cell propagation and formation of numerous cell clusters within 4-5 days in a serum-rich medium (200 μ FBS).

These cell clusters were 100 to 200 µm in diameter and similar to that of isolated pancreatic islets. However, homogenates of these cell clusters were negative for insulin. This is in contrast to 10 µ FBS containing media in which clusters were not formed and 90% of the cells died within 5 days.

In the present study, the onset of fibroblast like cell formation could be observed approximately during 7 to 14 days. A similar finding was declared by Gao et al., (2008)[7] & Zhang et al., (2009)[15]. However, Chang et al., (2007)[1] observed that placenta derived MSCs usually appeared as spindle-shaped cells within 7 days.

Flowcytometric analysis of insulin positive cells of the present work revealed that 1.1% of undifferentiated cells and 40% of differentiated cells were insulin positive. In the contrary of, Gao et al., (2008)[7] who noticed that 1.11% of preinduced cells were insulin-positive, whereas 25.2 % of differentiated cell were insulin-positive.

Chao et al., (2008)[2] estimated the mean content of total cellular insulin in both undifferentiated (0.14 ng/mg protein) and differentiated cells (12.28 ng/mg protein). In the present work, the mean content of total cellular insulin in undifferentiated was (0.75 ng/mg protein) and in differentiated cells was (14.28 ng/mg protein).

In conclusion, this study shows that human UCB-MSCs are capable of differentiating into insulin-secreting cells in vitro by using high glucose media and exendin-4. Obviously, more work is needed to increase efficiency of the stem cell cultures and to evaluate the therapeutic potential of UCB-MSCs in management of DM.

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REPRINT

BENHA MEDICAL JOURNAL

DIFFERENTIATION OF HUMAN UMBILICAL CORD BLOOD MESENCHYMAL STEM CELLS INTO PANCREATIC ISLETS LIKE CELLS : IN VITRO STUDY

Adel A. Elhawary MD, Fathy A. El-Ghany MD, Mohamed Abdo MD. and Mohamed H. Elsherbiny M.Sc

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CHANGES IN DESTRIBUTION OF THE NEURAL STEM CELLS IN THE HIPPOCOMPUS OF THE RAT AT DIFFERENT POSTNATAL AGES.

Fathy A. Ibrahim MD, Mohammed I. Abdou MD, Raouf F. Bedeer MD, Hamdino M. Attia MD*, Al Sayed A. Abd Elhady M.Sc.*

*Departments of Anatomy, Mansoura Faculty of Medicine and Al-Azhar Faculty of Medicine (Damietta)**

Abstract

Neural stem cells (NSCs) were self-renewing, multipotential progenitor cells. A single NSC can give rise to a wide variety of central nervous system (CNS) cells, including neurons, astrocytes, and oligodendrocytes. New neurons were continuously generated in the dentate gyrus of the mammalian hippocampus and in the sub ventricular zone of the lateral ventricles throughout life. The origin of these new neurons is believed to be from multipotent adult neural stem cells. There were extensive and prospective In Vitro studies in the field of NSCs, yet there were no or limited In vivo studies regarding the effect of aging on the distribution of NSCs in different brain regions. The objects of our study demonstrate that the density and distribution of neural stem cells in hippocampus of the rat at different postnatal ages. Rats were divided into 7 age groups as follow: Group1: at postnatal day 1(neonatal period); Group 2: at one week age (early childhood); Group 3: at one month age (late childhood); Group 4: at 3 month age (adolescence); Group5: at 6 month age (adulthood); Group 6: at one year age (adulthood); Group 7: at two years age (senility). Neural stem cells were detected by means of immuno-histochemical staining and then were subjected to morphological and morphometric studies to determine their density in hippocompus. Nestin, sox2 positive stem cells were assessed in hippocampus in all studied age groups. These positive stem cells differs from one age

group to another but present throughout life in all age groups, The present studies highlighted the fact that the hippocampus of rats contains neural stem cells that present from early life (neonatal day one) and gradually increase up to six months reach maximal number then gradually decrease at adult age.

Introduction

Neural stem cells (NSCs) were self-renewing, multipotential progenitor cells. A single NSC can give rise to a wide variety of central nervous system (CNS) cells, including neurons, astrocytes, and oligodendrocytes[25]. New neurons were continuously generated in the dentate gyrus of the mammalian hippocampus and in the sub ventricular zone of the lateral ventricles throughout life. The origin of these new neurons is believed to be from multipotent adult neural stem cells[40]. Adult (NSCs) have been isolated from various regions of adult mammalian brain, where the highest densities of (NSCs) have been found in the hippocampus, and the sub ventricular zone. It seems that adult (NSCs) have the ability to develop into functional mature neurons. These regions were of special interest as they reveal spontaneous neurogenesis throughout the entire lifetime, suggesting to play a functional role in physiological cell replacement in aging, learning and cognition, as well as proposing a therapeutic potential in neurological diseases, including neurodegenerative disorders like Alzheimer's and Parkinson's disease, cerebrovascuolar insults such as stroke, or developmental impairments[37]. Neurogenesis is known to decline during aging and, to the extent that neurogenesis is required for normal CNS function, this may contribute to neurodegenerative disease. Decreased neurogenesis could result from loss of NSCs or dysfunction at some later step, and distinguishing these possibilities is important for understanding the cause of the decline [2]. Although, there were extensive and prospective In Vitro studies in the field of NSCs, yet there were no or limited In vivo studies regarding the effect of aging on the distribution of NSCs in different brain regions[18]. And

hence study of the plasticity and normal distribution of NSCs and effect of aging on these processes were of extreme importance for proper and successful In Vitro studies and as it is still lacking in the literature, our study will be subjected to cover different aspects of proliferation, integrity, migration and distribution of NSCs in developing rats. Also we will follow these aspects within different age groups up to senility hoping at clarifying this neglected area in the field of NSCs research.

Materials and Methods

70 Albino rats were obtained from (animal house, Cairo faculty of medicine). Rats were housed in standard individual maternity cages under controlled temperature and humidity with free access to water and food. All rats were perfused transcardially with salin. Albino rats were divided into groups which were chosen representing different stages of postnatal development. Rats were divided into 7 age groups as follow: Group1: at postnatal day 1(neonatal period); Group 2: at one week age (early childhood); Group 3: at one month age (late childhood); Group 4: at 3 month age (adolescence); Group5: at 6 month age (adulthood); Group 6: at one year age (adulthood); Group 7: at two years age (senility). Neural stem cells were detected by means of immunohistochemical staining and then were subjected to morphological and morphometric studies to determine their density in hippocompus. Data were collected, and then analyzed statistically. The method of immunostaining applied in this study is the indirect immunohistochemistry using avidin-biotin complex (ABC) [39].

Primary Antibody (Nestin), (sox^2) : The antibody used as a marker for detection of neural stem cell was Polyclonal mouse anti nestin, (aa254-270) Antibody - LS-C40764 - LifeSpan, BioSciences). Mouse anti-nestin (optimal concentration was 1:100) and anti sox^2 (optimal concentration was 1:50.

Secondary Antibody: The anti-mouse (IgG) serum was diluted to 1:200 in 1% rabbit serum diluent. Incubation with rabbit anti-mouse (IgG) solution for 30 minutes at room temperature. The slides were

washed in PBS three times for 5 minutes each on the shaker.

ABC-preparation : ABC reagent was prepared according to the instructions of the manufacture in advance (30 minutes before use) by adding two drops of reagent A (Avidin) to 10 ml PBS in a mixing bottle. Then two drops of reagent B (biotin) were added to the solution. The tube was covered with aluminum foil and kept on ice. The specimens were incubated with the ABC solution in a well sealed humid chamber, covered with aluminum foil for one hour at room temperature.

DAB: Gloves/face mask and goggles were wearied when weighing DAB powder. 100 mg DAB tetra hydrochloride (Sigma) were added to 100 ml phosphate buffered (PB) in a beaker and stirred for 10 min. 2 mls of 0.4% ammonium chloride (400 mg/100 mls) were added. 2 mls of 20% D-Glucose (Sigma) (20g / 100 mls) were added. Stirring until DAB (pinkish solution) is dissolved. The solution was filtered through Whatman #1 paper. The solution was poured into a graduated cylinder and PB was added until getting the required 200 ml. The clear solution was ready for use. Washed sections were transfer into 20 mls DAB mix per vial, sections were placed on orbital mixer for 20 min once the cells started turning brown, washing twice in distilled water was done for 5 minutes each on the shaker [29] .

Dehydration: The slides were dipped in 70% and 95% alcohol for 3 minutes each. The slides were dipped in 2 changes of 100% alcohol for 3 minutes. The slides were dipped in 3 changes of xylene for 3 minutes [28].

Mounting: Mounting was done in a synthetic resin. Nitrile gloves were weared while handling xylene-containing materials. After dehydrating the sections in absolute alcohol and xylene, rack of slides and trough of xylene were transferred the to a fume cupboard. A glass syringe was removed from a trough of xylene, kept inside the fume cupboard using metal forceps and half-fill with DPX resin. Wetting the plunger with xylene and inserting it into the barrel of the syringe was done.

The appropriately sized cover slips were selected to fit the section and placed on a sheet of fiber-free post lip paper. A drop of DPX was applied to the cover slips. Using forceps, a slide from the rack was removed and surplus xylene was drained onto a paper tissue; the slide containing the section was gently lowered onto the drop of DPX so that the section is sandwiched between its slide and the covers slip. The slide was turned over and the DPX was allowed to spread between the section and covers lip. Using a paper tissue, surplus DPX was wiped off around the cover slip and left to dry. When the DPX was hardened sufficiently, a slide label was applied to the opposite end of the number and the correct number and name was entered on the slide/ specimen form. The labeled slides were placed in a slide tray with the matched specimen form [30].

Cover Slips: Paramount covers lip solution was drizzled onto covers lips or slides. Covers lip was applied to slide. The slides were let dry overnight.

The slides were studied using: A Zeiss Axioscope transmitted light microscope providing total magnifications of 50X, 100 X, 200X and 400X.

Morphometrical studies: Systematic random sampling was used for selection of morphometrically studied sections[33]. The brain sections (10-15 μm) at body, the brain segments were cut by rotatory microtome at interval of 1 ml thickness. Every 3rd section was taken. The density of the neural stem cells was evaluated in this section using computer based image analysis system. A Zeiss Axioscope transmitted light microscope was connected by means of a camera to a PC computer and, by using the Northern Eclipse version 6.0 morphometric analysis software (Empix Imaging Co., Mississauga, ON, Canada), point counting of the nestin, sox2 positive cells was conducted. The densities of nestin, sox2 positive cells the hippocampus, were determined under magnification (100X).

Statistical analysis: the collected data were organized, tabulated and statistically analyzed us-

ing Statistical Package for Social Sciences (SPSS) version 16, running on IBM compatible computer with Windows 7 operating system. For quantitative data, mean and standard deviation (SD) were calculated and for comparison between two means, the student (t) test was used. For comparison between more than two means, the one way analysis of variance (ANOVA) tests was used. For interpretation of results, the p value ≤0.05 was considered significant.

Results

Morphological and morphometric assessment of immunostained sections:

Nestin, sox^2 positive stem cells density could be detected in the hippocampus, in all studied age groups. Their density differs from one age group to another but present throughout life in all age groups, the density of positive cells increase with age until reach its maximal number, density in 3rd.6th month, then slow decline with further age, fig (1),table(1).

Neonatal (groups 1):

Nestin, sox^2 positive stem cells could be detected in the hippocampus, in all examined sections at these age groups. In hippocampus nestin positive stem cells present in all layer mainly granular layer of dentat gyrus and appeared mainly as small bipolar cells, stained positive cytoplasm (brown color), fig (2). Sox2 positive stem cells present in scanty aggregation in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei fig (3).

Early childhood periods (group 2):

There was gradual increase of nestin, sox^2 positive stem cells in the hippocampus, in all examined sections at this age group, compared by previous group. In hippocampus nestin positive cells present in all layer mainly granular layer of dentat gyrus, cells appear as elongated bipolar cells and others star shaped cells, stained positive cytoplasm (brown color) fig (4). Sox^2 positive stem cells gradual increase in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei fig (5).

Late childhood periods (group 3):

There was gradual increase of nestin, sox^2 positive stem cells in the hippocampus, in all examined sections at these age groups in comparison to previous groups. In hippocampus nestin positive cells in all layer and granular layer of dentat gyrus, cells appear in the form of bipolar cells, star to ramify, with small branching dendrite, and others star shaped, stained positive cytoplasm (brown color), fig (6). Sox^2 positive stem cells increase in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei fig (7).

Adolescence 3rd and 6th months ages (groups 4 and 5) :

The density, number of nestin, sox^2 positive stems cells reaches to maximum in the hippocampus, in all examined sections at these age groups in comparison to previous groups. In hippocampus nestin positive cells present in all layer and granular layer of dentat gyrus cells appear in the form of multipolar cells, with large branching dendrite, stained positive cytoplasm (brown color), fig (8&10). Sox^2 positive stem cells reach to maximal density in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei fig (9&11).

One year age (group 6):

There was decline of nestin, sox^2 positive stem cells in the hippocampus, in all examined sections at these age groups. In hippocampus nestin positive cells present mainly in granular layer of dentat gyrus, in the form of multipolar cells, with long process, as astrocyte, stained positive cytoplasm (brown color), fig (12). Sox^2 positive stem cells decline in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei fig (13).

Two years age (group 7):

There was more decline of nestin, sox^2 positive stem cells in the hippocampus, in all examined sections at these age groups. In hippocampus nestin positive cells present mainly in granular layer of dentat gyrus, in the form of star shaped, with thick process, stained positive cytoplasm (brown color) fig (14). Sox^2 positive stem

cells very scanty in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei fig (15).

Table (1) : Comparison between mean values of numerical densities of nestin / sox2 positive cells at hippocampus at different postnatal ages .

Variables	Groups							F*	P
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7		
Hippocampus									
Mean	347.1	514.5	592.2	606.4	686	249.9	164.6	47.59	0.000 HS
SD	92.3	108.8	89.3	101.9	90.2	91.5	52.7		

HS = highly significant SD = standard deviation *= ANOVA TEST

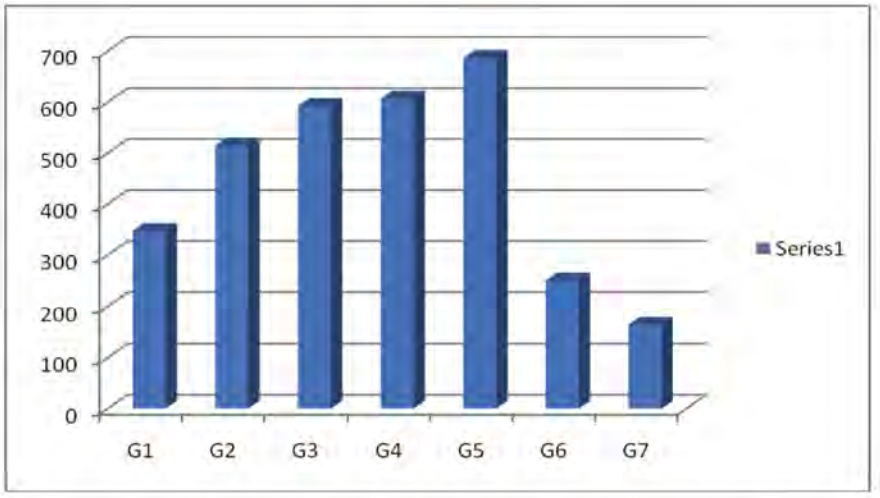

Figure (1) showing number of neural stem cells in hippocampus in all studied groups

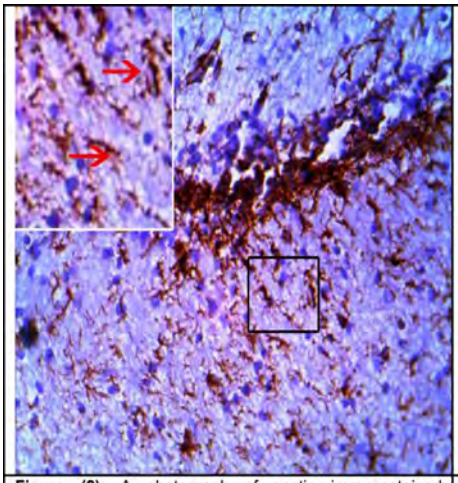

Figure (2): A photograph of nestin immunostained transverse section in the hippocampus of (1 day) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200).** Inset showing higher magnification. **(x400)**, demonstrated the bipolar nature of most of cells (arrow).

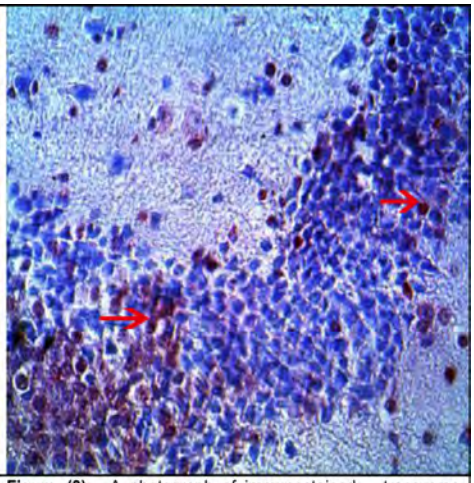

Figure (3): A photograph of immunostained transverse section in the hippocampus of (1 day) albino rat showing scanty aggregation of Sox^2 positive cells in hippocampus, (arrow). **(X400)**

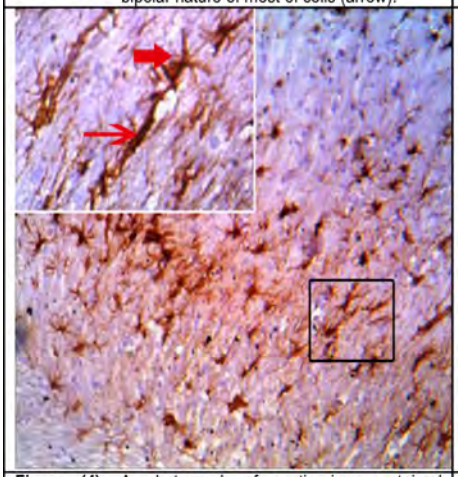

Figure (4): A photograph of nestin immunostained transverse section in the hippocampus of (1 w) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200).** Inset showing higher magnification. **(x400)**, demonstrated the bipolar cells apper with larger process(arrow), and few cells acquier multiple process (thick arrow).

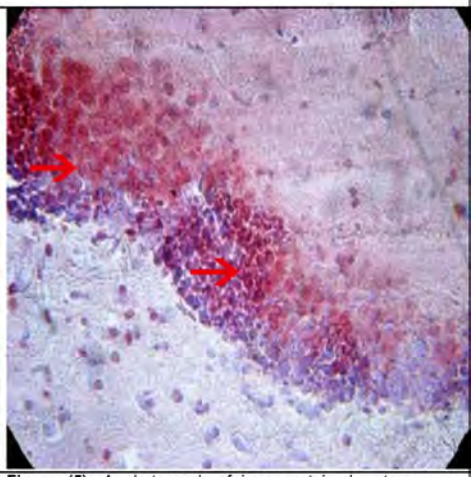

Figure (5): A photograph of immunostained transverse section in the hippocampus of (1 w) albino rat showing gradual increases aggregation of Sox^2 positive cells in hippocampus, in comparison to previous group, (arrow). **(X400).**

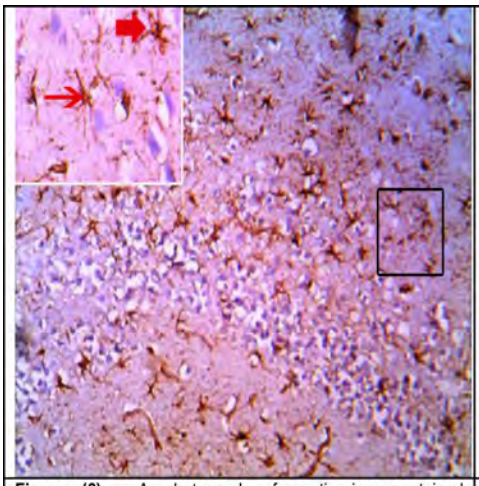

Figure (6): A photograph of nestin immunostained transverse section in the hippocampus of (1 m) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200).**Inset showing higher magnification.**(x400)** ,demonstrated the bipolar cells apper with larger process(arrow), and few cells acquier multiple process (thick arrow).

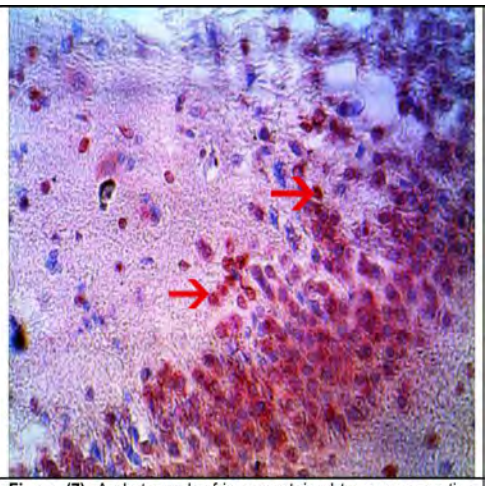

Figure (7): A photograph of immunostained transverse section in the hippocampus of (1 m) albino rat showing increase aggregation of Nestin positive cells in granular layer of dentat gyrus in comparison to previous group (arrow). **(X400).**

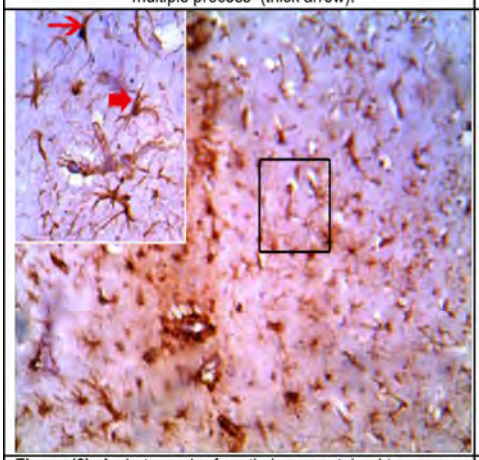

Figure (8): A photograph of nestin immunostained transverse section in the hippocampus of (3 m) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200).**Inset showing higher magnification.**(x400)** ,demonstrated the bipolar cells acquire process(arrow), and 0ther cells showing multiple process (thick arrow).

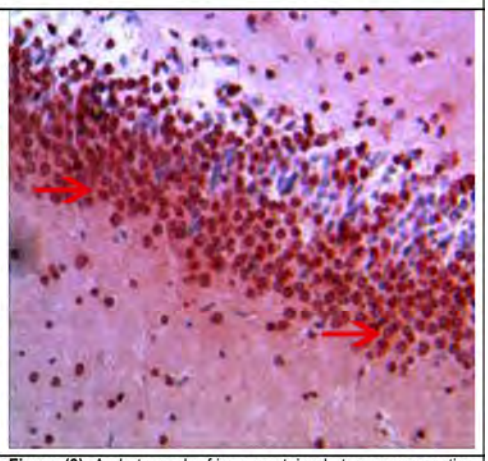

Figure (9): A photograph of immunostained transverse section in the hippocampus of (3 m) albino rat showing high density aggregation of Sox2 positive cells in hippocampus, in comparison to previous group (arrow). **(X400)**

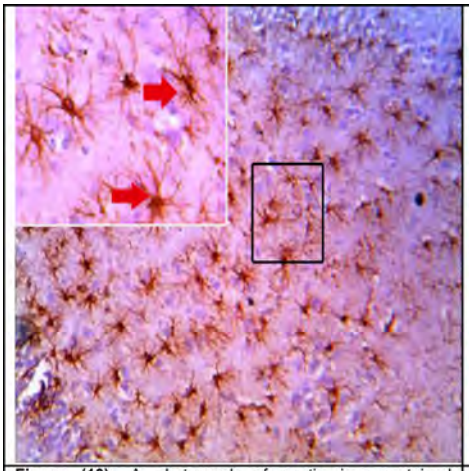

Figure (10): A photograph of nestin immunostained transverse section in the hippocampus of (6 m) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200).**Inset showing higher magnification. **(x400)** , demonstrated that the most of cells , multipolar.

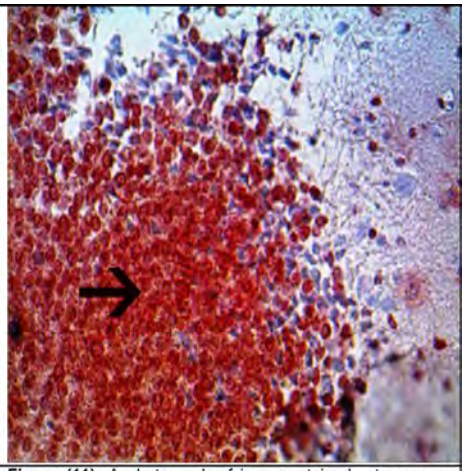

Figure (11): A photograph of immunostained transverse section in the hippocampus of (6 m) albino rat showing high density aggregation of Sox2 positive cells in hippocampus, in comparison to previous group (arrow). **(X400)**

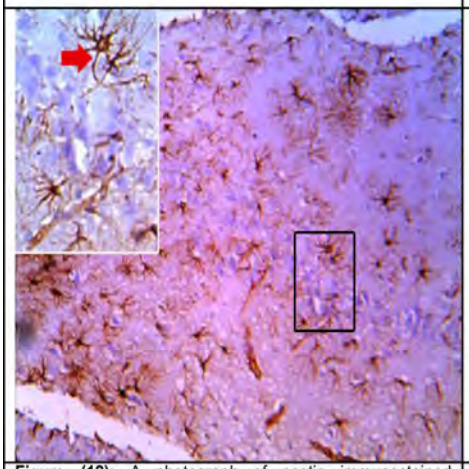

Figure (12): A photograph of nestin immunostained transverse section in the hippocampus of (1 y) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200).**Inset showing higher magnification.**(x400)** ,demonstrated that the most of cells ,multipolar with long thin processes.

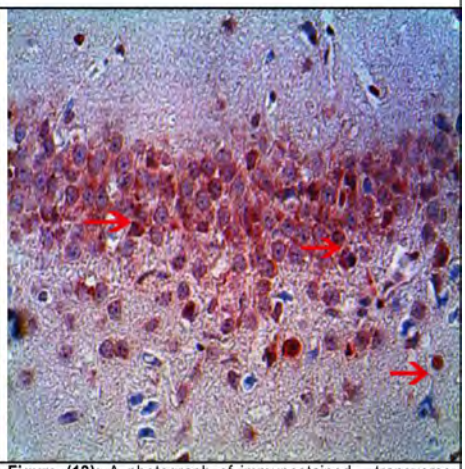

Figure (13): A photograph of immunostained transverse section in the hippocampus of (1 y) albino rat showing decreased aggregation of Sox2 positive cells in hippocampus, in comparison to previous group (arrow). **(X400)**

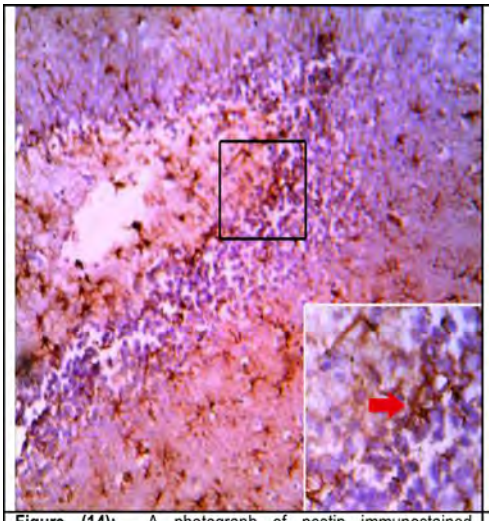

Figure (14): A photograph of nestin immunostained transverse section in the hippocampus of (2 y) albino rat showing aggregation of Nestin positive cells in granular layer of dentat gyrus, **(X200)**.Inset showing higher magnification.**(x400)** ,demonstrated that the most of cells ,multipolar with Ithick processes.

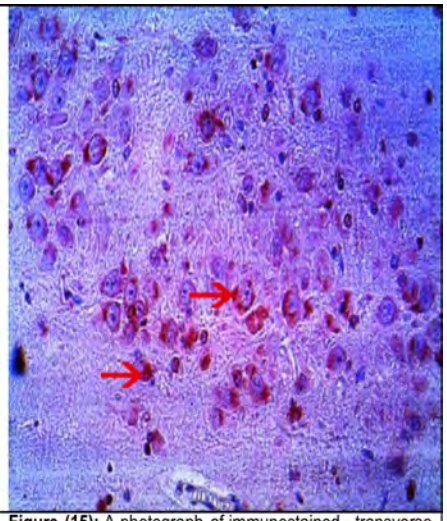

Figure (15): A photograph of immunostained transverse section in the hippocampus of (2 y) albino rat showing very scanty aggregation of Sox2 positive cells in hippocampus, in comparison to previous group (arrow). **(X400)**

Discussion

The present study showed that nestin, sox^2 positive stem cells density could be detected in hippocampus in all studied age groups. Their density differs from one age group to another but present throughout life in all age groups, the density of positive cells increases with ageing until reaching its maximal density in 3rd and 6th month, then shows slow decline with further aging.. In hippocampus nestin positive cells were detected in all layers mainly granular layer of dentat gyrus, cells appear as bipolar cells and star shaped cells, stained positive cytoplasm (brown color). Sox^2 positive stem cells were detected in all layer mainly granular layer of dentat gyrus and appeared as small red color nuclei

Our results were coinciding with (Temple)[35]. Who showed that in mammals, adult neurogenesis occurs at two principal sites, the sub ventricular zone (SVZ) of the ventricles, which generates olfactory bulb neurons, and the hippocampus. Both regions harbor

neural stem cells that can be cultured in vitro in the presence of growth factors such as epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).

Neurogenesis in 2-year-old mice declines to approximately half the level found in 2-month-old animals[17,36]. Our results coincide with this result.

To the extent that neurogenesis within the adult contributes to the maintenance of normal CNS function, the loss of functioning NSCs can be expected to lead to age-related neurodegeneration [7],[32]. This neurodegeneration was a potential contributing factor to the development of Parkinson's and Alzheimer's diseases, each of which has strong age dependence in its onset. Stimulation of more rapid NSC division could in part compensate for the reduced number of functioning cells and was a potential therapeutic approach. However, a potential side effect of this approach that will need to be addressed was the possibility that such therapies also lead to an increase in the rate at which NSCs were lost because of replication-related errors.

In the present study we showed that the morphology of nestin positive stem cells assumes many shapes in hippocampus as bipolar or star shaped cells. This result in agreement with (Seri,et al)[31]: who showed that the hippocampus was the other major site of neurogenesis in adult mammals. Granule neurons in the dentate gyrus were born locally in the sub granular zone (SGZ), which lies between the granule cell layer and the hillus. In contrast to the extensive tangential migration undertaken by olfactory bulb neurons, hippocampal granule neurons move only a short distance into the granule cell layer. The SGZ contains SGZ astrocyte (B cells) and ultra structurally dark GFAP-negative D cells. As in the adult SVZ, astrocytes act as the in vivo primary precursors in the SGZ.

(Doetsch and Scharff,2001)[12]. who showed that In sub ventricular zone in early age cells mainly bipolar, the identity of neural stem cells and the lineage of new

neurons have been most clearly defined in the SVZ of adult mice. The SVZ is a layer of dividing cells that extends along the lateral walls of the lateral ventricle. Neurons were born throughout the SVZ and feed into a network of chains of tangentially migrating neuroblasts ('blasts' indicates they were still dividing) that coalesce to form the rostral migratory stream leading to the olfactory bulb, where they differentiate into two kinds of inhibitory neurons: granule and periglomerular cells.

In the present study showed that the density of nestin,sox2 positive stem cells increases gradually then declines with old age in agreement with (Palmer et al., & Kronenberg. et al)[27,19]. Who found that active neurogenesis continues well into adulthood in the hippocampus of all mammals. Adult neurogenesis in the dentate gyrus of the primate hippocampus undergoes a substantial decline with advancing age. The numbers of newly generated cells were significantly lower in older animals and declined linearly with age.. Similar to the dentate gyrus, the number of BrdU-labeled cells in the SVZ was significantly less in older animals and showed a linear decrease with age. Although some studies have reported reduced neurogenesis in the SVZ of aged animals.

Molecular, structural and functional criteria-that stem cells derived from adult hippocampus, like those from developing brains, retain the potential to develop into functional CNS neurons when provided a permissive environment. The manipulation of CNS adult neural stem cells in vitro allows a precise analysis of mechanisms at various stages in functional neurogenesis, including proliferation, cell fate specification and finally neuronal maturation and synapse formation [34].

(Doetsch et al)[11] (Alvarez-Buylla et al) [4], (Gray and Sanes,)[15] (Noctor et al)[24], (Alvarez-Buylla et al) [5]. explained that Astrocytes were derived from radial glia during fetal and early postnatal development. Both SGL astrocytes and SVZ astrocytes may maintain their neurogenic potential because of their derivation from radial glia. In some verte-

brate species, radial glia persist in the adult brain, and, in songbirds, they divide and appear to function as the primary precursors for the continual generation of new neurons. Retroviral labeling experiments in the developing avian brain was consistent with the proposition that radial glia could function as neuronal precursors also during development. In mammals, they have been generally considered committed progenitors of astrocyte, but recent evidence in the developing mammalian brain indicates that radial glia can give rise to new neurons and may correspond to neural stem cells. Therefore, neural stem cells were probably contained within the lineage: "neuroepithelium-radial glia-astrocytes, which cannot be conducted in the present study.

That new neurons in the adult hippocampus originate from astrocytes. It is important to note that neural stem cells may also reside in non-neurogenic regions of the adult brain. Thus, subsets of astrocytes in other brain regions may also, under appropriate conditions, function as neural precursors. The identification of astrocytes with neural stem cell potential and the regulatory mechanisms that allow them to behave as neuronal precursors will have powerful implications for the experimental manipulation of adult neurogenesis [26].

In the present study aging process is associated with decline in density of nestin, sox^2 positive stem cells in contrary to other tissue like hematopoetic system whether the loss of stem cells contributes to the aging phenotype was an issue that has been considered for several somatic tissues previously. Within the hematopoetic system, a restriction on the replicative capacity of the stem cells has been demonstrated by serial transplantation studies. Additionally, cell-autonomous factors have been demonstrated to affect the longevity of these cells between different strains of mice, and it has been possible to map the locations of some of the relevant genes [14] Nonetheless, the number of hematopoetic stem cells does not appreciably decline, and in some strains may even increase, during aging in mice [23]. Hence, it has been argued that ge-

netic changes occurring within hematopoetic stem cells primarily affect the quality of the stem cells (i.e., their ability to function) rather than the quantity [14].

NSCs within the SVZ were lost during aging. Similar age-related declines have also been documented for epidermal stem cells [20], and skeletal muscle satellite cells [10]. At least three factors could contribute to the difference between these systems and the hematopoetic system. First, the reserve capacity of the hematopoetic stem cell population may be greater. Second, the rate at which stem cells were replaced by symmetric divisions, resulting in two daughter stem cells may be higher for hematopoetic stem cells. Finally, there may be tissue-specific differences in the ability of the stem cells to respond to genetic damage, which affects whether the cells survive and accumulate damage or undergo apoptosis. Regardless of whether the outcome is the functional or actual depletion of stem cells, genetic damage within each of these stem cell compartments was likely to be a primary cause of aging-related phenotypes [13].

The Bergmann glia were a radial glial population residing in the Purkinje cells layer(PCL), with cell bodies located adjacent to Purkinje neurons and radial processes extending to the pial surface. Little was known about the developmental origin and biological functions of Bergmann glia beyond their documented scaffolding role for both the guidance of Purkinje cell dendrites and the migration of granule cell precursors (1).

Cerebellar stem cells exist in both the embryonic and adult cerebellum. While the existence of such a population in the embryonic and adult telencephalon was well recognized [6]. Cerebellar neurons and glia originate from two principal germinal zones, the upper rhombic lip (URL) and the VZ. Excitatory granule neurons and unipolar brush cells were generated by the RL whereas Purkinje cells and inhibitory neurons were generated from the VZ.Continuous generation of granule neurons and conserved migratory patterns in the adult zebrafish cerebellum In mammals and

birds, the granule precursors migrate from the URL and pile up over the cerebellar surface to form a highly proliferative second germinal zone, the external granule layer (EGL). In zebrafish, the granule cells leave the germinal zone in the upper rhombic lip and migrate and differentiate [38].

(Milosevic)[21] & Hachem, et al.)[16]. who found that Sox1-expressing cells were present outside of the PCL at early postnatal stages,). In the immature cerebellum, Sox[1] expression was widespread across all tissue layers from the white matter to the molecular layer. This suggests a migration path of Sox1-expressing cells, from the ventricular zone toward the cortical layers, which was consistent with that of Bergmann glia precursors during cerebellar formation although the possibility of Sox1 expression being sequentially turned off and on in different populations during development cannot be formally excluded at this stage

These findings on Bergmann glia were notable in the light of the recent report describing the multipotency of quiescent cortical astrocytes. These cortical glial cells, which can also undergo reactive gliosis in vivo, have been shown to form multipotent neurospheres in vitro even though their in vivo potential appears more limited. The fact that matures Bergmann glia can also change from quiescent state to reactive gliosis in reponse to injury [8].

The perinatal cerebellum, embryonic as well as adult cerebellar-derived neurospheres generated cells with characteristics of multiple neuronal and glial cerebellar cell types. Both embryonic and adult NSCs were observed to give rise to neurons that resemble GABA-ergic interneurons, granule cells, oligodendrocytes and astroglia. In addition, at low frequencies transplanted embryonic cerebellar NSCs gave rise to Purkinje cells, while adult cerebellar NSCs generated cells that resemble Lugaro neurons, indicating that the developmental potential of neurosphere-derived NSCs might get restricted over time, as has been shown for acutely dissociated cells grafted into the developing cerebellum [9].

(Miyata et al) [22], (Altman and Bayer)[3]. After a second wave of proliferation, the EGL precursors generate granule cells that migrate radially inwards to form the internal granule cell layer. Postmitotic granule precursors in the inner EGL express the neuronal differentiation. The subsequent tangential ventrolateral migration in zebrafish resembles the migratory behavior of GCPs in the EGL of rodents. In the EGL of rodents, GCPs first migrate tangentially in a medial to lateral direction before turning into the deep layers of the EGL where they migrate radially along Bergmann glia inwards to the IGL. However, in zebrafish we do not detect GCP migration along radial glia in the IGL.

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CHANGES IN DESTRIBUTION OF THE NEURAL STEM CELLS IN THE HIPPOCOMPUS OF THE RAT AT DIFFERENT POSTNATAL AGES.

Fathy A. Ibrahim MD, Mohammed I. Abdou MD, Raouf F. Bedeer MD, Hamdino M. Attia MD, Al Sayed A. Abd Elhady M.Sc

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CARTILAGE SHIELD FOR REPAIR OF THE TYMPANIC MEMBRANE PERFORATION WITH POOR EUSTACHIAN TUBE FUNCTION : FUNCTIONAL AND ANATOMICAL EVALUATION

Khaled M. Mokbel MD, Yasser W. Khafagy MD and El Saeed Thabet MD*

Department of ENT, Aaudiology unite, Faculty of Medicine, Mansoura university, Mansoura city, Egypt*

Abstract

The objective of this study is to evaluate the effectiveness of the thin cartilage shield in comparison to full thickness cartilage and temporalis fascia, in reconstruction of tympanic membrane perforation in cases associated with poor Eustachian tube function. This study was done at Mansoura University Hospital, ENT Department between uly 2003- September 2010 and included 140 patients with Tympanic membrane perforation with poor Eustachian tube function. Repair of the tympanic membrane perforation was done by thin cartilage (0.3 m) in 50 cases, full thickness cartilage in 50 cases and temporalis fascia alone in 40 cases. Results: study included 65 males and 75 females. They aged 12-56 years. The mean follow up was 18 months. The take rate was better in both cartilage groups than that in fascia group. The hearing was better in the thin cartilage group in comparison to that in the other groups. Conclusion: Eustachian tube dysfunction necessitates the use of cartilage grafts and more better the thin cartilage which obtains a more better take rate and hearing results than that obtained by the full thickness cartilage and fascia grafts.

Introduction

The Eustachian tube (ET) is a part of a system of contiguous organs that include the nose, palate, nasopharynx, middle ear and mastoid air cells. The Eustachian tube is not actually a tube but rather an organ consisting of lumen with mucosa, cartilage, surrounding soft tissue, Peritubal muscles and superior bony support (sphenoid sulcus). Eustachian tube has at least three physiological functions; ventilating

function to equilibrate gas and atmospheric pressure in the middle ear, protection from nasopharyngeal pressure and drainage of secretions produced within the middle ear into the nasopharynx [1]. The Eustachian tube plays a chief role in the pathogenesis of different middle ear diseases; tympanosclerosis, and chronic suppurative otitis media (Cholesteatoma and non cholesteatoma) [2]. Subjects with chronic central perforation (CCP) also demonstrate a poor capacity to equilibrate an over or under pressure induced during provocation tests of ET function [3]. Ears with CCP have a middle ear pressure (ME) equal to ambient pressure. However, if these ears were to be temporarily sealed off from ambient air and the ME pressure measured continuously, it might be possible to detect a failure of pressure regulation possibly relevant to the etiology and/or continuation of the disease. Many authors found that the success rate of myringoplasty in good Eustachian tube function was better than in poor function. They concluded that Eustachian tube function could predict the results of tympanoplasty [4,6].

The use of temporalis fascia in reconstruction of the tympanic membrane perforation is more subjected to negative middle ear pressure postoperatively in poor Eustachian tube function. This will promote early graft retraction and eventually failure and re perforation[7]. So the use of less compliant material as cartilage graft may be beneficial for reconstruction of the perforation. As the cartilage has more rigid quality it tends to resist resorption and retraction especially in Eustachian tube dysfunction [8]. Autologous cartilage grafts fulfill the parameters of an ideal graft material for reconstruction of the tympanic membrane [9]. The cartilage has a very low metabolic rate that enables the cartilage to withstand long periods in ischemic situations. It can be harvested easily from the operative field in adequate amount. It has a normal curvature similar to that of a normal tympanic membrane[10]. Also, stiffness of the cartilage allows precise manipulation and positioning of the cartilage and prevents retraction of the tympanic

membrane in poor function of the Eustachian tube [8]. Salen and Jansen[11] first reported the use of cartilage composite grafts for tympanic membrane reconstruction. Over the past twenty years autologous cartilage-perichondrium grafts have come into use to prevent recurrence of retraction pockets and cholesteatoma in patients with Eustachian tube dysfunction. Cartilage is also thought to reduce the rate of extrusion of prostheses used for ossicular chain reconstruction as well as reduction of postoperative atelectasis [12]. Cartilage grafts are usually used in combination with temporalis fascia grafts[13]. Cartilage has also been used to graft the entire tympanic membrane [8]. Hearing results after cartilage tympanoplasty have been shown to be comparable to temporalis fascia and perichondrium [14].

Material and Method

Inclusion criteria were; inactive chronic suppurative otitis media, central perforation, no middle ear pathology, no mucosal changes, dry for at least three months without any episode of otorrhea and conductive hearing loss correlating with the perforation and with poor Eustachian tube function. 140 consecutive patients that fulfilled the inclusion criteria were selected from our outpatients' clinic of Otolaryngology Department, Mansoura Univesity from July 2003 to september 2010. Otomicroscopy was performed for all patients. Pure tone audiometery (air conduction and bone conduction sthresholds at frequencies 250- 8000 HZ), speech reception thresholds (SRT), speech discrimination scores (SDS) and Eustachian tube function were carried out preoperatively, postoperatively (3 months, 6 months and 1 year) and at the last available follow up. Eustachian tube function was done using a Madsen immittancemeter as follow: negative pressure of -200 mm H2O was established in the external canal and middle ear, then the patient was asked to swallow and the residual pressure was measured. Eustachian tube function was classified into: a) Good: when the residual pressure was reduced to >-100mm H20. b) Fair: when patient can reduce the -200 to a residual pressure between -100 and -200 H2O. c) Poor: when the residual

pressure was not reduced below -200mm H2O with swallow[15]. Reconstruction was done by full thickness cartilage graft in 50 cases, partial thickness in 50 cases and temporalis fascia graft in 40 cases. Data were evaluated using an IBM computer and statistical analyses were done through the SPSS program version 10. The values were considered statistically significant when p value was <0.05.

Surgical technique:

Local anesthesia with good sedation was used in most cases (110 patients). General anaesthesia was done in uncooperative cases (30 patients). The vascular strip was created (through postauricular approach) by making two incision in the canal wall over the tympanomastoid and tympanosquamous suture 3mm from the annulus and extends laterally just medial to the bony cartilaginous junction, then the two incisions are connected by another incision to create a laterally based flap. The vascular strip was elevated and incorporated in the blade of the mastoid retractor and held foreword with the auricle. Using a fine needle and a cup forceps to fresh the margins of the perforation was done. The skin from the bony canal is elevated down to the bony annulus. Once middle ear has been exposed any adhesions, tympanosclerotic patches, and thickened mucosa around Eustachian tube orifice were removed. The cartilage graft was harvested from the scapha at the upper part of the auricle by a separate small incision on the medial surface of the auricle. The cartilage was excised alone without the perichondrium. A notch is done in the cartilage to fit the manubrium of the malleus. In this study, we used full thickness and partial thickness (0.3 mm) where thinning of the cartilage was done by a special instrument called Conchotome (Kurz Co. Germany). The cartilage was placed by underlay fashion. Anteriorly the graft is placed under the remnants of the tympanic membrane and medial to the annulus. The malleus is fitted into the notch in the cartilage. A piece of temporalis muscle fascia was used to cover the cartilage shield and extended under the posterior skin flap. Gel foam was packed under the anterior annulus to

support the graft in this area. Gel foam is packed in the canal with antibiotic ointment gauze pack. The pack is removed after ten days and gel foam remnants after three weeks. In the 3rd group we used temporalis fascia alone to repair the tympanic membrane.

Results

This study was conducted on 140 patients suffering from inactive chronic suppurative otitis media. They were 65 males and 75 females. The mean age at operation was 25.4 years with a range from 12 to 56 years (standard deviation ± 11.7). The mean follow up period was 18 months. The take rate was more significantly high in cartilage groups than in fascia group (Table 1).

The mean preoperative air conduction threshold (500-8000) was 42.3 dB. The mean postoperative bone conduction threshold (500-4000) was 28.9 dB. There was significant hearing gain of the average pre and postoperative air bone gap in cartilage and fascia cases ($P<0.001$), but there was no significant difference in hearing results between the fascia and cartilage groups. The mean pre and postoperative hearing results are shown in table (2). The postoperative tympanogram revealed that, type 'A' tympanogram was obtained in partial cartilage grafts more than others (Table 3).

Table 1 : Take rate in the three groups; FTC= full thickness cartilage, PTC= partial thickness cartilage, TF= temporalis fascia.

	FTC	PTC	TF
Take rate	47 (94%)	47 (94%)	16 (40%)
Failure rate	3 (6%)	3 (6%)	24 (60%)
Total	50	50	40

Table 2 : The mean pre and postoperative hearing.

	Pure tone average dB			Air bone gap dB		
	Prep	**Postop**	***P***	**Preop**	**Postop**	***P***
FTC	44.5	30.8	<0.001	27.1	14.9	<0.001
PTC	45.9	29.6	<0.001	26.9	14.3	<0.001
TF	46.8	31.5	<0.001	26.3	14.2	<0.001

Table 3: Postoperative tympanogram .

	Type A	Type B	Type C
Full thickness	16	24	7
Partial thickness	32	9	6
Temporalis Fascia	2	7	7

Discussion

The main goals of tympanic membrane reconstruction are, first closure of the perforation, second obtaining a more stable tympanic membrane to resist pressure changes caused by ET dysfunction, and the third goal is to obtain a new tympanic membrane with acoustic qualities similar to that of normal tympanic membrane[16]. Since the introduction of tympanoplasty by[17], numerous graft materials were used for reconstruction of the tympanic membrane perforation, but the temporalis fascia remains the most commonly used for myringoplasty with success rate about 90% of primary tympanoplasty [8].

However, in poor Eustachian tube function, there will be negative pressure changes in the mid-

dle ear and the new thin tympanic membrane will not bear these changes leading to retraction and atrophy with failure of myringoplasty. Belal[18] stated that Eustachian tube dysfunction is one of the main non technical causes of failure in myringoplasty.

So the use of less compliant and more rigid material as composite cartilage and perichondrial graft may be beneficial. Although it is similar to the temporalis fascia in being mesenchymal tissue, its more rigid quality tends to resist resorption and retraction even in the presence of poor Eustachian tube function[8]. In our study we used two different graft materials, the standard temporalis fascia and the cartilage graft of two thicknesses, the full thickness and the partial thickness. Cartilage in our study was used in two thickness, full thickness and partial thickness (0.4mm). Partial thickness cartilage can achieve the same acoustic properties similar to normal tympanic membrane. Thinning of the cartilage was done by using a special instrument (Conchotome) to obtain the exact thickness of the cartilage. This agrees with Zahnert et al[16] who used the same instrument and reported that cartilage with thickness less than 0.5 mm had the same acoustic properties as the normal tympanic membrane and temporalis fascia.

Regarding the take rate in our study, there was statistical significant difference between the cartilage and fascia, where the take rate was high in cartilage group. These due to the rigid quality of the cartilage which resist the decreased middle ear pressure due to Eustachian poor function. We found that the postoperative gain was statistically significant in cartilage and fascia cases, but there was no significant difference in hearing results between the fascia group and the cartilage groups. These results agree with the results of Geber et al[14] and Zahnert et al[16]. By comparing the postoperative air bone gap of different thickness cartilage graft, we found that the least postoperative improvement was obtained with full thickness cartilage as compared to the partial thickness cartilage. However, these differences were not statistically significant

Regarding the postoperative Tympanometry, the partial thickness cartilage most commonly produced type "A" tympanogram, on the other hand the full thickness cartilage grafts produced mostly type "B" tympanogram. Fascia cases produced both types A and B tympanogram equally. This finding agree with Zahnert et al[16] who stated that partial thickness cartilage graft offered approximately the same mechanical stability at the atmospheric variations. They showed the same pressure displacement curves similar to that of the tympanic membrane, reflecting essentially a similar stiffness of the cartilage was increased. So the cartilage does not act as an immobile graft but bit has the same acoustic properties as the normal tympanic membrane and vibrates in response to sound waves.

Conclusion

We conclude that Eustachian tube function should be assessed preoperatively in all cases of chronic suppurative otitis media. Poor Eustachian tube function mandates the use of either full thickness or partial thickness cartilage shields for repair of tympanic membrane perforation to obtain better results than that of temporalis fascia alone. Partial thickness cartilage has better acoustic properties than the full thickness cartilage.

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BENHA MEDICAL JOURNAL

CARTILAGE SHIELD FOR REPAIR OF THE TYMPANIC MEMBRANE PERFORATION WITH POOR EUSTACHIAN TUBE FUNCTION : FUNCTIONAL AND ANATOMICAL EVALUATION

Khaled M. Mokbel MD, Yasser W. Khafagy MD and El Saeed Thabet MD

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POSTNATAL CHANGES IN DISTRIBUTION OF THE NEURAL STEM CELLS IN THE SPINAL CORD OF THE RAT

Adel A. Bondok MD, Huda M. Eltahry MD, Adel A. Elhawary MD, Hamdino M. Attia MD* and Fawzy M. Abou-Galala M.Sc*

Departments of Anatomy, Mansoura Faculty of Medicine and Al-Azhar Faculty of Medicine (Damietta).*

Abstract

Background: *The developed mammalian spinal cord seemingly contain neural stem cells (NSCs), which can give rise to neurons and glial cells when they are placed in appropriate environment, however, the location of NSCs in the spinal cord and the regions to which their progeny migrate in order to differentiate remain unresolved.*

Aim of the work: *the present study was designed to detect and localize nestin positive cells in spinal cord of albino rat at different postnatal ages.*

Material and Methods: *60 albino rats were divided equally into 6 age groups varies from neonatal (one day age), early childhood(one week age), late childhood(one month age), adolescence (3 months age), adulthood(6months age), and senility (one year age). Neural stem cells were detected by immune-histochemical stain and then were subjected to morphological and morphometric studies to determine their density in different regions of the spinal cord. The method of immunostaining applied in this study is the indirect Immunohistochemistry using avidin-biotin complex (ABC). Data were collected and then analyzed statistically.*

Results: *Nestin positive cells were detected in one day age then gradually decreased till the age of 6 months and became negative at one year. In addition, the densities of nestin positive cells were higher in cervical region than both lumbosacral and thoracic region, the latter shows the lowest density at all studied age groups. The densities of nestin positive cells were higher in white matter, followed by posterior horn,*

zone around the central canal and anterior horn in order.

Conclusion: *It could be concluded that spinal cord of rats contain neural stem cells that present from early life (neonatal day one) up to six months then disappeared at old age. Further efforts should be devoted to delineate the differentiation mechanism of nestin positive cells and their functional capacity in the repair and remodeling of injured spinal cord.*

Key Words: *Spinal cord, neural stem cells, nestin positive cells, postnatal ages.*

Corresponding author: *Adel A. Elhawary, Anatomy Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. Mobile: 0161826052,01001254135,E-mail: Adelhawary@ hotmail.com.*

Introduction

Stem cells play a critical and essential role in the human body not only by providing the starting material for organs and tissues but also for their continual maintenance, growth and renewal through life [1].

The spinal cord as a part of the central nervous system (CNS) forms from the neural tube. This is initially a simple epithelium from which through finely orchestrated events including proliferation, differentiation and morphogenesis the CNS emerges in all its complexity[2]. Rakic[3] stated that the spinal cord, like most structures of mammalian brain, belong to the class of non renewable epithelium. However, small numbers of cells that line the central canal remain mitotic[4]. It has been demonstrated recently that the adult rat spinal cord contains large number of dividing cells that give rise to glia but not neurons [5]. Sakakibara et al.[6] reported that, in mammalian spinal cords, no neurogenesis has been observed after initial development. However, they added that, developed mammalian spinal cords seemingly contain neural stem cells, which can give rise to neurons and glia cells when they are placed in appropriate environment.

The earlier conventional idea that all neural precursors cells (NPCs) become progressively more

restricted and disappear with age, consequently neural tissue could not renovate after damage, has been challenged by much evidence for the presence of multipotent neural precursors throughout development and in adulthood [2].

Many reports has provided proof that NPCs exist in CNS of adult animals as subventricular zone (SVZ) and subependymal zone (SEZ)[7,8,9]. Adult (NPCs) were detected in rat and mouse spinal cord[5,10]. Lois et al.[11] in their in vitro study, stated that, postnatal spinal cord retains the capacity to generate functional neurons and added that the presence of (NPCs) in postnatal spinal cord may offer new therapeutic approaches for restoration of function to individuals with spinal cord injuries.

The present theories suggested that NPCs present in the spinal cord can recover proliferation and differentiation potency and can be induced to differentiate into certain special neural cells and can substitute damaged neural cells in special pathological conditions [7,9]. Xu et al.[10] stated that endogenous NPCs could be better therapeutic alternative in spinal cord injury because by the time exogenous NPCs transplantation has not acquired an effective result yet.

NPCs distribution in spinal cord is an essential primary work to evaluate the therapeutic use of endogenous NPCs in repairing of spinal cord damage. Therefore, the present study is intended to detect and localize the neural stem cells in the spinal cord of rat at different levels and postnatal ages.

Material and Methods

Albino rats were divided into 6 groups. Each group contained 10 animals which represented different stages of postnatal development as follow: Group1 : at postnatal day one; Group 2 : at one week age; Group 3 : at one month age; Group 4 : at 3 months age; Group 5 : at 6 months age; Group 6 : at one year age. Animals were housed in standard individual maternity cages under controlled temperature and humidity. Under deep Phenobarbital anaesthesia (100mg/Kg), rats were perfused transcardially with

saline containing heparin (2U/ml), and subsequently with a fixative consisting of 4% paraformaldehde in Phosphate Buffered Saline (PBS). The spinal cords were dissected out and immediately put into the same fixative over night at 4°C and subsequently cut into transverse paraffin sections with a thickness of (10-50µm) at 120 µm interval between two sections from rostral to caudal.

Histological study : sections from different regions of the spinal cord were stained by Haematoxylin and Eosin [12].

Immunohistochemistry: Sections from different regions of the spinal cord were incubated with primary antibody (NESTIN Rabbit anti-Human Polyclonal Antibody - LS-C40764 - Lifespan Biosciences) for 5 minutes. The sections were then incubated with biotinylated anti-mouse secondary antibody followed by the avidin-biotin complex (ABC)[13]. Finally, the sections were visualized with 3, 3-diaminobenzidine (DAB) (optimal concentration was 1:100).

Morphometrical studies : Morphometric assessment of the nestin positive cells were evaluated by using computer based image analysis system. The image pro plus program (image pro plus, v6.3, Media cybernetics, USA) were used for calculation of positive cells.

Statistical analysis: The collected data were organized, tableulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 16, running on IBM compatible computer with Windows 7 operating system. For quantitative data, mean and standard deviation (SD) were calculated and for comparison between two means, the student (t) test was used. For comparison between more than two means, the one way analysis of variance (ANOVA) tests was used. For interpretation of results, the p value ≤0.05 was considered significant.

Results

I- Histological results :

Light microscopic examination of the spinal cord sections of the six age groups that stained with

haematoxylin and eosin showed that, the structure is basically similar throughout its whole length. The volume of grey matter is much enlarged in the cervical and lumbar regions (fig, 1). In transverse sections, the central mass of gray matter has the shape of a butterfly, the ventral horns being more prominent than the dorsal horns. The central canal lies in the central commissure of gray matter (fig. 1). The neurons were recognizable by their large nuclei, with prominent nucleoli, and extensive basophilic granular cytoplasm; one or more processes of which may be visible. Oligodendroglia has small round condensed nuclei. In grey matter oligodendrocytes are not only scattered between the nerve cell bodies along with the astrocytes but also tend to be aggregated around the neuron cell bodies (fig, 2).

II- Immunostained results :

Nestin positive cells were detected only from the first day after birth to the age of 6months. These immunostained positive cells were detected in the white matter, zone around the central canal, posterior and anterior horn of gray matter (figs, 3 ~24). The number of these nestin positive cells differed from one age group to another and from one region of the spinal cord to another. These cells decreased with aging till the age of 6months. The numerical densities of nestin positive cells were assessed in different levels of the spinal cord in the studied age groups (tab 1).

A- One day age (groups 1): Nestin positive cells were detected in the white matter, posterior horn, anterior horn and zone around the central canal at the different spinal cord levels. The densities of nestin positive cells were higher in the cervical region than both the lumbosacral and thoracic regions; the later showed the lowest density (Table 1) and (fig, 5). The nestin positive cells were higher in the white matter as compared to all other regions followed by the posterior horn then area around the central canal then anterior horn in order (Table 1).These cells were seen in small and large aggregations especially around the central canal (Fig.3). Different morphology of the cells were detected; most cells had a common rounded appearance

with small round dark nuclei and brown cytoplasm (Figs, 3, 4, 6).

B- One week age (group 2): The numerical density of nestin positive cells at all segments of spinal cord showed significant decrease as compared with group one (table 1). There was a statistically significant increase in density of nestin positive cells at cervical region in comparison with thoracic and lumbosacral region (Tab 1) and (fig, 10). At this age group, the densities of nestin positive cells in the white matter were higher than all other groups followed by posterior horn, central canal and anterior horn in order (table 1). The cells attained different shapes that vary from bipolar to multiple process bearing cells. These cells showed rounded nuclei dark in color and cytoplasmic staining brown color (figs, 7, 8, 9). The cells around the central canal in this age group had a common rounded appearance and appeared more scattered compared with similar cells in the previous age (Fig, 3 & 7).

C- One and three months age (group 3 and 4): Marked decrease in the density of nestin positive cells was detected at all levels (figs, 11~18). The densities of these cells were higher in the cervical region than both lumbosacral and thoracic regions. (Table 1) and (figs, 14 & 20). Also the densities of these cells were higher in the white matter as compared to all other regions, followed by the posterior horn, area around central canal and anterior horn in order (table 1). Marked increase in multiple process bearing cells was showed as compared with previous age groups (figs, 12, 13, 16, 17, 18). Also marked decreases in the densities of these cells around the central canal with dispersion were detected. These cells had rounded appearance (Fig, 11 & 15). Also some nestin positive cells seen in the white matter arranged in radial manner towards the pial surface. (Fig, 16).

D- Six months age (group 5): The density of nestin positive cells reached to minimum in the cervical, thoracic and lumbosacral regions at all examined sections as compared to the previous ages (table 1). There was a statistically significant increase in the density

of nestin positive cells in cervical region in comparison to thoracic and lumbosacral regions (table 1) and (fig, 24). The densities of the nestin positive cells in the white matter were the highest of all other groups followed by posterior horn, area around central canal and anterior horn in order (table 1). The nestin positive cells appeared in scattered manner at this age (Figs, 19, 21, 22, 23). Some nestin positive cells seen in the white matter arranged in radial manner towards the pial surface (Fig, 22).

E- One year age (group 6): No detection of the nestin positive cells at all regions of the spinal cord of this group (Figs, 25 & 26).

Table (1): The correlation between mean values of numerical densities of nestin positive cells at different regions of spinal cord at different postnatal ages.

		One day		One week		One month		Three months		Six months	
		Mean	**S. D**	**Mean**	**S. D**	**Mean**	**S. D**	**Mean**	**S. D**	**Mean**	**S. D**
Cervical	Zone around CC	31.10	5.56	28.40	5.10	15.00	3.74	8.50	2.75	5.20	1.61
	Anterior horn	25.90	5.17	14.50	4.62	5.00	1.76	6.70	2.35	4.00	1.15
	Posterior horn	51.80	6.03	36.70	3.83	40.00	14.90	38.10	4.74	10.00	5.01
	White matter	100.70	9.62	82.60	6.56	81.00	20.13	66.90	17.43	30.00	2.74
Thoracic	Zone around CC	23.60	3.97	19.50	2.54	5.00	1.76	6.10	1.66	2.00	1.49
	Anterior horn	21.30	4.00	13.30	5.12	5.00	2.05	3.50	1.08	2.00	1.49
	Posterior horn	47.90	5.50	25.00	5.55	20.00	5.77	17.00	3.05	5.00	1.76
	White matter	96.20	9.93	75.00	2.94	55.80	18.73	55.80	14.39	20.00	5.79
Lumbosacral	Zone around CC	25.00	5.61	24.90	2.92	8.20	1.54	8.20	1.98	3.00	1.24
	Anterior horn	26.50	4.27	19.10	3.14	8.00	1.15	5.60	2.45	4.00	1.15
	Posterior horn	51.30	5.29	34.80	4.49	30.00	5.67	28.00	2.35	7.00	2.26
	White matter	104.00	8.44	71.90	22.49	65.00	17.05	52.90	18.25	26.00	4.78

SD= stander deviation, CC= central canal.

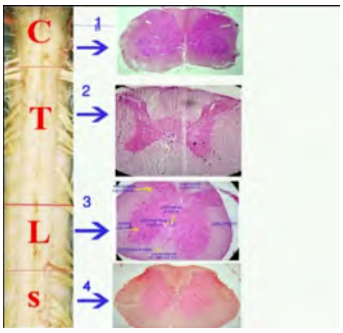

Fig. (1) : A photomicrograph showing transverse sections at different levels of spinal cord of albino rat stained by H& E, C&1= cervical . T& 2= thoracic. L& 3= lumber. S& 4= sacral. (x100).

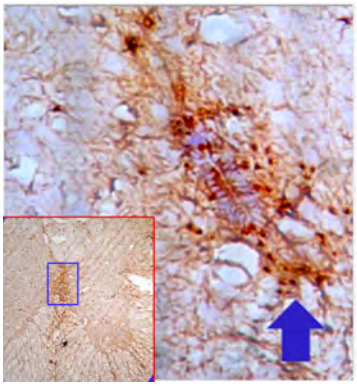

Fig. (3) : A photomicrograph of immunostained transverse section in the thoracic region of spinal cord of (one day) albino rat showing aggregation of rounded nestin positive cells around the central canal (arrow). (X400). (Low magnification view in the inset x100).

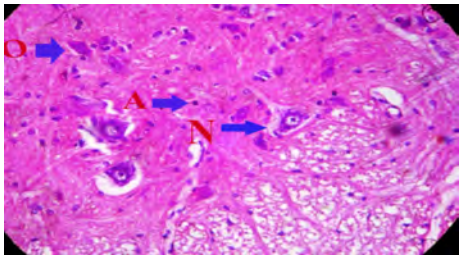

Fig. (2) : A photomicrograph of transverse section in the spinal cord of adult albino rat at cervical level stained with (H&E X400). Neuron (N), oligodendrocytes (O) and astrocytes (A).

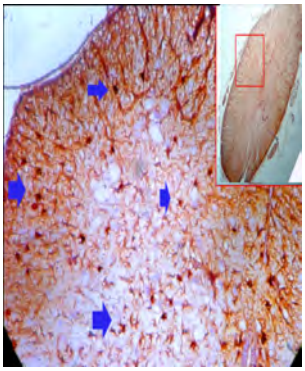

Fig. (4) : A photomicrograph of immunostained transverse section in the cervical region of spinal cord of (one day) albino rat showing dispersed nestin positive cells in the anterior horn and white mater (arrows). (X400).

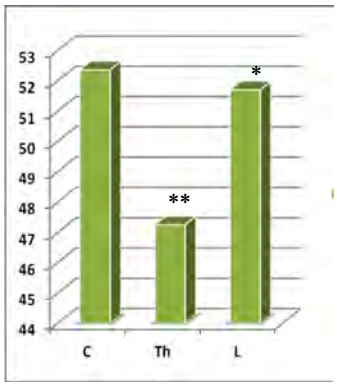

Fig. (5) : A histogram representing the density of nestin positive cells between at regions of spinal cord at age of (one day) albino rat (data represented by means ± SD). PV>0.001 is significance (*). PV > 0.0001 is high significance (**). C= cervical. Th= thoracic. L= lumbar.

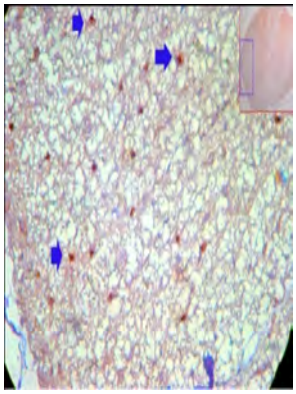

Fig. (6) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (one day) albino rat showing rounded nestin positive cells in the white matter (arrows) (X400).

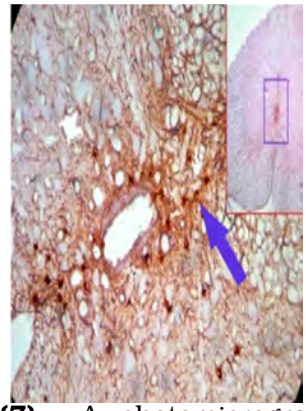

Fig. (7) : A photomicrograph of immunostained transverse section in the cervical region of spinal cord of (one week) albino rat showing dispersion of rounded nestin positive cells in the zone around central canal (arrow). (X1000).

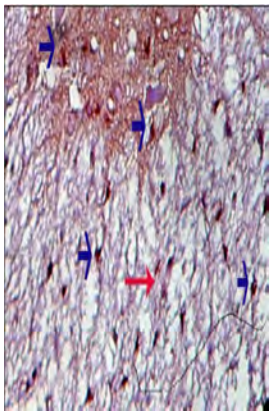

Fig. (8) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (one week) albino rat showing nestin positive cells (fusiform in shapes) in the anterior horn and white matter (arrows) (X400).

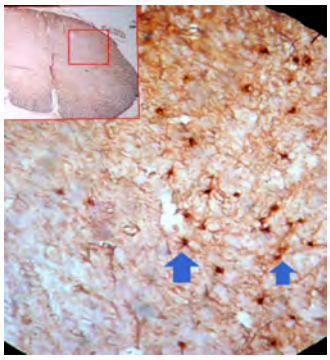

Fig. (9) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (one week) albino rat showing aggregation of nestin positive cells in the posterior horn (arrows). (X400). Note: a few cells have multiple processes.

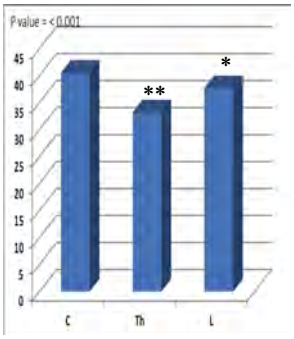

Fig. (10) : A histogram representing the density of nestin positive cells at different regions of spinal cord of (one week) albino rat. (Data representing by means ± SD). PV>0.001 is significance (*). PV > 0.0001 is high significant (**).C= cervical. Th= thoracic. L= lumbar.

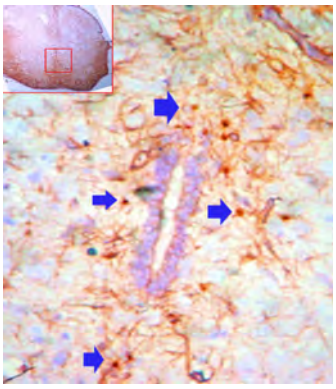

Fig. (11) : A photomicrograph of immunostained transverse section in the cervical region of spinal cord of (one month) albino rat showing dispersed nestin positive cells in the zone around central canal (arrows) (x1000).

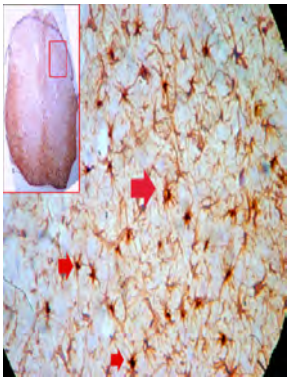

Fig. (12) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (one month) albino rat showing aggregation of nestin positive cells (with multiple processes) in the posterior horn (arrows). (x400).

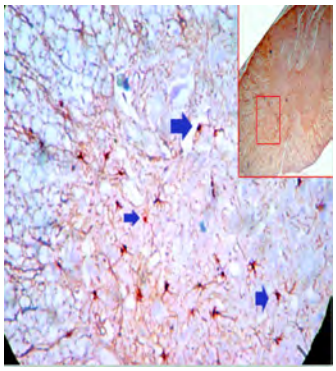

Fig. (13) : A photomicrograph of immunostained transverse section in the thoracic region of spinal cord of (one month) albino rat showing nestin positive cells(with multiple processes) In anterior horn . (x400).

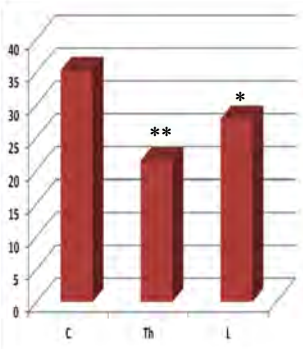

Fig. (14) : A histogram representing the density of nestin positive cells at different regions of spinal cord (one month) albino rat (data representing by means ± SD). PV>0.001 is significance (*). PV > 0.0001 is high significant (**).C= cervical. Th= thoracic. L= lumbar.

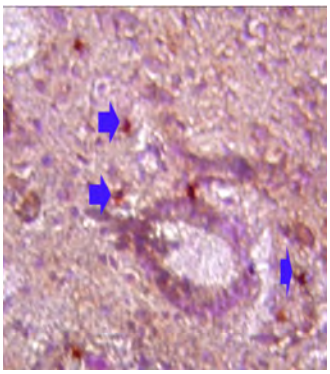

Fig. (15) : A photomicrograph of immunostained transverse section in the cervical region of spinal cord of (3months) albino rat showing dispersion of nestin positive cells in the zone around central canal (arrows with appearance of (dispersed rounded) cells. (x1000).

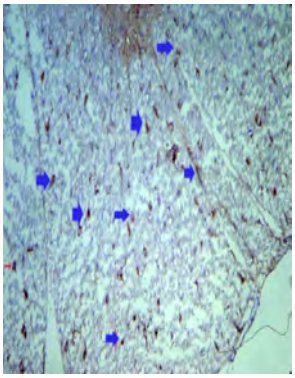

Fig. (16) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (3months) albino rat showing nestin positive cells in white matter (arrows). NB: some cells arranged in a radial manner towards the pial surface. (x400).

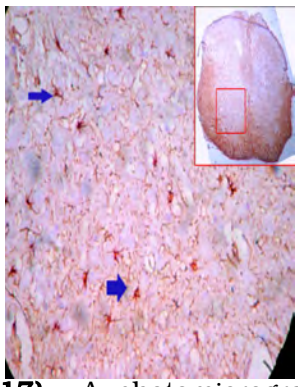

Fig. (17) : A photomicrograph of immunostained transverse section in the thoracic region of spinal cord of (3 months) albino rat showing nestin positive cells in the anterior horn (arrows). (X400). Note: most of these nestin positive cells have multiple processes.

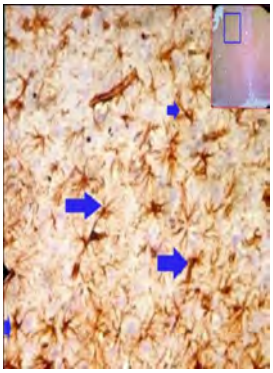

Fig. (18) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (3months) albino rat showing aggregation of nestin positive cells (with multiple processes) in the posterior horn (arrows). (X400).

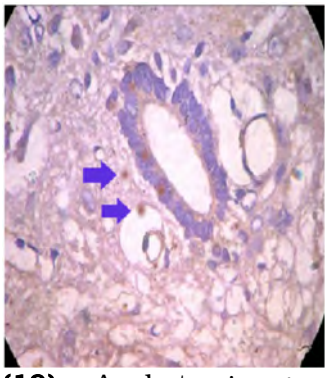

Fig. (19) : A photomicrograph of immunostained transverse section in the lumbar region of spinal cord of (6months) albino rat showing nestin positive cells in the zone around central canal (arrows). (x1000).

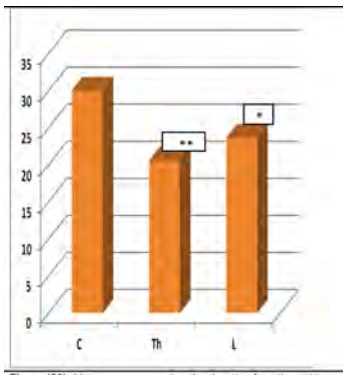

Fig. (20) : histogram representing the density of nestin positive cells at different regions of spinal cord of (three months) albino rat (data representing by means ± SD. PV>0.001 is significance (*). PV > 0.0001 is high significant (**).C= cervical. Th= thoracic. L= lumbar.

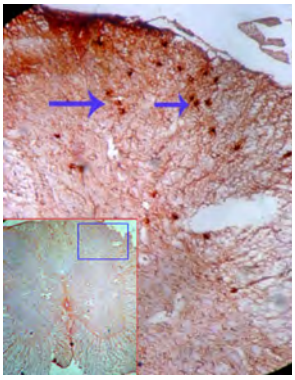

Fig. (21) : A photomicrograph of immunostained transverse section in the posterior horn of cervical region of spinal cord of (6months) albino rat showing relatively few number of nestin positive cells (arrows) (x400).

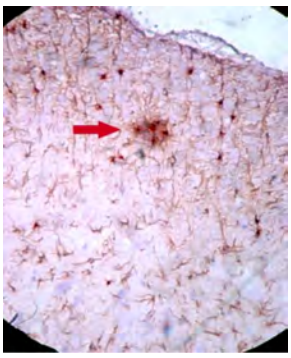

Fig. (22) : A photomicrograph of immunostained transverse section in the white matter of thoracic region of spinal cord of (6months) albino rat showing relatively few number of nestin positive cells (arrow). Note: some cells radially arranged towards the pial surface. (x400).

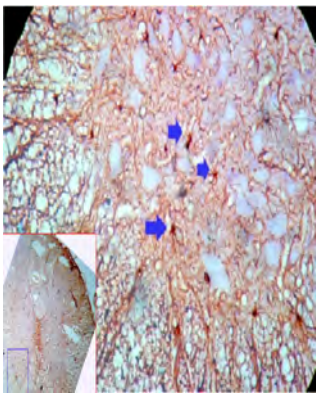

Fig. (23) : A photomicrograph of immunostained transverse section in the anterior horn of thoracic region of spinal cord of (6months) albino rat showing scattered nestin positive cells (arrows). (x400).

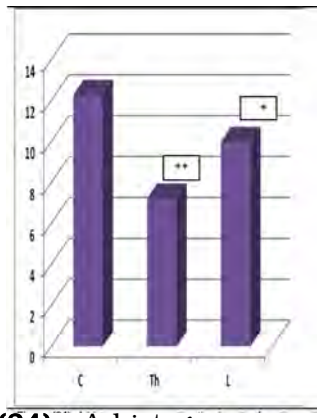

Fig. (24) : A histogram representing the density of nestin positive cells at different regions of spinal cord (6months) albino rat. (Data representing by means ± SD). PV>0.001 is significance (*). PV > 0.0001 is high significant (**).C= cervical. Th= thoracic. L= lumbar.

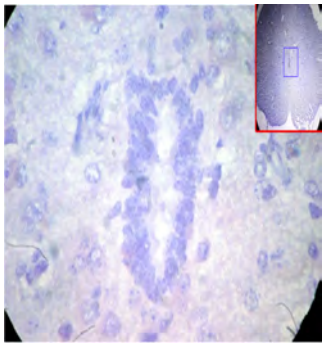

Fig. (25) : A photomicrograph of immunostained transverse section in the spinal cord of (one year) albino rat showing absence of nestin positive cells around the central canal (X1000).

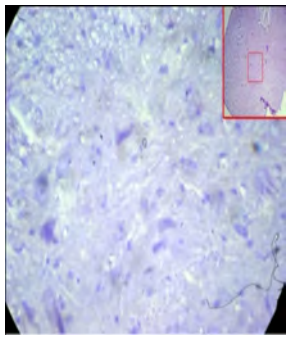

Fig. (26) : A photomicrograph of immunostained transverse section in the anterior horn of spinal cord of (one year) albino rat showing absence of nestin positive cells (x400).

Discussion

Neural stem cells (NSCs) are self-renewing, multipotential progenitor cells. A single NSCs can give rise to a wide variety of central nervous system (CNS) cells, including neurons, astrocytes, and oligodendrocytes[14]. Reynolds and Weiss[15] were the first to isolate neural progenitor and stem cells from the striatal tissue, including the subventricular zone, one of the neurogenic areas of adult mice brain tissue. Since then, neural progenitor and stem cells have been isolated from various areas of the adult CNS, including non-neurogenic areas, such as the spinal cord, and from various species including human [16]. NSCs have the capability of persistent dividing and reproductive activity, and may differentiate into neuron, astrocyte and oligodendrocyte[17,18]. Therefore, NSCs become the winsome choice for the repairing and remodeling of mammalian CNS. NSCs which were transplanted into CNS showed enough plasticity, participate in neurogenesis of embryonic, adult and senile stage, and could differentiate neuron, astrocyte, oligodendrocyte, but it has

not been enough experimental studying proof whether they could substitute the died and dysfunction neural cell [18,19]. Some studies indicated that exogenous NSCs could live in the transplanted regions and differentiate neural cells, but could not effectively construct functional synaptic linkage and microenvironment of neuron living, such as producing neurotrophic factor, could not do normal physiologic function and effectively rebuild neural dysfunction, and brought a lot of side effects of tissue transplantation [10]. Proliferation, differentiation and directing immigration induction of endogenous NSCs is a possible breakthrough of neural recovery, possibly can surmount defects of exogenous NSCs transplantation. Thus, to induce endogenous NSCs to proliferate, migrate to damaged CNS regions and differentiate the special neural cells is the best method of neural repairing [7]. The study of NSCs distribution in spinal cord is an important work to study the repairing of spinal cord damage by endogenous NSCs. Therefore the present work was intended to study the distribution of NSCs in spinal cord of rat at different postnatal ages by observing nestin positive cells distribution.

The intermediate filament nestin has been reported as a marker for stem cells as well as precursor populations of specific cell types in the developing mammalian CNS giving rise to both neurons and glia [20,21], also nestin is a well-establelished marker for NSCs and proliferating precursor cell populations in some tissues, such as CNS [22]. Nestin is classified as type IV neurofilament, which together with microfilaments and microtubules constitutes a major component in the cytoskeleton. In contrast to other more general cytoskeletal elements, nestin is expressed in a cell type specific manner, and major differentiation steps are marked by the transition from one intermediate filament type to another. With the onset of neurulation, neuroectodermal cells starting to express nestin, the expression is dramatically down regulated when NPCs differentiate and become postmitotic cells[21,23,24]. Nestin also has been reported to be expressed in the developing myotome[25], skele-

ton muscle precursors[21,25], mesenchymal pancreatic cells [26], intestine [27], and cranial ganglia [28]. Nestin has been investigated extensively in mammalian systems, including rat, mouse, human, as well as chick.

The present study revealed that, nestin positive cells were abundant in early ages (one day) then decreased gradually and significantly till the six months and then became undetectable and this attitude was observed in all parts of spinal cord and in all examined zones. These findings were consistent with previous reports in rats, mice, and tree shrews, suggesting that a decline in adult neurogenesis during midlife is a common feature of mammalian species, including primates[29]. Also the gradually decrease in density of nestin positive cells from one day to 6 months age may be explained by differentiation of these cells into glia, this finding was in agreement with Warf et al.[30] who reported that, differentiated glia were present in the rodent cervical spinal cord by El6 and the number of spinal cord glia continues to increase up to 12 days postnatally. Arnold et al. [31] added that, Glia cells were long considered end products of neural differentiation, specialized supportive cells with an origin very different from that of neurons. New studies have shown that some glia cells and specific subpopulation of astrocytes in adults' mammals have a function similar to that a primary progenitor or neural stem cells [32].

In this study, the neural stem cells were more abundant in cervical region followed by lumbosacral region and were least at thoracic region; also the white matter had abundant stem cells followed by posterior horn, then area around central canal and finally anterior horn regardless of age with statistically significant difference.

In this study, the nestin positive cells were detected along the different segments and parts of spinal cord of the rat from birth up to six months age but were not detected after that. However, Doetsch et al. [33] and Cassidy & Frisen [34] reported that, in the adult CNS, NSCs were not detect in the spinal cord but founded

only in certain locations such as SVZ of telencephalic lateral ventricles and subgranular zone (SGZ) of hippocampal dentate gyrus. This may be explained by the fact that, their studies did not included the early postnatal.

In this work, the nestin positive cells were detected in the intact spinal cord of the rat. This is in disagreement with Shan[7], who stated that, NSCs were detected in regions near injury site only and might last for days to months, and de novo neurogenesis has been detected in regions distal to the injury site. The adult rodent spinal cord, however, has the potential to make neurospheres that give rise to neurons, astrocytes and oligodendrocytes, and contains progenitor cells that can differentiate into neurons when transplanted into the hippocampus, which seems to provide a neurogenic environment[4,35]. Thus, cells that has the potential to proliferate and differentiate preferentially to glial cells in vivo, but also to neurons in a favorable and artificial condition seemingly remain in the developed mammalian spinal cord. Such cells have been shown to be distributed widely throughout the adult rat spinal cord. In their study, Horner et al.[5] reported that, the adult rat spinal cord contain large numbers of dividing cells in vivo that give rise to glia but not neurons. In addition, it was reported that, neural precursor cells were isolated from the postnatal day 15(P15) to P16[36] and adult rat spinal cord [37]. Some study demonstrated the presence of progenitor cells within the gray and white matter of the adult spinal cord as well as in the central canal. A percentage of these mitotic cells appear to be a source of newborn astrocytes and oligodendrocytes but not neurons within the intact cord [5].

In this study, the nestin positive cells were detected outside the central canal at all postnatal ages till 6months. This finding is in agreement with that reported by Ayako et al. [38] who stated that, nestin positive cells were expressed in the ependymal layer in prenatal human spinal cord and were seen up to 14 months after birth and also the nestin positive cells were detected outside the central canal. However, Hockfield

& McKa [20] reported that cells in the ependymal layer express nestin in the developing rat spinal cords from embryonic day 11 to postnatal day 6.

In this study, the nestin positive cells were showed variable shapes; from oval or rounded at the early age groups to star like shaped up to multiple process bearing cells at the adult age and the majority of these cells were detected in the white matter. This were explained by Wei [39] who studied the distribution of nestin positive cells and glial fibrillary acidic protein glial (GFAP) in proliferative regions of central nervous system of postnatal developing and adult mice and found that, nestin positive cells were predominately distributed in certain proliferative regions, such as cerebral cortex as well as ependymal and subependymal zones of the brain and spinal cord from P2 up to P60. The majority of nestin positive cells, characterized by astroglial profiles of multiple and radial processes, showed a partial overlapping distribution with that of GFAP-immunoreactivity astroglial cells. Double immunofluorescence confirmed that about 77% of these nestin positive cells exhibited GFAP- immunoreactivity, indicating that large percentage of nestin positive cells may have committed to astroglial cells.

In this study, the nestin positive cells were detected in both gray and white matter. This is in agreement with Horner et al. [5] and Yamamoto et al. [35] who reported that, the NSC were scattered throughout the parenchyma as well as around the central canal.

In the present work, the nestin positive cells showed a statistically significant increase in density in cervical zone in comparison to lumbosacral and thoracic zones, the later showed the lowest density. This finding may explain by the presence of brachial plexus in the cervical region and lumbosacral plexus in the lumber region. This is in agreement with Fujita [40] and Altman & Bayer [41] who proposed that gliogenesis begins after the onset of neurogenesis and proceeds in a rostral-to-caudal direction as neurogenesis.

Regarding the distribution of nestin positive cells, the present study showed that, the density of NSCs showed a high significance increase in the posterior horn compared to the anterior one. This was explained by the fact that the dorsally originated glial precursors dominate that of the ventral origin in late prenatal life. This finding is in agreement with results of Nornes & Das [42] and Nornes & Carry [43] who proposed that, ventro-to-dorsal gradient exists in neuron production in which neurons first develop in the more ventral aspect of the developing spinal cord followed by neuron production in the dorsal spinal cord. These cells migrate to their final positions along the processes of a transient population of glial cells termed radial glia [3]. However He added that, radial glial cell bodies are found in the ventricular zone and their processes extend laterally to the pial surface of the neural tube. Warf et al. [30] and Noll & Miller [44] reported that, oligodendrocyte and astrocyte precursors have been founded in the ventral neural tube and suggest that they migrate laterally and dorsally to populate the surrounding white matter, However, Cameron-Curry & LeDouarin, [45] showed that oligodendrocytes and astrocyte were generated in both the ventral and dorsal neural tube and migrated in the ventrodorsal and the dorsoventral directions.

The results of the of the present study were in contradiction to that stated by Xu et al. [10] who stated that, the NSCs in gray matter were more than that in white matter, while in the present study it was more in the white matter. This may be attributed to the fact that, their work was done by different technique in the form of analyzing the distribution of the nestin positive cells in spinal cord of adult nestin second-intron enhancer controlled LacZ reporter transgenic mice (pNes-Tg) with LacZ staining. However, these results were in agreement with Wei [39] who reported that, nestin in the CNS was largely expressed in astrocytes which are more common in white matter.

In short, the present study highlighted the fact that spinal cord of rat contains nestin positive cells that present from early life

(neonatal day one) up to the age of six months then disappeared at old age. These present results can suggest the use of endogenous neural stem cells in young age and exogenous neural stem cells in old age during management of spinal cord injuries. However further efforts should be devoted to delineate the differentiation mechanism of nestin positive cells and their functional capacity in the repair and remodeling of mammalian spinal cord.

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POSTNATAL CHANGES IN DISTRIBUTION OF THE NEURAL STEM CELLS IN THE SPINAL CORD OF THE RAT

Adel A. Bondok MD, Huda M. Eltahry MD, Adel A. Elhawary MD, Hamdino M. Attia MD and Fawzy M. Abou-Galala M.Sc

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ADJUVANT PELVIC RADIOTHERAPY VERSUS SEQUENTIAL CHEMORADIOTHERAPY IN HIGH RISK STAGE I-II ENDOMETRIAL CARCINOMA

Hanan A. Wahba MD, Hend A. El-Hadaad MD, Anas M. Gamal MD* and Tamer Dawod MD**

Department of Clinical Oncology and Nuclear Medicine, Obstetrics and Gynecology, **Medical physics, Mansoura University, Mansoura, Egypt*

Abstract

Objective: *To assess whether addition of adjuvant chemotherapy of paclitaxel and carboplatin to radiotherapy confers an advantage for overall and progression-free survival and for the incidence of relapse over standard pelvic radiotherapy and also to assess its related toxicity in high risk stage I-II endometrial carcinoma. Patients and methods: Medical records were retrospectively reviewed to identify high-risk stage I-II endometrial carcinoma treated in Clinical Oncology and Nuclear Medicine department during the period between 2002-2008 by adjuvant radiotherapy alone (arm I) (57 patients) or by sequential carboplatin (AUC5-6), paclitaxel (135-175 mg/m^2) with radiotherapy (arm II) (51 patients). Radiotherapy was delivered through four-field box technique at dose of 45- 50 Gy (1.8 Gy/day x 5 days/ week). Results: Toxicity was easily manageable and predominantly hematologic with grade 3 neutropenia and thrombocytopenia in 9.8% and 6% respectively with no febrile neutropenia. All patients lost their hair. Chemoradiotherapy (arm II) was associated with lower rate of relapse (9.8% vs 22.7%). After median follow up of 48 months, five-year overall survival (OAS) and progression-free survival (PFS) rates for chemoradiotherapy-treated patients were significantly more favorable than those who did not receive chemotherapy (P =0.02 and 0.03 respectively). In arm I, OAS and PFS rates were 73.7% and 66.7% in contrast to 90.2% and 84.3% in arm II. Conclusion: Adjuvant chemoradiation with paclitaxel and carboplatin improved survival rates and decreased recurrence rate in patients with high risk stage I-II endometrial carcinoma. Chemotherapy was associated with an acceptable rate of toxicity. However, prospective studies with*

larger number of patients have to be performed to define a proper standard adjuvant treatment for high-risk stage I-II endometrial carcinoma.

Key-words: *Stage I-II high risk endometrial cancer; adjuvant radiotherapy; adjuvant sequential chemoradiotherapy.*

Introduction

Endometrial cancer is the most common gynecological malignancy in Europe and North America. It is the 7th most common cause of death from cancer in women in Western Europe[1]. The International Federation Gynecology of Obstetrics and (FIGO) surgical stage is considered the most important independent prognostic indicator of progression-free or overall survival and has a significant impact on treatment decisions[2]. Some independent prognostic factors have been identified in early stages (stage I-II) including lymphovascular space involvement, histologic grade 3, aggressive pathologic subtypes (uterine papillary serous carcinoma and clear cell carcinoma), deep myometrial invasion, cervical invasion and age>60 years[3]. Patients exhibiting any of these features are often characterized as having high risk endometrial cancer for which adjuvant therapy is often recommended. Prior clinical trials using adjuvant radiotherapy has been shown to reduce the risk of local relapses but have not improved overall survival due to the development of distant metastases[4,5].

Jolly et al, [6] reported that vaginal brachytherapy alone has similar overall survival and cumulative recurrence rates to standard external pelvic radiotherapy in stage I-II endometrial cancer. Trials of chemotherapy have demonstrated that combination of adriamycin and cisplatin was more effective than single agent therapy[7,8]. Substitution of carboplatin for cisplatin may improve tolerability without sacrificing efficacy. Myelosuppression may be more frequent but nephrotoxicity, neurotoxicity and emesis were all less frequently reported and were milder with carboplatin than those in cisplatin–based regimen[9]. Other agents such as paclitaxel have shown promising survival and response rates in endometrial cancer. Paclitaxel has been associated with favorable rates either as a single agent or in combination

with platinum based chemotherapy[10].

Given the risks of both local and distant relapse, interest in giving combined chemotherapy and radiation either concomitantly or sequentially has continued to be an attractive option. The Duke group reported their findings of a large retrospective study of chemotherapy vs radiation vs concomitant chemoradiation in women with stage III or IV uterine cancer[11]. Their analysis suggested that chemoradiation improved progression-free and overall survival. So, we conducted this study to assess whether addition of adjuvant chemotherapy of paclitaxel and carboplatin to radiotherapy confers an advantage for overall and progression free survival and for the incidence of relapse over standard pelvic radiotherapy and, also, to asses its related toxicity in high risk stage I-II endometrial cancer.

Materials and Methods

This retrospective study included 108 patients with endometrial cancer presented to the department of Clinical Oncology and Nuclear Medicine, Mansoura University Hospital during the period from January 2002 to December 2008. Demographic and treatment data as date and pattern of progression, date of death or last follow up and toxicities were collected by checking patients' files.

Eligible criteria:

All patients underwent total abdominal hysterectomy with bilateral salpingo-ophrectomy (TAH, BSO) with no residual disease. Other eligible criteria were:

(1) Non metastatic patients with histologically confirmed FIGO stage I-II endometrial cancer (2009) [12] with one or more of the following risk factors: lymphovascular space invasion, histologic grade 3, aggressive pathology (papillary serous and clear cell carcinoma) and age >60 years.

(2) Patients who received adjuvant pelvic radiotherapy (RT) (arm I) or chemoradiotherapy (arm II).

(A) Arm I: Included 57 patients who received pelvic RT at dose of 45-50 Gy (1.8 Gy/d x 5 days/ week) through four-field box technique. The upper border of pelvic

field was at L4-5; lower border at lower border of vagina by marker; lateral border at 1.5 cm lateral to pelvic brim; posteriorly at S3; anteriorly at symphysis pubis.

(B) Arm II: Included 51 patients treated by 4 cycles of paclitaxel 135-175 mg/m^2 and carboplatin AUC 5-6 at 3-week interval following completion of pelvic RT.

The primary end points were progression-free survival (PFS) and overall survival (OS) rates. The secondary end point was treatment related toxicities. Systemic toxicity of chemotherapy was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0[13].

Statistical methods:

Data were entered coded and using Statistical Package for Social Sciences (SPSS) version 15.0. Because the data were non-normally distributions, results were expressed as number, percentage and medians. Categorical variables were compared using Fisher test. Survival functions (OAS and PFS) were estimated using Kaplan Meier test. Log rank test was used to analyze the difference between the curves. All statistical tests were two-sided, with a p-value of <0.05 to indicate statistical significance.

Results

We identified 108 women who met the eligibility criteria with 57 patients assigned for RT alone (arm I) and 51 patients for combined chemoradiation (arm II). Risk factors were well balanced between both arms (table 1). Median age was 65 years and 62 years (in arm I and II respectively), as expected, endometrioid carcinoma was the most common pathological type, grade 3 was high in both arms (49% and 54.9%) and most patients were of stage II (54.4% and 55%). Apart from alopecia that occurred in all patients, hematologic toxicities with grade 3 neutropenia and thrombocytopenia were higher in arm I than arm II (9.8% and 6% respectively) (table 2). No patients developed febrile neutropenia. No reports of hospitalization during therapy and the majority of cycles (91%) were delivered without delay. There were no treatment related deaths.

After median follow-up of 48 months, relapse rate was higher in radiotherapy only (22.7% vs 9.8%) (table 3). In arm I, OAS rate was 73.7% compared to 90.2% in arm II (fig.1) while PFS rates were 66.7% and 84.3% in arm I and arm II respectively (fig.2). Patients in arm II had significantly better OAS and PFS rates than those in arm I (P=0.02 and 0.03).

Table (1): Patients' characteristics

Character	Arm I(n=57)	Arm II (n=51)	P
Age:			
Median	65 years	62 years	0.72
Range	55-72 years	57-70 years	
Pathological type:	No (%)	No (%)	
Endometrioid carcinoma	44 (77.2)	41 (80.4)	0.81
Adenosquamous carcinoma	3 (5.3)	2 (3.9)	1.0
Papillary serous carcinoma	5 (8.7)	6 (11.7)	0.75
Clear cell carcinoma	3 (5.3)	1 (2)	0.62
Adenoacanthoma	2 (3.5)	1 (2)	1.0
Grade			
1	6 (10.5)	4 (7.8)	0.74
2	23 (40.4)	19 (37.3)	0.84
3	28 (49.1)	28 (54.9)	0.57
FIGO			
IA	8 (14)	9 (17.6)	0.79
IB	18 (31.6)	14 (27.4)	0.68
II	31 (54.4)	28 (55)	1.0
Lymphovascular space invasion	9 (15.9)	13 (25.5)	0.24

Table (2): Chemotherapy- related toxicity

Toxicity	Grade			
	1 No (%)	2 No (%)	3 No (%)	4 No (%)
Anemia	4 (7.8)	7 (13.7)	2 (3.9)	0
Neutropenia	5 (9.8)	13 (25.5)	5 (9.8)	0
Thombocytopenia	4 (7.8)	11 (21.6)	3 (6)	0
Hypersensitivity	2 (3.9)	0	0	0
Neuropathy	8 (15.7)	6 (11.8)	1 (2)	0
Alopecia	0	51 (100)	0	0
Nausea and vomiting	10 (19.6)	2 (3.9)	0	0

Table (3): Pattern of relapse

Site of relapse	Arm I	Arm II	P
Local No (%)	4 (7%)	2 (3.9)	0.68
Distant No (%)	6 (10.5%)	1 (2%)	0.28
Both local and distant No (%)	3 (5.2%)	2 (3.9%)	1.0

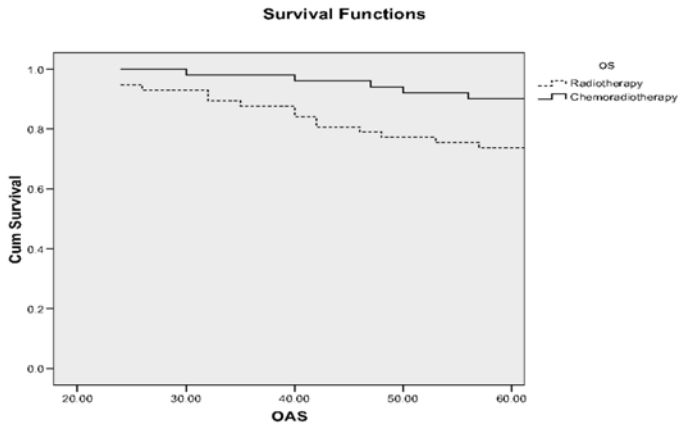

Fig. 1 OAS

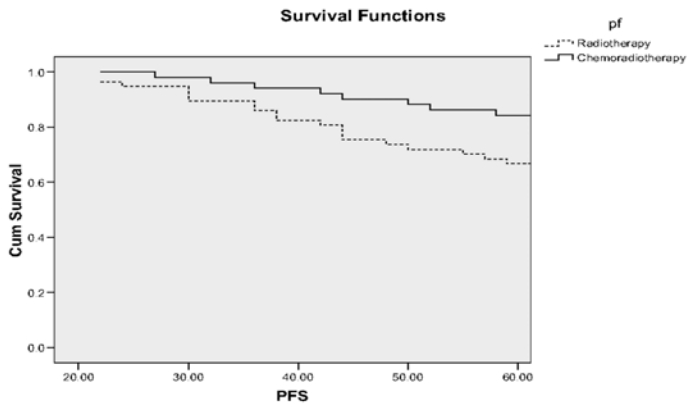

Fig. 2 PFS

Discussion

Optimal adjuvant therapy with high risk endometrial cancer is poorly defined. Randomized clinical trials comparing adjuvant pelvic RT with brachytherapy or observation showed significant reduction of the risk of locoregional relapse with pelvic irradiation but without clear trend toward prevention of distant metastases or improvement of overall survival[4,5,14].

Chemotherapy has a role in the management of advanced and recurrent endometrial cancer but with no consensus regarding the optimal chemotherapy. Combination of cisplatin plus doxorubicin was commonly used[15]. Paclitaxel attracted attention for use in patients with endometrial cancer because of its success in ovarian and breast cancers. When paclitaxel was used as single agent in advanced or recurrent cases, response rates was 36%-43% and activity was demonstrated in truly platinum resistant patients[16,17]. Combination of paclitaxel with platinum analogue was proved to be more effective regimen as reported in many studies[18,19,20]. In the present study, chemotherapy was associated with an acceptable rate of toxicity; the most common toxicity was hematologic, a findind consistent with that reported by Michener et al,[21]. All patients lost their hair which is similar to that observed by Hoskins et al[18]. The incidence of grade 3 hematologic toxicity was 21.7% and no grade 4 toxicity in contrast to Lupe et al,[22] who reported grade 3-4 toxicity in 27%. This higher rate can be explained by the increased number of cycles used in their study (6 cycles).

Fader et al,[23] mentioned that, addition of platinum and taxane to adjuvant RT was associated with decrease risk of relapse which was the case in our work but Kuoppala et al, [24] found that, adjuvant chemotherapy with cisplatin, epirubicin and cyclophosphamide and RT failed to lower recurrence rate. Five year-overall survival (OAS) rate were significantly higher (P=0.02) in arm II (90.2% versus 73.7%) and so was the progression free survival (PFS) (84.3% in arm II and 66.7% in arm I) (P=0.03). These

finding were comparable to that reported by Susumu et al[25] and Hogberg et al,[26].

Conclusion

Adjuvant chemoradiation with paclitaxel and carboplatin improved survival rates and decreased recurrence rate in patients with high risk stage I-II endometrial carcinoma. Chemotherapy was associated with an acceptable rate of toxicity, However, prospective studies with larger number of patients have to be performed to define a proper standard adjuvant treatment for high-risk stage I-II endometrial carcinoma.

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DOUBLE ROW SUTURE BRIDGE TECHNIQUE FOR REPAIRING ROTATOR CUFF TEARS

Hosam Elbegawi MD

Department of Orthopedic, Faculty of Medicine,

Benha University, Egypt

Abstract

Introduction: *Tears of the rotator cuff are among the most frequent causes of pain and dysfunction around the shoulder. Arthroscopic rotator cuff repair allows excellent visualization of rotator cuff tear geometry, facilitates precise soft-tissue releases to mobilize the rotator cuff and allows for a tension-free repair.*

Purpose; *To report outcomes of arthroscopic repair of rotator cuff tears using double raw suture bridge technique.*

Patients and Methods; *Seventeen patients underwent arthroscopic repair for rotator cuff tears using anchors with a double-row suture bridge technique. Mean age was 37.7 years (range 18-45) had been followed up for a mean of 16 months (range 12-24 months). The extent of the tear was classified as large, medium or small. Functional outcome was assessed using the UCLA (University of California at Los Angeles) score system.*

Results; *According to the UCLA score system of evaluation, by the end of follow up period the main preoperative score was changed significantly ($P \leq 0.05$) from 11.6 (range 6-18) points to 30.4 (range 16-35) points.*

Conclusion; *Arthroscopic repair of rotator cuff tears using double-row suture bridge technique showed good clinical outcome in patients with rotator cuff tear.*

Key wards: *rotator cuff-double row- repair.*

Introduction

Tears of the rotator cuff are one of the most frequently encountered causes of pain and dysfunction in the shoulder. [1] Over the last few years arthroscopy has been introduced to repair rotator cuff tear (RCT). Despite continual

improvement in surgical techniques and instrumentation, retear of the sutured tendons is the major limitation and does still occur in significant percentage of patients [2]. Arthroscopic rotator cuff repair allows excellent visualization of rotator cuff tear geometry, facilitates precise soft-tissue releases to mobilize the rotator cuff and allows a tension-free repair.[3]

As repair techniques have continued to advance, many authors have sought a method that restores normal anatomy and to identify the factors and techniques that promote postoperative cuff integrity, such as double row repair and more recently suture bridge techniques [4]

Biomechanical studies of double-row repair have shown increased load to failure, improved contact areas and pressures, and decreased gap formation at the healing site. Clinical studies, however, have not yet demonstrated a substantial improvement over single-row repair with regard to either the degree of structural healing or functional outcomes [5]

The aim of this study is to evaluate the results of double row suture technique in repair of rotator cuff tears.

Patients and Methods

Between June 2008 and March 2010, 17 patients (14 males and 3 females) were enrolled in the current study in Benha University Hospital. The mean age of the patients was 37.7 years ranging between 28-45 years. The right side was affected in 9 patients (53%) and the left side was affected in 8 patients (47%). The dominant hand was affected in 13 (76.4%) patients. Twelve patients (70.5%) were included in heavy work. Inclusion criteria include: Symptomatic full thickness rotator cuff tear patients approved by MRI and failed conservative treatment for at least 6 months.

Exclusion criteria include: Previous surgery on the affected shoulder, arthritic changes in glenohumeral joint, presence of massive cuff tear (defined as > 5 cm or more than 2 tendons torn), Severe fatty infiltration (Goutelier grade 3 and 4), Presence of other shoulder pathology e.g. SLAP le-

sion, instability, marked stiffness or frozen shoulder and symptomatic acromion-clavicular joint pathology. The mean follow up was 16 months (range 12-24 months).

Surgical technique:

Under general anesthesia, one gram broad spectrum antibiotic in the form of third generation encephalsporins, the patient was positioned in the beech chair position. Standard 30 degree angled 4 mm scope was used with automatic pump set at 55mm hg. Using the VAPR radiofrequency (DePuy, Mitek), the thickened subacromial bursa is cleaned, acromioplasty was then done till flat acromion in all the cases. The delaminated edges of the cuff tear are cleaned and the extent of rotator cuff tear is clearly visualized from anterior to posterior.(figure1 A) Tear size was classified according to Cofield classification[6] into Small (<1cm), medium sized (1-3cm), large (3-5cm), and massive tears (>5cm). Massive tears were excluded from the study. There were 11 cases with medium sized crescent shaped mobile tears, and 7 cases with large adequately mobile tears. All of them involving SS and IS with no subscapularis tear in any of the cases.

The footprint of the cuff insertion was prepared with a motorized burr to bring about a bleeding surface (figure 1B). Long head of biceps (LHB) tenotomy was done at this stage if more than 50% degenerative tear is present. It was done in 7 patients. Double row suture bridge technique was used in all patients with two 5.5mm Healix anchors (DePuy, Mitek) (figure 1 D) were used in medial row and one Versalok (DePuy, Mitek) anchor in the lateral row (figure 1F,G,H) . Suture passing instrument; Expressew II (Mitek) (figure 1E) was then used to pass sutures in the cuff using the lateral portal. The sutures were then tied through the lateral portal using dunkan sliding knot and 2 half hitches. (Figure 1 I)

Post operative follow up:

The patient is put in a sling with abdominal belt, no abduction slings were used. The patient is discharged from the hospital the next day of the surgery and exercises to the wrist and the elbow is

given to the patient. Passive exercises of the shoulder were started in the next visit after one week. The patient was trained to do pendulous exercises for two weeks, then passive range of motion for four weeks. The patient started to do active exercises for six weeks. The patient was allowed to return to his job after 3-6 months.

Evaluation:

All patients were evaluated preoperative and postoperative at the end of follow up period. Patients were assessed according to the UCLA (University of California at Los Angeles) score.[7] The UCLA Shoulder Score is a 35-point scale consisting of 10 points for pain, 10 points for function, and 5 points each for motion, strength, and patient satisfaction. A higher score indicates increased shoulder function. The UCLA shoulder score is expressed as excellent (34 or 35 points), good (29-33 points), fair (25-28 points), and poor (≤24 points). [8]

Statistical analysis:

Results were expressed with descriptive methods (median, range). Non-parametric analysis was performed with the Wilcoxon signed rank test for comparison of UCLA scores. The Pearson correlation coefficient was used for correlation analysis. $P \leq 0.05$ was considered statistically significant.

Results

According to the UCLA score system of evaluation, by the end of follow up period the main preoperative score was changed significantly ($P \leq 0.05$) from 11.6 (range 6-18) points to 30.4 (range 16-35) points.

The pain score was changed from 3.6 (range 0- 6) points to 8.8 (range 4-10) points. The daily living activity score was changed from 4.2(range 2-6) points to 8.6 (range 6-10). The range of motion in the form of active forward flexion was changed from 2.3 (range 1-3) points to 4.47 (range 3-5) points. Also the strength of muscle action was compared to the other healthy side and changed from 2.5 (range 2-3) points to 4.1 (range 3-5) points. Fifteen patients became satisfied with the result of the operation and only two patients were not satisfied

although they got better than before, Table (1).

There were 15(88%) patients with good and excellent scores (29-35 points) and only two (12%) patients with fair and poor scores post operative, Table (2).

Complications:

There were no reported intraoperative or postoperative complications in term of nerve damage, superficial or deep infection. Only two (12%) patients suffered from symptoms like rotator cuff retear.

Table (1) the UCLA score preoperative and postoperative.

Score	Preoperative	postoperative
Pain(10points)	3.6 (0-6)	8.8(4-10)
Activity of daily living(10 points)	4.2(2-6)	8.6 (6-10)
Range of motion (5 points)	2.3(1-3)	4.47 (3-5)
Power of Muscle strength (5 points)	2.5 (2-3)	4.1 (3-5)
Patent satisfaction (5 points)	0	4.4 (0-5)
Total score (35 points)	11.6 (6-18)	30.4 (16-35)

Table (2) the postoperative results.

UCLA score	Number of patients	percentage
Excellent (34-35points	7 patients	41%
Good (29-33 points)	8 patients	47%
Fair and poor (< 29 points)	2 patients	12%

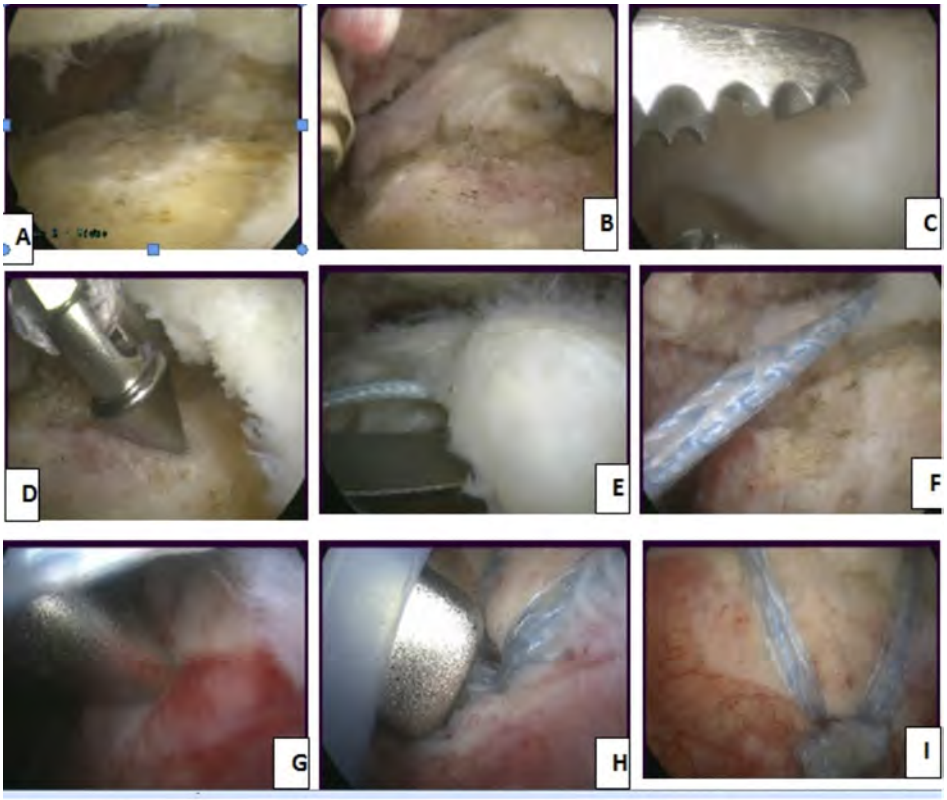

Figure (1) Ssteps of double row suture technique A- Identification of the tear B- Preparation of footprint and creation of bleeding surface C- Mobilization of the tendon D-Insertion of the medial anckor E- Expressew suture passer to pass suture through the tendon. F, G,H- The lateral anckor. I- Tying the suture.

Discussion

Comparative effectiveness of operative approaches was assessed in 2 recent systematic reviews, with the most frequent comparison was mini-open vs. arthroscopic rotator cuff repair; this comparison provided moderate evidence for no statistical or clinically important difference in function or cuff integrity between the two approaches; however, there was some evidence suggesting an earlier return to work by approximate-

ly 1 month for arthroscopic repairs [9,10].

Arthroscopic rotator cuff repairs have been performed in increasing numbers during the past 2 decades, with an exponential increase occurring during the past few years. Some major advantages for arthroscopic repair include decreased surgical morbidity, the potential for improved rehabilitation, and the ability to identify and treat associated pathology, such as labral lesions, as well as, biceps pathology, and loose bodies. In addition, the arthroscope allows enhanced visualization of the tear size, retraction, and tissue quality without the need to take down the deltoid as is done in open repairs. Releases, so much a part of open rotator cuff repair, can also readily be performed with arthroscopic technique, facilitating low-tension repairs. The final factor in the argument for an arthroscopic approach is that of patient preference.[11]

Several technical evolutions have recently occurred in arthroscopic rotator cuff repair. A controversial advance from single-row to double-row and recently to double-row suture-bridge repair took place.[12]

Nho SJ et al., reported meta-analysis and concluded that both single and double row rotator cuff repair resulted in significant improvement shoulder function and satisfactory clinical outcome. The data in the published literature did not support the use of double row suture anchor fixation to improve clinical outcome, but there are some studies that report that double row suture anchor fixation may improve tendon healing [13]. In vitro studies showed that contact area is greater with double row techniques showing greater fixation strength [14, 15].

In our study, there was marked increase in the UCLA score of the patients at the end of follow up period. Fifteen patients (88%) became satisfied with the result of the operation. These results are comparable with the results of other techniques like Mason Allen suture technique by Castagna et al [16] in which the postoperative results were 86%. These results are

comparable with the results of El-Azab et el (80%) [12] and with that of Pauly et al (73%)[17].

Double-row repairs are associated with increased consumption of material and surgery time as well as with a demanding learning curve regarding technical skills of the surgeon. On the other hand, new techniques may help to achieve high initial fixation strength with only a small extension of operating time [17].

There were two patients (12%) with bad results and symptoms suggesting failure of repair. This complication rate is also comparable with that of El Azab et al (20%) [12].

Conclusion

Arthroscopic repair of rotator cuff tears using double-row suture bridge technique showed good clinical outcome in patients with rotator cuff tear but it needs more skills and consume more materials. This was a short term study and this technique needs to be evaluated for a long time and on more patients.

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DOUBLE ROW SUTURE BRIDGE TECHNIQUE FOR REPAIRING ROTATOR CUFF TEARS

Hosam Elbegawi MD

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DIAGNOSTIC AND PREDICTIVE POWER OF PCT FOR SEPSIS IN CRITICALLY ILL PATIENTS AFTER MAJOR ORTHOPEDIC SURGERIES

Ghada F. El-Rahmawy MD and Lamia Tawfik MD

Department of Anesthesiology, Faculty of Medicine ,

Mansoura University, Ain-Shames, Cairo, Egypt.

Abstract

Background: *Procalcitonin (PCT) is an innovative marker for the diagnosis of bacterial infections in critically ill patients postoperatively.*

Objective: *To quantify the diagnostic role, sensitivity, specificity and predictive power of procalcitonin (PCT) for sepsis and septic shock diagnosis in critically ill patients admitted to ICU after major orthopedic surgery. Patients and methods: This prospective observational study was conducted over 12 month period, on 90 patients who were admitted for intensive treatment in surgical ICU after major orthopedic surgery as a result of severe underlying disease or potential serious postoperative complication with clinical picture of systemic inflammatory syndrome. PCT, CRP and WBC were investigated upon admission and for three days and compared. Specificity, sensitivity, positive predictive value, negative predictive value and receiver operating characteristic (ROC) analysis, were carried out.*

Results: *The values of PCT, CRP and WBC were significantly higher in infected group than non-infected group, with significant positive correlation between PCT, CRP and WBCs in sepsis group. The PCT and CRP level in septic shock patients were significantly higher than SIRS subgroup. PCT was significantly by decreased in both groups in 2^{nd} and 3^{rd} days when compared with the 1^{st} day. Moreover, it was significantly increased in infected than non infected group. In infection and septic shock, PCT had higher specificity, positive and negative predictive value with lower sensitivity than C-reactive protein.*

Conclusion: *PCT had diagnostic and discriminative role between SIRS and infection. PCT has higher specificity, positive and negative*

predictive power and lower sensitivity than C reactive protein in SIRS, infection, severe sepsis and septic shock diagnosis.

Keywords; *PCT, Sepsis, SIRS, septic shock, Critically ill patient.*

Introduction

Sepsis is the commonest cause of mortality in elderly critically ill patients[1]. Recognition of sepsis in critically ill patients is difficult because no single variable of inflammation allows establishment of the diagnosis[2]. There are a plenty markers of sepsis have been studied for its ability to differentiate between SIRS and sepsis including; C-reactive protein (CRP), tumor necrosis factor (TNF-α), IL-1β, IL-6 and IL-8[3-6]. Procalcitonin (PCT) CT-messenger RNA CALC-I gene ion is ubiquitously expressed from various extrathyroid neuroendocrine tissues throughout the body during bacterial infection[7]. PCT is one of calcitonin prohormones, with elimination half-life between 22 and 35 hours[8]. C-reactive protein (CRP) rises slowly and peaks 36 hours after an endotoxin challenge[9]. High PCT serum levels after surgery contributes too many causes include sepsis, surgical trauma, and bacterial contamination during operation or cytokines released during wound healing[10,11]. This prospective clinical study was designed to assess the role and correlation between PCT, C-reactive protein (CRP) and white blood cell in diagnosis of sepsis in critically ill patients admitted to ICU after major orthopedic surgeries. We also hypothesized that PCT level has higher sensitivity and specificity power for diagnosis of sepsis.

Patients and Methods

Study population

After approval of this prospective observational study protocol by the local ethical committee of the hospital over 12 months. Ninety patients their age ranging from (60-70 years) with ASA physical status III were admitted for intensive treatment in surgical ICU at Al-Razi orthopedic Hospital in Kuwait within 24 hours after major elective orthopedic surgery (total knee and hip replacements), as the result of severe underlying disease or potential serious postoperative complication. Informed

consent was obtained from patients before enrollment from conscious patients and unconscious patients from next of kin.

Study design

On arrival to surgical intensive care unit (ICU) and for three days after admission the attending physician evaluated all the study patients using American College of Chest Physicians/Society of Critical Care Medicine consensus classification[12] for diagnosis of SIRS, sepsis and septic shock.

Definitions: the main terms used in this study: systemic inflammatory response syndrome (SIRS) without any infection signs, is accompanied by at least two of four clinical criteria, includes: body temperature <35.6°C or >38.3°C, tachycardia (>90 beats/min), ventilatory frequency >20 bpm or PaCO2 <4.3 kPa (unless the patient was mechanically ventilated), a white cell count>12x10^9/liter or <4x10^9/liter, or >10% immature neutrophils (bands). Sepsis is defined as SIRS with infection, severe sepsis was defined as sepsis with evidence of at least one of the following criteria: in adequate organ function or perfusion, acute alteration of mental status without sedation (Glasgow coma score ≥3, elevated plasma lactate >2, unexplained metabolic acidosis (arterial pH <7.3), hypoxemia (PaO2 <10 kPa breathing room air, or an acute drop in PaO2 of >2 kPa below baseline breathing room air or hypoxemia requiring mechanical ventilation), oliguria (output less than 30 ml/hour) and hypotension defined as systolic arterial pressure <90 mmHg or a decrease of >40 mmHg from baseline. Septic shock was defined as hypotension in addition to sepsis syndrome persisting despite adequate fluid resuscitation and requiring vasoactive amine support. Severity of disease was estimated by the Acute Physiology and Chronic Health Evaluation (APACHE-II) score[13]. Two groups of patients were observed: Group (I) Non-infected group, with no evidence of infection clinically and the patients were not given any antibiotic therapy. Group (II) Infected group, patients had a definite source of infection, positive blood, sputum or urine culture and received specific antibiotic. Pneumonia was diagnosed clini-

cally by productive cough, dyspnea, chest pain, radiography by infiltrates on the chest radiograph and a positive culture from sputum or bronchial fluid. Abscesses were diagnosed by ultrasonography or CT scan, together with pathogenic bacteria growth from aspirated pus. Urinary tract infections were diagnosed by the evidence of dysuria, frequency of micturation, leucocyturia and growth of pathogen in urine culture. Standard supportive care, surgical procedures (drainage of abscesses, etc.) and broad-spectrum antibiotics were provided to all septic patients.

The clinical and laboratory investigations

The clinical data were collected included age, sex, type of surgery, criteria of admission diagnosis, infection site and data required for acute physiology and chronic health evaluation score (APACHE II) was collected.

PCT was measured using commercially available immunoluminometric assay using 20 ml of serum or plasma in an immunoassay with a sandwich technique and a chemiluminescent detection system (LumiTest PCT, Brahms Diagnostica, Berlin, Germany). Limit of assay detection was 0.3-0.5 ng/ml. Also, CRP was measured using a routine turbidimetry assay (ILAD-900; Instrumentation Laboratory, Milan, Italy); a value greater than 10mg/l was considered to be abnormally elevated. In addition, results of routine blood analyses (serum chemistry, WBC, blood gases analyses) were recorded.

Statistical analysis

Statistical software program (SPSS version 10.5) Quantitative and qualitative data were expressed as (means ± SD), or median and percentage respectively. Categorical variables were compared using the chi-square or Fischer's exact test as appropriate. Quantitative variables were compared using the Student t-test or the Mann-Whitney non parametric test as appropriate. The best cut-off value of parameters for the diagnosis of sepsis was determined according to the Youden's index method. Predictive accuracy, the ability of test to discriminate diseased patients from normal was

evaluated by performing receiver operating characteristic (ROC) analyses[13]. Sensitivity and specificity were calculated for the cutoff that represented the best discrimination as derived from AUCS. Furthermore, the areas under the receiver operating characteristic curve (AUCs) were determined, as well as the positive/negative predictive values. The correlations between WBC count, and serum CRP and PCT levels were tested using the Spearman correlation coefficients ($p< 0.05$) was considered significant in all tests.

Table (1): Admission diagnosis (diseased systems) data were expressed in number

	No of patient
Respiratory:	
Pneumonia	21
COPD	10
Acute bronchial asthma	4
Pulmonary edema	4
Pneumothorax	1
Cardiovascular:	
Pulmonary embolism	7
Myocardial infraction	6
Heart failure	5
Central nervous system	
Ischemic stroke	3
Epilepsy	2
Intracerebral hemorrhage	1

Table (2): Patients Study population characteristics Infected and non infected group data are expressed in mean (SD) and number (%)

	Non Infected Group	Infected Group
Age (years) (mean±SD)	66±8.2	64.8±7.8
Sex (M/F)	39/11	27/13
APACHE II (mean±SD)	22.2±10.5	26.1±10.2
No. of patients	50 (56%)	40 (44%)
Weight of patients (Kg) (mean±SD)	88±4.2	92±3.5
Total hip surgery	24	10
Total knee surgery	26	30
Epidural and general anesthesia	25	23
Combined spinal epidural anesthesia	25	17

Table (3): Infection sites (number)

Respiratory tract	18
Urinary tract	10
Wound	5
Abdominal	2
Miscellaneous	3
Unknown	2

Table (4): Microbiology (number)

Organisms	No. of patients (Total =40)
Gram +ve	
• Staph aureus	8
• Streptococcus pneumonia	2
• Enterococcus fecalis	2
Gram –ve	
• Pseudomonas aeruginosa	6
• Klebssiella species	3
• Escheriria coli	2
• Aciobacter baunaii	2
• Proteus mirabilis	3
• Hemophilus influenza	1
• Pseudomembranous colitis	1
• Candida albicans	1

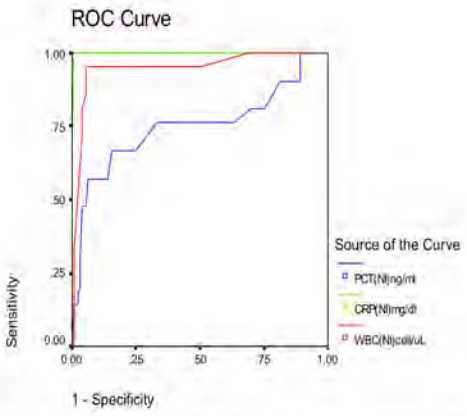

Figure (1): receiver-operating -curve analysis of serum procalcitonin, precursors C-reactive protein and WBC for infection diagnosis

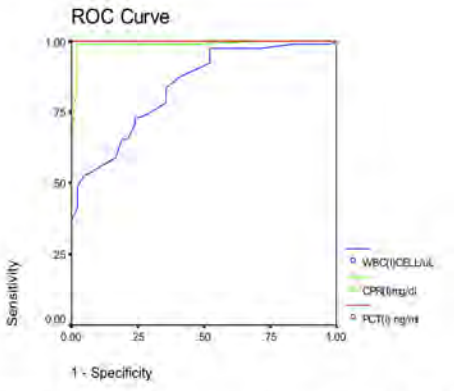

Figure (2): receiver-operating –curve analysis of serum procalcitonin, C-reactive protein and WBC precursors for septic shock diagnosis

Table (5): PCT (ng/ml), CRP (mg/dL) and WBC (cell/μL) in non infected, infected groups, SIRS, sever sepsis and septic shock (Values are median "min-max"):

	Non infected Group	Infected Group
SIRS		
• PCT (ng/ml)	1.5 (0.6-100)	3 (0.5-150) #
• CRP (mg/dL)	30 (3-100)	26 (19-200) #
• WBC (cell/uL)	13.1 (6-20)	17.5 (6-.25) #
Severe sepsis		
• PCT (ng/ml)	20 (4-100)	27.5 (2-150) #
• CRP (mg/dL)	50 (20-100)	35 (20-200) #
• WBC (cell/uL)	17 (6-20.9)	20.3 (7.9-27.30) #
Septic Shock		
• PCT (ng/ml)	25 (4-100)	80 (3-150) #
• CRP (mg/dL)	70 (20-100)	45 (25-200) #
• WBC (cell/uL)	18.9(8.2-22.4)	22.9 (9.3-30) #

#significant increased when compared with other group

Table (6): PCT (ng/ml), CRP (mg/dL) and WBC (cell/uL) in non infected and infected groups in first three days after ICU admission (Values are in mean± SD)

	Non infected Group	Infected Group
1st day		
• PCT (ng/ml)	3.6(14.7)	13.3(33.6) #
• CRP (mg/dL)	26.8(33.3)	37.2(19.7) #
• WBC (cell/uL)	13.3(3.7)	17.7(2.7) #
2nd day		
• PCT (ng/ml)	1.1(3.1) *	4.3(12.3)* #
• CRP (mg/dL)	20.1(11.9) *	27.48(16.6) *#
• WBC (cell/uL)	12.1(2.9) *	15.6(2.6) *#
3rd day		
• PCT (ng/ml)	.5(.4) *	1.1(1.4)* #
• CRP (mg/D	10.1(7.7) *	13.1(12.4) *#
• WBC (cell/uL)	8.4(1.8) *	10.8(2.7) *#

*significant decreased when compared with e first day
#significant increased when compared with other group

Table (7): Diagnostic level, Cerum C-reactive patients and procalcitonin level for diagnosis of infection and septic shock in critically ill patients after major orthopedic surgery.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
PCT cutoff value (1 ng/ml) for infection diagnosis	68%	83%	77%	71%
PCT cutoff value (3 ng/ml) for septic shock	69%	97%	93%	75%(0.42)
CRP best cutoff value for infection diagnosis (20mg/L)	90%	46%	36%)	54%(0.12)
CRP best cutoff value for septic shock diagnosis (30mg/L)	94%	49%	41%	58%

Results

Study population characteristics

Ninety patients were included in these study, of whom 50 patients were classified in group I (non infected group) (56%) and 40 patients in group II (infected group) (44%). The admission diagnoses of both groups are presented in table (1).

The two groups were comparable in age, body weight, sex, APACHE II score, type of surgery and anesthesia with no statistically significant differences in between both groups (Table 2). In 40 patients of infected group 31 (77.5%) had identified etiological microorganisms and bacteremia was diagnosed in 9 patients (22.5%). The commonest site of infection was the respiratory tract followed by the urinary tract and lastly the wound and soft tissue. There was no difference in gram positive and gram negative bacteria proportion (Table 3). Patients who acquired more than two SIRS criteria on admission 47 (94%) in non infected group and in infected group 39 (98%). Severe sepsis was reported in non-infected group in 5 patients (10%) and in infected group in 11 patients (28%). Septic shock was recorded in 1 patient (2%) in non-infected group and in infected group in 5 patients (13%).

Laboratory data

The values of PCT, CRP and WBC were significantly higher in infected group than non-infected group. Also, there was statistically significant positive correlation between; PCT and CRP (r=0.223), PCT and WBCs (r=0.182) and CRP with WBC (r=0.366) in sepsis group for three days after ICU admission. The PCT, CRP and WBC level in septic shock patients were significantly higher than in patients with SIRS ($p<0.001$) (Table 5). However, there was significant decrease in PCT level in 2nd and 3rd day compared with 1st day in non infected and infected group. The cutoff value of PCT for infection diagnosis in both groups according to Youdin Index 1 ng/ml that had sensitivity (68%) (95% confidence interval (CI) 52.3-83.9) and specificity 83% (95%CI 66.2-95.1) (AUC=.902; 95% confidence interval .844-1.000). On the other hand, cutoff value of CRP for in-

fection diagnosis 20mg/ml with sensitivity 90% (95%CI 74.3-98.6) and specificity 46% (CI 27.5-52.6) (AUC: 0.380; 95% confidence interval 0.143-0.566). The cutoff value of PCT for diagnosis of septic shock 3 ng/ml with sensitivity 69% (95% CI 55.1-77.2) and 97% (95% CI 99.7-85.9) specificity (AUC: 0.995; 95% confidence interval 0.983-1.006) with higher positive predictive value(93%) than negative predictive values (75%).

Discussion

Our present study performed on critically ill patients admitted to ICU with suspected sepsis after major orthopedic surgery confirmed the diagnostic and discriminative roles of PCT in SIRS, severe sepsis and septic shock that improve management of patients. High serum PCT concentrations were first reported in children with severe bacterial infections, and were suggested to be a specific marker for bacterial infection[15]. PCT was a more reliable marker in the diagnosis of sepsis than other measures[6,16]. Al-Nawas and coworkers found that the patients with clinically documented infection had higher PCT levels than in those fulfilling the criteria for SIRS[17]. Also, Our results confirmed by Muller and colleagues (3) whom found that PCT is more reliable marker of sepsis than C-reactive protein, IL-6 and lactate levels. Moreover, Brunkhorst et al[18] documented that PCT measurement appears to be useful to discriminating between sepsis and severe sepsis in contrast to C-reactive protein, WBC and body temperature. Interestingly, Clech et al [19] found that in surgical group of patients levels of PCT were significantly higher in patients with than patients without septic shock.

Present work documented the correlation between PCT and other markers of inflammation as CRP and WBC in sepsis. In the same line of our result, Harbarth et al reported the excellent correlation of PCT with the other biological markers of sepsis as cytokines, CRP and white blood cells (5).

Additionally, this study reported that PCT had significant sensitivity, specificity, positive and negative predictive power but the

specificity and positive predictive power are higher in SIRS, severe sepsis and septic shock diagnosis. Although, C-reactive protein had more sensitivity in infection and septic shock prediction than PCT, PCT had higher specificity, positive and negative predictive value in infection and septic shock. PCT is much more useful for its negative predictive value. As it has no value in the assessment of fungal or viral infections and shows no response to intracellular microorganisms (such as Mycoplasma) or in local infections with no systemic response[20]. In the previous studies that compared CRP, IL-2, IL-6, and TNF-α and PCT separately for differentiating SIRS from sepsis. PCT had the highest sensitivity and specificity for differentiating SIRS from sepsis when compared with IL-2, IL-6, IL-8 and TNF-α [21]. In bacteramia identification, Jones and colleagues study found that PCT had higher sensitivity (76%) and specificity (70%)[22]. On the other hand, we found that PCT cutoff level 3 ng for septic shock diagnosis had significant sensitivity and specificity with high positive predictive like hood ratio 19.7. Contradictory to our results, clinical study compared PCT and CRP in ICU patients and found that PCT had poorer sensitivity, specificity than did CRP as a marker of sepsis [23]. Clec'h et al [18] reported that at a cutoff value 9.7 ng for septic shock diagnosis, PCT had significant sensitivity 91.7 and specificity 74.2 with high positive predictive like hood ratio 3.55. In controversy to our study which reported that PCT had lower sensitivity 69% at cut off value of 3ng. Surprisingly, Ugrate and colleagues [16] and reported that CRP had sensitivity of 71.8% and specificity 66.6% for infection identification (at cutoff level of 79 mg/ml) in critically ill patients. Chan and coworkers[24] demonstrated that high sensitivity of CRP 67.2% in contrast to our study that had poor specificity (46%) (at cutoff level 20mg/ml).

The possible reason for this controversy may be attributed to the fact that our study was done on critically ill patients after major orthopedic surgery, on smaller number of patients and the samples were taken on three days after admission but in the other

studies only single sample was taken within the 12-24 hours after SIRS or shock. Also, PCT assay must be a quantitative one with an appropriate functional sensitivity (0.06μg/L) to detect low levels of PCT. The use of semiquantitative test kits is likely to be problematic, with their high threshold (0.5μg/L) increasing the risk of false negatives (3). Sepsis is not the only cause of increased PCT level postoperatively, but may be induced by transient bacterial contamination or by translocation of bacterial endotoxins due to gut wall congestion or ischemia during the operation. Additionally, PCT could be induced by cytokines that are released during normal postoperative wound healing process, these explained persistent high PCT level 48-72 hrs after surgery. Also, there are many unknown pathogenic pathways reflected by the differences in the level of PCT according to the type and extent of surgery [25].

Conclusion

Present study demonstrated that PCT had diagnostic and discriminative role between SIRS and infection. PCT has higher specificity, positive and negative predictive power and lower sensitivity than C-reactive protein in SIRS, infection, severe sepsis and septic shock diagnosis. More studies are needed to determine more precisely the diagnostic cut off value of PCT in larger number of population with different types of surgery which more useful for its negative predictive value.

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DIAGNOSTIC AND PREDICTIVE POWER OF PCT FOR SEPSIS IN CRITICALLY ILL PATIENTS AFTER MAJOR ORTHOPEDIC SURGERIES

Ghada F. El-Rahmawy MD and Lamia Tawfik MD

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INTERSCALENE BLOCK, SHOULDER BLOCK : WHICH IS PREFERABLE FOR POSTOPERATIVE PAIN RELIEF AFTER ARTHROSCOPIC SHOULDER SURGERY?

Dalal E. M. Soud MD, Neven M. Gamil MD, and Mohamed Abdeen MD*

Departments of Anesthesia and Intensive Care and Orthopedic Surgery, Faculty of Medicine, Zagazig University, Egypt.*

Abstract

Background: *Postoperative pain still represents a major problem for patients and doctors. Opioids were used in the past to relieve pain, but they cause many side effects. So, searching for an alternative method was mandatory. Nerve blocks are a recent era to replace the opioids for postoperative pain relief. Objectives: This prospective study was designed to compare the efficacy of interscalene block and shoulder block (suprascapular and axillary circumflex nerve block) for postoperative pain relief after arthroscopic shoulder surgery. Patients and methods: Sixty patients ASA class I or II scheduled to undergo arthroscopic shoulder surgery were randomly, allocated to three equal groups; group I (general anesthesia group) received general anesthesia alone, group II (interscalene group) in which interscalene block was performed before induction of general anesthesia and group III (shoulder block group) in which shoulder block was performed before induction of general anesthesia. The nerve block was done guided by nerve stimulator. In all groups, induction of anesthesia was achieved by IV atropine 0.01 mg/kg, propofol 2 mg/kg, fentanyl 1 ?g/kg and atracurium 0.5 mg/kg, then patients intubated and ventilated. Maintenance of anesthesia was achieved using isoflurane 1.5-2% in oxygen and 0.1 mg/kg atracurium. The group I received additional doses of fentanyl intraoperatively to maintain the stability of hemodynamics. All patients were continuously monitored. Pain was assessed in recovery room, one hour, 2 hours, 4*

hours, 8 hours and 12 hours postoperatively using VAS score during rest and passive movement. The first sensation of pain, the first call for analgesia, total analgesic requirements for 12 hours, satisfaction score, simplicity of the technique and side effects were also noted. Results: The VAS showed highly significant decrease ($p < 0.001$) in the interscalene and shoulder block groups in comparison to group I in most of the studied times during rest and passive movement. Also, there was highly significant increase in the analgesic time achieved by both interscalene and shoulder blocks compared to the general anesthesia group (10.5 ± 2.3 and 9.6 ± 3.1 versus 1 ± 0.3). The total analgesic used also showed highly significant increase in the general anesthesia group (90 ± 15.5) in contrast to the other two groups (15 ± 4.5 and 17.5 ± 7.5 respectively). There was highly significant satisfaction in interscalene and shoulder block groups than that of group I (8.8 ± 0.6 and 7.8 ± 0.8 versus 1.5 ± 0.5). The interscalene group showed significant increase in complications and side effects as Horner's syndrome, weakness of the arm, hoarseness, and difficulty of breathing, but group I showed highly significant increase in nausea and vomiting than the other two groups. Conclusion: Shoulder block was effective as interscalene block for postoperative analgesia but superior than interscalene as it had minimal side effects. So, shoulder block can be used as an alternative technique for pain relief after arthroscopic shoulder surgery if there is any contraindication for interscalene block as chronic obstructive pulmonary disease.

Keywords: *interscalene block, shoulder block, suprascapular nerve block, shoulder surgery, pain relief.*

Introduction

Postoperative pain is a major problem after shoulder surgery especially on movement. This pain is due to nociceptive input from the joint tissues which causes deep somatic pain and reflex spasm of muscles supplied by the same spinal cord segments at the site of surgery[1].

Control of pain affects both the patient's wellbeing and the outcome of surgery and gives chance

for early rehabilitation which affects the success after major open orthopedic surgery[2].

In the past, this pain can be controlled by large-dose opioids, which causes nausea, vomiting, sedation or failure to control pain[3]. So, search for other techniques was started. General Anesthesia (GA) with a regional nerve block might reduce the postoperative pain. The brachial plexus supplies the motor and most of the sensory functions of the shoulder joint[4].

The shoulder joint is innervated by axillary, suprascapular, subscapular and musculocutaneous nerves. So, analgesia for shoulder surgery requires blockade of C5-6 nerve roots or superior trunk[5].

Different methods of nerve blocks were tried to control pain. Interscalene Block (ISB) was the commonly used technique for pain relief after shoulder surgery, but it was associated with many complications due to inadvertent injection of local anesthetic into cerebrospinal fluid causes total spinal or into the epidural space or injection into the vertebral artery[6]. These complications were due to perpendicular direction of the needle to the cervical spine. So, many modifications in the direction of the needle to avoid these complications.

Although that, side effects still common due to unpredictable spread of local anesthetic to the near structures such as phrenic and vagus nerves and the stellate ganglion[7]. Also, there was a case report of severe hypotension after interscalene block for outpatient shoulder surgery[8]. So, it was necessary to search for another nerve block efficient as interscalene block but with less complications and side effects.

Suprascapular Nerve Block (SSNP) alone, affects the sensory innervation to the posterior shoulder joint and the surrounding tissues[9], as it supplies the supraspinatus and infraspinatus muscles with some branches to teres minor muscle[10].

But, this block does not cover the anterior portion of the shoulder joint, as the inferior, lateral

and anterior structures, deltoid and fibres of teres minor muscles are supplied by axillary nerve[10].

Recently, the block of axillary nerve [Articular Branch of Circumflex Nerve (ACNB)] in addition to the suprascapular nerve block, will provide complete shoulder joint analgesia[11].

On the basis of the study of Price[11], the hypothesis of this study was that the shoulder block (suprascapular nerve block and axillary nerve block "circumflex nerve") was effective as the interscalene block for postoperative pain relief after arthroscopic shoulder surgery, but with minimal side effects.

Patients and Methods

This prospective study was done in Zagazig University Surgical Hospitals between September 2009 to June 2011. After obtaining a written informed consent, sixty patients of both sexes (37 males and 23 females) ASA class I or II, aged 25-50 years and their weight ranges from 60-90 kg scheduled for elective arthroscopic shoulder surgery (frozen shoulder, recurrent shoulder dislocation, rotator cuff injury, supraspinatous tendonitis and subacromion bursitis) were entered in this study.

According to the outcome, 20 patients would be estimated to be included in each group as a sample size.

Patients were excluded if they had allergy to the studied drugs, refused regional anesthesia, pulmonary disease as chronic obstructive pulmonary disease, local infection, coagulation abnormalities, diabetes or previous neurological damage to the brachial plexus.

The sixty patients were divided randomly into three equal groups; each twenty patients: group I (control) in which patients received general anesthesia only, group II [Interscalene Block group (ISB)] in which patients had interscalene nerve block before induction of general anesthesia and group III [Shoulder Block group (ShB)] in which patients received shoulder block (suprascapular nerve block and axillary circumflex nerve

block) before induction of general anesthesia.

On arrival to the operating room, 18 gauge intravenous cannula was inserted into a peripheral vein in the arm not requiring surgery.

All patients were premedicated using dormicum (midazolam 0.05 mg/kg intravenous half an hour before operation).

The ISB and Shoulder Block (ShB) were performed before induction of general anesthesia guided by nerve stimulator (B-Braun-Stimuplex HNS 11-12218).

Technique of interscalene nerve block:

ISB technique was performed depending on Winnie's landmarks [12] and lateral modified technique of Borgeat[4]. The patient was supine, and the shoulder was pushed down. The neck of the patient was in neutral position, and the head slightly tilted to the opposite side. The anesthesiologist stood at the patient side to be blocked, to roll the fingers effectively. Ask the patient to lift his head up of the table to tense the sternomastoid muscle and allow identification of its lateral border. The fingers roll onto the anterior scalene muscle and down to the interscalene groove, this grove along the horizontal plane passed through the cricoid cartilage (at the level of transverse process of C6) where the brachial plexus traverses the neck perpendicular to that plane. Under sterile condition, 22 gauge 5 cm short bevel needle is inserted, directed caudally, posteriorly and medially. The needle is introduced slowly till parasthesia and the position was considered right when contraction of triceps muscle was obtained with current less than 0.5 mA.

Then, aspirate to avoid intravascular injection or cerebrospinal fluid injection, after that 30-40 ml 0.5% bupivacaine was injected.

Technique of shoulder block (suprascapular nerve block and axillary circumflex nerve block)[13]:

Suprascapular nerve block was described by Meier[14], this technique was achieved while the

patient was sitting with his arm in full adduction and his hands on knee, his head and shoulder were flexed. The anesthesiologist stood behind him and divided the spine of scapula into medial $^{3}/_{4}$ and lateral $^{1}/_{4}$, then drew line from this point parallel to the midline (vertebral column). Another line was drawn in the middle of the angle between this line and the outer $^{1}/_{4}$ of the spine. Then, 22 gauge needle was entered about 5 m under sterile condition and was directed downwards and medially. Aspirate to avoid intravascular injection, then inject 10 ml 0.5% bupivacaine.

Axillary nerve block[13,15] occurred at the point where the nerve passed through the quadrilateral space to lie posterior to the humerus. The patient is seated with the arm in full adduction. Take a point in the middle of aline connecting the anterior acromion with the inferior angle of the scapula and extend it laterally, this line represented the horizontal axis (quadrilateral space). Vertical axis was drawn from the posterolateral part of acromion down to the horizontal axis. 22-gauge needle introduced 2-3 cm through the skin at point of intersection the vertical axis with the horizontal axis. Correct position of the needle was achieved when there was contraction at the deltoid muscle or at supraspinatous and infraspinatous muscles led to abduction and external rotation of the arm, then 10 ml 0.5% bupivacaine was injected.

The block (ISB or shoulder block) was considered succeeded if there was sensory block (loss of cold sensation) along the distribution of the nerves.

After doing the block, all patients received general anesthesia with atropine 0.01 mg/kg, propofol 2 mg/kg, fentanyl 1 µg/kg and atracurium 0.5 mg/kg. Endotracheal tube was inserted and controlled ventilation was started. Anesthesia was maintained with isoflurane 1.5-2% in oxygen and 0.1 mg/kg atracurium to maintain blood pressure and heart rate with normal range (not exceeding 20% increase than the baseline values). The general anesthesia group (control group) needed supplementary doses of fentanyl 0.5 µg/

kg IV till 10 minutes before the end of surgery to maintain hemodynamics within normal limits.

At the end of surgery, anesthesia was stopped and the muscle relaxant was reversed with neostigmine 0.02 mg/kg and atropine 0.01 mg/kg.

All patients continuously monitored using standard monitors (pulse, blood pressure, oxygen saturation, capnograph and ECG) till completed the surgery and in the recovery room.

Visual Analog Scale score (VAS) from 0 = no pain to 10 = worst pain was used to assess the postoperative pain at rest and on passive movement in the following times: in recovery room; one hour, two hours, four hours, eight hours and twelve hours postoperatively.

The patient's need for analgesia was also recorded (when VAS > 3). Analgesia was achieved by incremental dose of IV pethidine. Also, the first time of feeling pain, 1st time calling for analgesia, total analgesic requirements during 12 hours and satisfaction score were recorded.

Satisfaction score was assessed using VAS score at 12 hours postoperatively (0 = not satisfied and 10 = full satisfaction).

Any complication during performance of the block e.g. pneumothorax, Horner's syndrome and hematoma was recorded.

Also, motor weakness, nausea and vomiting, hoarseness and simplicity of the technique were also assessed.

Statistical analysis:

Data were checked, entered and analyzed by using SPSS version 17. Data were expressed as mean ± SD for quantitative variables and number and percentage for categorical one. ANOVA and post hoc test, paired t test, chi-square (X2) and Fischer exact tests were used when appropriate. $p < 0.05$ was considered statistically significant.

Results

As regard the demographic data of all patients, there was no

significant difference between groups regarding the age, weight, sex and duration of surgery (table 1).

Table 2 represented the pain score during rest and passive movement between the three studied groups. There was highly significant decrease in the VAS score ($p < 0.001$) in ISB and ShB groups mostly in all the studied times in comparison to the control group. But, there was no significant difference in VAS score between ISB and ShB groups.

Table 3 showed early sensation of pain in the general anesthesia group comparable to ISB and ShB groups. Regarding the analgesic effect, there was highly significant increase in the analgesic time was achieved by ISB and ShB (10.5 ± 2.3 and 9.6±3.1 respectively) in comparison to the control group (1 ± 0.3).

Also, there was increase in analgesic effect of ISB group (10.5 ± 2.3) compared to the ShB group (9.6 ± 3.1), but this increase was not statistically significant. The total analgesic used allover 12 hours was highly significantly increased in the control group (90 ± 15.5) compared to the other two groups (15 ± 4.5 and 17.5 ± 7.5 respectively) as shown in table 3.

Table 3 showed also the satisfaction score which was highly significantly increased in the ISB group (8.8 ± 0.6) similar to the ShB group (7.8 ± 0.8) in comparison to the control group (1.5 ± 0.5).

Regarding the technical problems and its side effects, table 4 showed that there was significant increase in the side effects (Horner's syndrome "15%", muscle weakness "35%" and hoarseness "10%") in ISB group than the other two groups, but nausea and vomiting more in the control group (nausea "60%" and vomiting "15%").

The failure of block did not occur in groups II and III and this may be due to the use of nerve stimulator.

Also, the general anesthesia without block was the simplest technique than the other two groups. The ShB group showed

more difficulty in the application due to the use of axillary nerve block which was a new technique and lack of technical experience as the other techniques, also the need for two separate blocks.

Table (1): Demographic data between the studied groups

	Control group I (GA) (n = 20)	Group II (ISB) (n = 20)	Group III (ShB) (n = 20)	p
Age (years)	35.3 ± 7.4	33.8 ± 7.1	37.6 ± 8.2	0.29
Weight (kg)	72.3 ± 10.7	70.8 ± 8.5	71.9 ± 9.5	0.88
Female/male	9/11	7/13	7/13	0.75
Duration of surgery (minutes)	96 ± 30	102 ± 30	84 ± 36	0.39

Data was expressed as mean ± SD or numbers.
GA = General Anesthesia
ISB = Interscalene Block
ShB = Shoulder Block
$p < 0.05$ was considered significant

Table (2): Pain score during rest and passive movement at different times of the studied groups

	Control group I (GA) (n = 20)	Group II (ISB) (n = 20)	Group III (ShB) (n = 20)	p
VAS_R				
Recovery room	2 ± 1**	0	0	< 0.001
One hour	3 ± 0.5**	0	0	< 0.001
2 hours	4 ± 1.5**$^+$	0	0	< 0.001
4 hours	3 ± 0.5*	1 ± 0.5	0	< 0.01
8 hours	4 ± 1**$^+$	1 ± 0.5	2 ± 1	< 0.001
12 hours	5 ± 1.5**$^+$	3 ± 1$^+$	4 ± 1.5$^+$	< 0.001
VAS_M				
Recovery room	3 ± 0.5**	0	0	< 0.001
One hour	4 ± 1.1**	0	0	< 0.001
2 hours	4 ± 1.2**	1 ± 0.4	0	< 0.001
4 hours	5 ± 1.5*$^+$	2 ± 0.5$^+$	3 ± 0.6$^+$	< 0.01
8 hours	5 ± 1.5*$^+$	3 ±0.5$^+$	4 ± 1$^+$	< 0.05
12 hours	6 ± 2*$^+$	4 ± 0.5$^+$	5 ± 1$^+$	< 0.01

VAS_R = Visual Analog Scale at Rest
VAS_M = Visual Analog Scale at passive Movement
*$p < 0.05$ was considered significant.
**$p < 0.001$ was considered highly significant.
$^+p < 0.05$ when compared with the reading of recovery room

Table (3): Supplementary analgesia and satisfaction score in the studied groups

	Control group I (GA) (n = 20)	Group II (ISB) (n = 20)	Group III (ShB) (n = 20)	p
1st time of feeling pain (hours)	0.7 ± 0.4*	6.5 ± 1.7	7 ± 0.87	< 0.001
1st time to call analgesia (hours)	1 ± 0.3*	10.5 ± 2.3	9.6 ± 3.1	< 0.001
Total analgesic used over 12 hours (pethidine, mg)	90 ± 15.5*	15 ± 4.5	17.5 ± 7.5	< 0.001
Satisfaction score	1.5 ± 0.5*	8.8 ± 0.6	7.8 ± 0.8	< 0.001

Data was expressed as mean ± SD
*p < 0.001 was considered highly significant.

Table (4): Side effects in the three groups

	Control group I (GA) (n = 20)		Group II (ISB) (n = 20)		Group III (ShB) (n = 20)		p
	No	%	No	%	No	%	
Pneumothorax	0	0	0	0	1	5	0.36
Hoarseness	1	5	2	10	0	0	0.34
Difficulties in breathing	0	0	1	5	0	0	0.36
Horner's syndrome	0	0	3	15	0	0	0.04*
Weakness in the arm	0	0	7	35	1	5	0.002*
Nausea	12	60	1	5	1	5	< 0.001**
Vomiting	3	15	1	5	1	5	0.41

Data was expressed as number and percentage
*p < 0.05 was considered significant
**p < 0.001 was considered highly significant.

Discussion

Some of orthopedic surgeries require immediate postoperative movement at the site of operation to avoid deterioration as frozen shoulder surgery. But, the movement causes severe pain. So, the need for analgesics is mandatory, but they may cause many side effects. Now, the nerve block is an effective technique for postoperative pain relief with minimal side effects and allows early and efficient rehabilitation which causes decrease in postoperative adhesion, capsular retractions and formation of fibrous tissues[2, 4].

This study showed the application of new technique "shoulder block including the block of both suprascapular nerve and axillary nerve" which might achieve sufficient analgesia after arthroscopic shoulder surgery as that provided by the single-dose interscalene block and with minimal complications.

This study showed that the pain score at rest and on passive movement was low in shoulder block group, similar to that of interscalene group and in contrast to the higher level in control group. These results were in accordance with the results of Price[11] who found that suprascapular nerve block when combined with an axillary (circumflex) nerve block, provided complete shoulder joint analgesia.

Also, Singelyn et al.[16] provided that suprascapular nerve block achieved significant postoperative pain relief compared with placebo but provided less analgesia comparable to interscalene block.

The analgesic effect achieved by single injection or continuous infusion ISB was proved by many studies[4,11,13,16,17], and this was in contrast with the study of Haltiavaara et al.[18] who found that ISB failed to provide preemptive analgesia after shoulder surgery.

Also, this study showed that there was prolongation in the analgesic time in ISB and shoulder block groups compared to the control group, where there was increased in the time of 1st pain sensation and the time of 1st call of analgesia in ISB and

shoulder block groups than that in the control group.

The total analgesic used over 12 hours were less in both the interscalene and shoulder block groups comparable to the control group.

These results go hand in hand with the results of Price[11] who found that low morphine consumption postoperatively might be due to augumentation of suprascapular nerve block by axillary nerve blockade causing pain relief similar to that of interscalene block.

Regarding the satisfaction score of the patients, it was significantly increased in both the interscalene and shoulder block groups in comparison to the control group. The control group showed lower satisfaction score because of early sensation of pain, and early calling for analgesics, also there was higher incidence of nausea in 12 patients and vomiting in 3 patients due to severity of pain and greater postoperative use of analgesics.

But, the interscalene block and the shoulder block provided low incidence of postoperative nausea and vomiting in comparison to the general anesthesia group, and these results confirmed such efficacy as in studies done by Al-Kaisy et al.[3] and Laurila et al.[19].

In spite of the shoulder block achieved analgesia similar to that of the interscalene block, but it was superior than interscalene block (regarding the complications). The interscalene block caused Horner's syndrome in 3 patients and motor weakness in the arm in 7 patients but shoulder block does not cause motor weakness except in the muscles of the upper arm only and one patient in ShB group complained of weakness in the arm. These results were in agreement with the results of[7,16] who found that extensive paralysis of the muscles of the upper limb is considered a sign of effective interscalene block, but causes discomfort for patients while the motor weakness of shoulder block affects only the posterior three rotator cuff muscles (teres minor, supraspinatus

and infraspinatus) and the deltoid muscle.

Also, the interscalene block might cause other complications as hoarseness, Horner's syndrome as observed by Singelyn et al.[6] and ipsilateral hemidiaphragmatic paresis as observed by[6,20,21]. Also, Urmey and Jloegler [22] found that the forced vital capacity decreased by 41 ± 12%. But, the interscalene block and the shoulder block reduced the postoperative nausea and vomiting which were due to the excess usage of analgesics as with the control (general anesthesia) group.

The shoulder block in this study showed minimal complications in comparison to the interscalene block, and these results go hand in hand with the results of Singelyn et al.[16] who found that pneumothorax can occur if the needle traverses the suprascapular notch and puncture the pleura. So, many researchers[23, 24] found that the use of ultrasound with supraclavicular brachial plexus nerve block caused low incidence of complications. Also, possibility of intravascular injection or nerve injury may result.

Limitations

- The follow up of VAS scale was observed not more than 12 hours.

- We did not measure the cortisol level intraoperatively to assess the stress response and the analgesic effect of the block.

Conclusion

Shoulder block [suprascapular nerve block with axillary (circumflex) nerve block] was effective as interscalene block for postoperative pain relief but with minimal side effects. So, it is considered the technique of choice when there is contraindication for interscalene block (as respiratory disease and COPD patients).

Recommendations

The need for research about the efficacy of the shoulder block as a sole anesthetic for surgery as that of interscalene block.

Also, to do the shoulder block guided by ultrasound after training on the anatomical landmarks.

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INTERSCALENE BLOCK, SHOULDER BLOCK : WHICH IS PREFERABLE FOR POSTOPERATIVE PAIN RELIEF AFTER ARTHROSCOPIC SHOULDER SURGERY?

Dalal E. M. Soud MD, Neven M. Gamil MD, and Mohamed Abdeen MD

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NEOADJUVANT CHEMOTHERAPY IN ESOPHAGEAL CANCER : SINGLE INSTITUTION EXPERIENCE

Hany Eldeeb MD, David Hunter MD*, Waleed Abozeed MD and Shaffi Shaikh MD*****

Departments of Oncology Centre, Surgery, Northampton General Hospital, Cliftonville, Northampton, NN1 5BD, United Kingdom.*
***Department of Clinical Oncology and Nuclear Medicine, Mansoura University Hospital. Mansoura, Egypt.*
****Department of Family and Community Medicine, College of Medicine, King Saud University, Saudi Arabia*

Abstract

Background: *esophageal cancer is a major clinical problem with a generally poor prognosis. As a result there has been interest in combining surgery with neoadjuvant chemotherapy to try to improve outcomes. Evidence for clinical benefit from preoperative chemotherapy exists but it is not clear which patients (stage, tumor location, histology) will most benefit from this preoperative treatment.* ***Methods:*** *This study prospectively analyzed the outcome of 71 patients with operable esophageal carcinoma treated at Northamptonshire oncology Centre - UK during the period from January 2001 till July 2008. Patients were treated with 2 cycles of neoadjuvant chemotherapy followed by surgery. Data were analyzed using Kaplan- Meier plots, Cox regression modeling and Chi-squared test.* ***Results:*** *Median patient's age was 64. Male patients represent 83% of the cases. Performance status 1 patients represent 63%. Surgical resection was done for 63 patients (88.7%). Two year OS in this cohort is 5.6%. Univariate analysis identified only surgical resection to be associated with better prognosis (P<.0001). Multivariate analysis identified surgical resection (Hazard Ratio (HR) = 44.03, 95% CI [13.15-147.3], P < 0.0001) and pathology type (HR = 15.05, 95% CI [2.1-107.7], P = 0.007 to be the significant independent prognostic factors for survival.* ***Conclusion:*** *Our survival data for operable esophageal cancer is*

poor even with use of neoadjuvant chemotherapy. Lack of dedicated upper gastrointestinal surgeon and unavailability of PET scan staging during the study period might attribute to the dismal outcome.

Key words: *Neoadjuvant chemotherapy, esophageal cancer, surgery.*

Introduction

Adenocarcionma (AC) and squamous cell carcinoma (SCC) are the two principle variants of esophageal cancer diagnoses and account for > 98% of caes [1]. .Historically, AC and SCC have been treated as a single disease entity with many older clinical trials not differentiating between the two histologies, even in the study populations [2]. Over the years, however, a great deal of evidence has been compiled to support the notion that AC and SCC represent two separate diseases based on their differing etiology, epidemiology, prognosis, and response to treatment[3-6].

Recent epidemiological data also show variations in the incidence of histological types since AC now accounts for more than 50% of newly diagnosed cases [7–9].

Debate regarding the current standard of care for the management of esophageal cancer is ongoing [10-12].

Surgery alone is only really curative for tumors confined to the esophagus itself and to the resectable peripheral tissues including the regional lymph nodes. Despite progress in surgical techniques, and the extension of surgical resection, survival has not improved, which highlights the need for additional therapies [13].

From a purely theoretical point of view, preoperative therapy should make it possible to eradicate micrometastatic disease and to shrink the tumor, even to the point of reducing its stage, thus making surgical resection easier. The objective of neoadjuvant treatment is to increase the chances of R0 resectability, to reduce the incidence of local relapse and to treat micrometastases early [12].

Adjuvant chemotherapy with cisplatin-based regimens com-

pared to surgery alone has been examined in three separate phase ? trials[14-16], with none of them reporting a statistically significant difference in OS, although Ando and colleagues have reported a 5-year disease-free survival (DFS) advantage (55% vs 45%, P = 0.037)[16]. In the neoadjuvant setting, there have been multiple randomized trials that have compared varying chemotherapeutic regimens to surgery alone[17-25]. Clinical complete responses based on direct visualization and an assortment of imaging modalities have ranged from 19% to 58%, but the rate of pathological complete response (pCR) at the time of surgery was a disappointing 2.5%-13%. This is an unsurprising trend considering the relative ineffectiveness of chemotherapy alone in the treatment of esophageal cancer [17-25].

Neoadjuvant chemoradiation remains a controversial strategy in the treatment of SCC and AC of the esophagus. Until recently, randomised studies mixed the two, often without any distinction.

To date, at least nine randomized phase ? clinical trials have compared neoadjuvant chemoradiotherapy with surgery alone[18, 26-34]. These trials incorporated multiple chemotherapy regimens, doses of radiotherapy used (20-50.4 Gy), and timing of radiotherapy with regard to chemotherapy (sequential vs concurrent), in addition to differing by surgical procedures performed and histological types of esophageal cancer enrolled (AC, SCC, or both). Only two of these trials have revealed a significant survival benefit that favored multimodality treatment, and neither was without its imperfections [29,33].

Patients and Methods

This study prospectively analyzed the outcome of 71 patients with operable esophageal carcinoma treated at Northamptonshire oncology Centre – UK during the period from January 2001 till July 2008. Histopathologic diagnosis was based on morphologic criteria according to WHO criteria. Patients were staged according to the TNM staging system for esophageal cancer with Corresponding American Joint Committee on

Cancer stage grouping. Data from the files included information about physical examinations, chest X-rays, Computed tomography scans of chest and abdomen, and upper endoscopy.

Clinical and pathological variables analyzed included patient age, sex, performance status, tumor stage, pathology type and treatment modalities. The chemotherapy received included cisplatin 80 mg/ m^2 on day 1 and infusional fluorouracil 1000 mg/m^2 daily on days 1-4, or cisplatin 80 mg/m^2 and oral capecitabine 800 mg/m^2 BID on days 1-14. The primary end point was overall survival. End points were calculated from the date of diagnosis.

Overall survival (OS) was evaluated using the Kaplan-Meier method and logrank tests. The Cox proportional hazards model was used to estimate the independent factors prognostic for OS. All analyses were carried out using SPSS software (version 17.0, SPSS Inc., Chicago, IL), and a significance level of 0.05 was used.

Results

Clinical features:

In this cohort (n = 71), the 2-year OS is 5.6%, and the median OS is 6.3 months, 95% CI 4.56-8.04 (Fig-1). Patient age ranged from 50 - 78 years with a median age of 64 years. Eighty three percent of the patients were males. Most of the patients (63%) had performance status 1 with median OS 7.1 months (95% CI 5.9-8.2) with no statistical significant difference according to performance status (P=.731). Esophageal adenocarcinoma represent about 52 %with median OS 5.7 months (95% CI 3.3-8) with no statistical significant difference with other histologic types (P=.79)). TNM staging of the tumors of this cohort Included 19 patients (26.8%) with stage I disease having median OS 5.6 months (95% CI 3.6-7.6), 17 patient (23.9%) with stage II disease with median OS 7 months (95% CI 2.8-11.2), and 23 patient (32.4 %) with stage III disease with median OS 6.3 months (95% CI 3.9-8.7) . However the difference between different stages is statistically non significant (P=.817) (fig-3). Fifty nine patients (83%) received cisplatin and 5 FU

with median OS 5.6 months (95% CI 3.7-7.4) versus 8.1 months (95% CI 5-11.2) for those received cisplatin and capecitabine (13 patients) and the difference is statistically non significant (P=.608). Surgical resection was done for 63 (88.7%) patients with median OS 7.1 months (95% CI 6.1-8.1) vs 1.6 month (95% CI 1.7-2.7) for those with no surgery(Fig-2), and the difference is highly significant (P<0.0001) (Table 1).

Univariate and multivariate analysis

Various clinicopathologic variables were also evaluated to identify potential prognostic factors for survival. Univariate analysis identified only surgical resection to be associated with the prognosis of the patients HR 26.24 (95% CI 8.9-77.3, P <.0001). in contrast, patient age (HR11.04, 95% CI 0.64-1.6, P = 0.88), sex (HR1.36, 95% CI 0.71-2.07, P= 0.34), stage 2 (HR1.14, 95% CI 0.58-2.22, P = 0.69), performance status 1 (HR .71, 95% CI 0.32-1.63, P = 0.43),SCC pathology type (HR 1.13, 95% CI 0.65-1.99, P =0.65) were not found to be prognostic for survival (table 2).

Cox proportional hazard regression analysis of patient survival based on clinical and pathologic factors was also performed. Multivariate analysis identified surgical resection (HR 44.03, 95% CI 13.15-147.3, P < 0.0001), and undifferentiated pathology subtype (HR 7.03, 95% CI 1.8-26.2, P = 0.004) to be the significant independent prognostic factors for survival. In contrast, patient age, sex, stage, and performance status was not found to be significant independent prognostic factors for survival (table 2).

Table 1: the clinical and pathological features of the esophageal carcinoma cases examined with the median survival time

variables	Case(%)	Median survival time (months)			X^2	P
		Estimate	95% CI			
Age						
≤65	37(52.1)	6.2	3.2	9.3	.02	.88
>65	34(47.9)	6.3	4.3	8.3		
Sex						
male	59(83.1)	6.3	4.8	7.8	.9	.34
female	12(16.9)	5.2	0	10.5		
Ps						
0	7(1.7)	4.4	3.7	5.1	1.2	.731
1	45(63.4)	7.1	5.9	8.2		
2	3(4.2)	5.2	3.4	6.9		
unknown	16(22.5)	5.2	2.8	7.5		
Stage						
1	19(26.7)	5.6	3.6	7.6	.93	.817
2	17(23.9)	7.0	2.8	11.2		
3	23(32.4)	6.3	3.9	8.7		
unknown	12(16.9)	5.2	3.6	7.9		
Pathology type						
adeno	37(52.1)	5.7	3.3	8.0	.24	.97
sqcc	19(26.7)	5.6	2.1	9.1		
undiff	9(12.6)	6.3	6.2	6.4		
unknown	6(8.4)	8.1	5.0	11.2		
Surgery						
yes	63(88.7)	7.1	6.1	8.1	71.8	.0001
no	8(11.3)	1.7	0.7	2.7		

Table 2: Cox proportional hazard regression analysis of patient survival

variables	Cox (OS)			
	Univariate		Multivariate	
	HR (95%CI)	p	HR(95%CI)	p
Age				
≤65	1.04 (.64-1.6)	.88	----------	------
>65	1.0			
Sex				
male	1.36 (.71-2.07)	.34	----------	--------
female	1.0			
Ps				
0	1.0			
1	.72 (.32-1.63)	.43	----------	---------
2	.66 (.17-2.62)	.56		
unknown	.91 (.39-2.32)	.91		
Stage				
1	1.0			
2	1.14 (.58-2.22	.69	----------	--------
3	.84 (.45-1.58)	.61		
unknown	1.05 (.51-2.2)	.88		
Pathology type				
adeno	1.0		1.0	
sqcc	1.13 (.65-1.99)	.65	.96 (.11-1.8)	.89
undiff	1.10 (.53-2.31)	.78	7.03 (1.8-26.2)	.004
unknown	1.0 (.41-2.42)	.99	15.1(2.1-107.7)	.007
Surgery				
yes	26.24 (8.9-77.3)	< .00001	44.03(13.1-147.3)	<.00001
no	1.0		1.0	

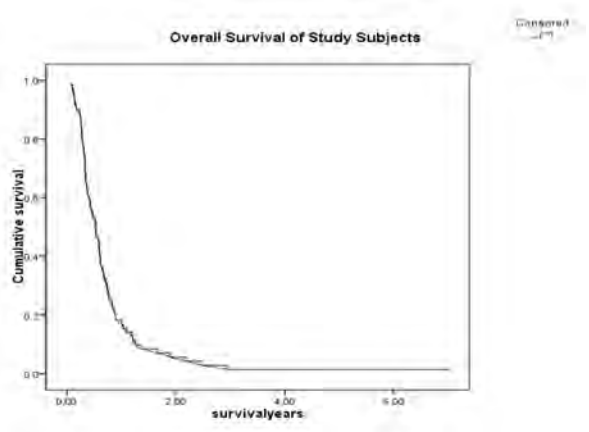

Fig 1. Kaplan-Meier Curve for allover survival

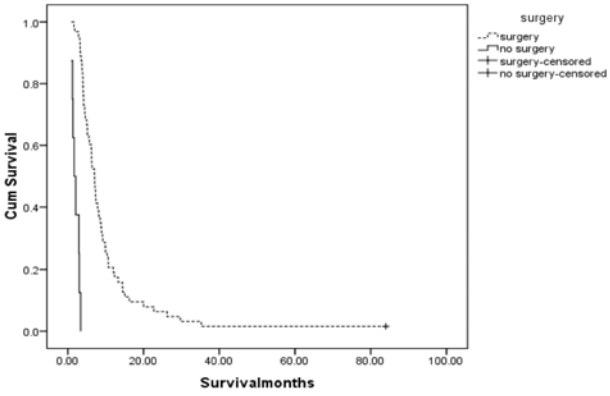

Fig 2. Kaplan-Meier Curve for OS in relation to surgery

Discussion

The management of esophageal carcinoma has been evolving over the past 30 years, despite recent improvement in detection and treatment, the overall survival still poor.

A dramatic shift of histology of cancer esophagus have been observed in USA and some parts of Europe[4], where SCC have become increasingly less common accounting for less than 30% of all esophageal malignancies which nearly corresponds to our finding in this study, where SCC accounts for about 27%.

The risk of SCC decreases substantially duo to smoking cessation, while adenocarcinoma increases duo to increase in gastric esophageal reflux disease and Barrett's esophagus which are major risk factors for adenocarcinoma [35].

The patient outcomes may correlates with initial stage of cancer at diagnosis, but the best correlation with survival is associated with the surgical pathologic stage. However, in our study the tumor stage did not affect the survival which may be explained by small number of the patients and lack of

statistical power, and inability to accurately stage about 17% of the patient's duo to lack file documentations.

Until recently, the therapeutic management of AC and SCC were similar and the results were considered together, often without distinction between the two. Yet the terrain, the nature of the evolution and the clinical presentation are very different. Nevertheless, large retrospective surgical series revealed a prognostic difference between SCC and AC [36], were survival was worse for SCC, which does not seems to correlate with our results were both histologies do the same. This can be explained by small number of patient in the study.

Multiple modalities have been employed for the treatment of esophageal carcinoma because of poor survival rates of patients who have been treated with resection alone. So chemotherapy has been investigated in the preoperative setting.

In our study, median OS is 6.3 months and the 2-year survival is 5.6%, which is nearly similar to that mentioned by Nygaord et al [18] and Schlag et al [19] (7 months), and is much lower than that mentioned by Maipang et al [20] and Law et al [21] (17 months) and Ancona et al [22] (25 months).

It is also much lower than that reported at the UK MRC trial that included 802 patients of all histologies, and randomized to 2 cycles of cisplatin and infusional fluorouracil vs surgery alone. A rather striking distinction of this trial compared to others was that clinicians could give their patients neoadjuvant radiotherapy (25-32.5 Gy) irrespective of randomization, and 9% of patients on each arm received radiotherapy. OS was improved in the neoadjuvant group (HR: 0.79, 95% CI: 0.67-0.93, P = 0.004), with a median OS of 16.8 mo vs 13.3 mo, respectively. However, several clinical methodological problems was found in this trial and 10% of the patients received off protocol preoperative radiotherapy, and the patients accrued from China were excluded. [23].

This low rate of median OS can

be explained by that about 11% of the patient did not perform surgery, about 32% of the patient had stage ??? disease, and accurate staging by PET scan was not available during the study period and unavailability of dedicated upper gastrointestinal surgeon at that time.

Another large trial by Kelsen et al [26] has evaluated neoadjuvant chemotherapy in the Intergroup (INT) 0113 study with 440 patients; however, no difference in OS was reported. Two large meta-analyses also have failed to demonstrate a survival advantage with neoadjuvant chemotherapy[37,38], although another meta-analysis by Gebski et al [39] has reported a statistically significant OS benefit with neoadjuvant chemotherapy (HR: 0.90, 95% CI: 0.81-1.00, P = 0.05), which corresponds to a 2-year absolute survival benefit of 7%. Caveats to this meta-analysis are that no statistically significant benefit was seen for patients with SCC treated with neoadjuvant chemotherapy (HR: 0.88, 95% CI: 0.75-1.03, P = 0.12) and that, although there was a benefit seen with AC (HR: 0.78, 95% CI: 0.64-0.95, P=0.014), these results were based solely on the single trial whose data were available for review - the MRC trial [23,39]. At least four separate trials have compared cisplatin based perioperative regimens (neoadjuvant and adjuvant chemotherapy) to surgery alone in esophageal cancer[17,40-42]. Those that focused solely on esophageal cancer did not reveal survival benefits[17,40], whereas the two trials that included patients with AC of the stomach and gastroesophageal junction (GEJ) did show such a benefit [41,42]. The largest of these, published by Cunningham and colleagues, randomized 503 patients with AC to three preoperative and three postoperative courses of epirubicin 50 mg/m^2 and cisplatin 60 mg/m^2 with infusional fluorouracil 200 mg/m^2 per day for 21 d vs surgery alone. Although the majority of patients had gastric AC, approximately 26% of the patients enrolled had AC of the GEJ or distal esophagus. Despite the fact that 58% of patients were unable to tolerate all six cycles of chemotherapy, the perioperative chemotherapy group had a statistically

significant higher likelihood of OS compared to those treated with surgery alone (HR: 0.75, 95% CI: 0.60-0.93, P = 0.009), with an improved median OS (24 mo vs 20 mo) and 5-year OS (36% vs 23%). Although postoperative complications were not increased (46% vs 45%), there was also no difference in the rate of R0 resection (69% vs 66%) or pCR (both 0%). Importantly, there was no evidence of heterogeneity of treatment effect based on the location of the primary tumor [41].

Conclusion

Our survival data for operable esophageal cancer is poor even with use of neoadjuvant chemotherapy. Lack of dedicated upper gastrointestinal surgeon and unavailability of PET scan staging during the study period might attribute to the dismal outcome.

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NEOADJUVANT CHEMOTHERAPY IN ESOPHAGEAL CANCER : SINGLE INSTITUTION EXPERIENCE

Hany Eldeeb MD, David Hunter MD, Waleed Abozeed MD and Shaffi Shaikh MD

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BOTULINUM TOXIN-A AS ANTICHOLINERGIC AGENT, DOES IT HAS A ROLE IN SECRET-MOTOR RHINITIS?

Mohamed A. M. Eltoukhy MD, Yehia Ebid MD* and Eman R. Aamer Ph.D**

*Departments of ENT, *Internal Medicine and*
***Clinical Pathology, Benha Teaching Hospital, Egypt.*

Abstract

Background: *Secret-motor or hyper reflexive rhinopathy or intrinsic rhinitis, is characterized by congestion and rhino rhea. Exacerbations are trigged by several factors including temperature changes, physical agents, certain odors, hot spicy foods and drug abuse as well as gastro-oesophageal reflux diseases. The patho-physiologic mechanism of secret-motor rhinitis is not known, yet, it is suggested to be the result of autonomic nervous system dysfunction. Treatment of nasal hyper-secretion of intrinsic rhinitis has no long-term efficacy.* ***Objective:*** *The aim of this study was to evaluate the role of Botulinum toxin type A (Botox-A) as anti-cholinergic agent in secret-motor rhinitis. Methods: Thirty adult patients of both sexes with secret-motor rhinitis who had negative skin prick test and normal or low IgE blood level were recruited in this study. They were divided randomly and equally into two groups A & B. In group A, 10 U Botox-A was injected into the inferior and middle turbinate of both nasal fosses of each case, while in group B as a control, saline was injected. Changes of symptoms were scored by the patients in four point scales.* ***Setting:*** *Benha Teaching Hospital.* ***Results:*** *There were a statistically significant reduction in symptoms scores of nasal rhino rhea and sneezing in group A compared with group B (all p <.05). Nasal congestion symptom was insignificant statistically lowered in group A, while it remained unchanged in group B.* ***Conclusion:*** *Intranasal injection of Botox-A as anti-cholinergic agent may have a role to ameliorate the symptoms of secret-motor rhinitis; especially rhino rhea*

and sneezing. It is a simple, safe, and effective symptomatic modality with a reasonable lasting effect

Introduction

Secret-motor rhinitis or intrinsic rhinitis is characterized by rhino rhea, sneezing, congestion and pruritis.[1] Exacerbations are triggered by several factors including temperature changes, emotional disturbance, physical agents, hot spicy foods and certain odors[2] as well as gastro-esophageal reflux diseases.[3] IgE-mediated hypersensitivity does not play a role in the etiology.[1]

The nasal airway patency is under control of the autonomic nervous system. The pathogenesis of Secret-motor rhinitis is unknown, but it is suggested to be an imbalance of the autonomic nervous system with a relative dominance of the parasympathetic nervous system in the nasal mucosa.[4] The treatment measures of intrinsic rhinitis includes: patients counseling, topical anti-histamines as azelastine nasal spray[5] and nasal anti-cholenergic as ipratropium bromide[6]. Yang et al[7] used topical corticosteroid and (Benninger & Ahmad)[8] proved the safety of its application in secret-motor rhinitis, yet, its efficacy has been not consistent[9]. Al-Samarrae[10] used local application of silver nitrate in intrinsic rhinitis with limited results. Oral vasoconstrictors in perennial non-allergic rhinitis had been used.[11] Prasanna & Murthy[12] managed hyper-reflex rhinopathy by sphenopalatine ganglion block. The safety of Botox-A has been approved with no reported visual complications after intra-turbinal injection.[13]

Botulinum toxin-A is a neurotoxin that inhibits the release of acetylcholine from the presynaptic nerve terminal at the neuromuscular and neuroglandular junction and it was suggested that local Botox-A may has a potential role in the treatment of intrinsic rhinitis.[14] Thus, it might be useful in blocking the cholinergic control of nasal symptoms. Recently, Botox-A is used in otorhinolaryngology for different dysfunctions like spasmodic dysphonia and tinnitus[15], gustatory sweating[16], primary headache[17],

dysphagia, sialorhea, facial and cervical movements disorders.[18] Gazerani et al [19] showed the suppressive effect of Botox-A on vasomotor reaction in human skin. Botulinum toxin-A has a better effect on allergic rhinitis symptom relief in terms of duration and degree than steroid injection.[7] Our aim was to evaluate the role of intranasal injection of Botulinum toxin type-A, as anti-cholinergic agent on the symptoms of secret-motor rhinitis.

Patients and Methods

Thirty adult patients with secret-motor symptoms for at least 4 months, age ranged from (18-56 years) of both sexes, with negative skin prick test for 5 common allergens in our locality (House dust mite extract, mixed pollen extract, mixed mould extract, dust extract, dog hair and cat fur) using modified skin prick test[20] to exclude allergic diseases. All the cases were exposed to: full ENT, medical exams to exclude allergy or other diseases that might be aggravated by anti-cholinergic therapy, anterior rhinoscopy and nasal endoscopy to exclude structural deformities or nasal pathology. Enzyme immunoassay for the quantitative determination of immunoglobulin E (IgE) concentration in the serum to exclude atopic allergic patients was done using Clinotech Diagnostics kits (Clinotech Diagnostics & Pharmaceuticals, Inc. Canada) and patients with normal (150 IU ml) or low total IgE were only enrolled in the study. The symptoms of the patients were scored through 0 to 4, with 0 as no, and 4 as severe. Patients were randomly and equally divided into 2 groups. Group A, consisted of 6 females and 9 males with mean age 32.6 years (range 18-52 yr) in which each patient was injected locally with 10 units of Botox-A (REFINEX, Pharmaceutical P.R. China) into the inferior and middle turbinate with a total dose of 20 units in each nasal fosse. One box of Botox-A contains 50 U which was diluted in 5 ml saline and the final solution of 1 ml contains 10 U of Botox-A. After topical lidocaine HCL 2%, one ml of Botox-A dose (10U) was injected into one point of anterior end of inferior and middle turbinate of left and right nasal cavities using 1-ml insulin syringe. Group B (control group) included 5 women and 10

men, with mean age 37.4 years (range 22-56 yr) in which 1ml of isotonic saline was injected into the same four points of the nasal fosses. All the patients have been instructed to stop any medical therapy and to record their subjective symptoms, number of facial tissues used daily at a nose diary throughout the clinical trial, starting one week before the injection, as well as any side effects after the local application such as dry nose, bleeding per nose or smell disorders. The patients were followed up week 1, 2, 4, 6, 10. The patient's subjective impression was recorded at each visit. Informed medical consent was taken from each case included in the study. Chi-square, paired t-tests were used for statistical analysis and Wilcoxon Rank-sum method to compare the nasal symptoms.

Results

All the patients had completed the study in group A, except 2 cases who were missed after the 4th week, while in the control group B, 3 cases did not follow up after the 2nd week. A considerable improvement of the total symptoms in both groups started after the first week and attained maximum subjective effect after 6 weeks follow up in group A. The total duration of the efficacy of Botox-A on nasal symptoms was up to 8 weeks, compared with saline which lasted one week. There was a statistically significant reduction of symptoms scores (rhino rhea, and sneezing) in group A compared with group B in all the follow up weeks with ($p = 0.011$, 0.008, 0.008, 0.009, and 0.029) at 1^{st}, 2^{nd}, 4^{th}, 6^{th} and 10^{th} weeks respectively. The subjective reduction of nasal rhino rhea was achieved by 40.4% in patients of group A, compared with 7% in group B. The sneezing symptom was reduced by 24.4% in patients of group A and lasted for 7weeks, compared with 4.2% in the control group which lasted 2 weeks. Nasal obstruction was relieved in group A, by 5.1% for one week and remained unchanged in group B. Itching symptom was improved by 10.5% in group A, with mean duration of 2 weeks, but only 2.1% in group B that lasted one week, with no statistically significant difference at the 10th control week ($p = 0.121$). In both groups, there

were no side effects such as nasal bleeding, allergic reaction, smell affection or dry nose; except two cases suffered from nasal itching, one case with mild erythema at the sites of injection and one female patient developed nasal irritation with mild crusting in the 2nd week whom managed locally with Triamcinolone Acetonide ointment 0.1%; and all were in group A.

Discussion

Secret-motor rhinitis or reflex-hyperactive rhinopathy is a common disorder that it has a diagnosis by exclusion with low or normal IgE level, negative allergic skin tests and lack of structural deformity or identifiable inflammatory signs.[21] The prominent symptoms of intrinsic rhinitis are rhino rhea, sneezing, and nasal blockage caused by sympathetic hypo activity.[22] The therapeutic options for intrinsic rhinitis include avoidance of non-specific stimuli, local decongestant, oral vasoconstrictor, topical steroid, topical anti-histamines, topical anti-cholinergic, and surgical measures.[23] Botulinum toxin-A is a neuro-toxin that inhibits the release of acetylcholine from the pre-synaptic nerve terminal at the neuro-glandular and neuro-muscular junction.[15] Nasal patency and secretions are mainly controlled by autonomic nervous system.[3] Yang et al[7] reported the potent anti-cholinergic effect of Botox-A on nasal mucosa regarding the symptoms of secretion, sneezing, and obstruction. Blocking the release of acetylcholine from the cholinergic nerve terminal in the nasal mucosa or preganglionic cholinergic nerve endings in spheno-palatine ganglion are the two suggested mechanisms of Botox-A action in the nasal cavity[14] Rohrbach & Laskawi[24] postulated the induction of apoptosis of submucosal seromucinous gland by Botox-A in the nasal cavity. Lance[25] attributed the relief of nasal congestion by Botox-A to its blocking effect, on the vaso-active polypeptides which are potent vasodilators.

Rohrbach et al[26] reported subjective reduction of nasal secretion with topical application of Botox-A with sponge to the nasal mucosa up to 46.4% in patients with intrinsic rhinitis and it was 24-42%

by local injection[14] while, we had incidence of 40.4% and theses variable scores might be due to the difference in the method of application. The mean duration of subjective improvement of nasal symptoms in our series lasted 8 weeks, compared with 12 weeks[26] using higher dosage and topical application. Unal et al[27] described significant reduction of sneezing in patients with allergic rhinitis with different dosages of Botox-A (40 or 60 units) and obtained similar results, while Kim et al[14] did not report significant reduction in this symptom with intrinsic rhinitis. We had reduction in sneezing symptom up to 24.4 % and this might indicate an important role of acetylcholine as a relevant neurotransmitter in sneezing reflex. Unal[28] showed significant reduction in the nasal congestion with Botox-A in patients with allergic rhinitis, Kim et al[14] proved no change in nasal stuffiness in patients with intrinsic rhinitis, while we had a reduction of this symptom up to 5.1%. This low improvement in nasal stuffiness may be due to the fact that nasal congestion is mainly regulated through the volume of nasal blood vessels. However, Botox-A may reduce the sensory neuron sensitivity of the nasal mucosa and can alleviate the subjective symptoms of nasal stuffiness, sneezing and itching.

The effective duration of Botox-A in secret- motor or intrinsic rhinitis is not constant and it shows a variation in different studies. While, Kim et al[14] had 4 weeks duration, Ozcam et al[13] got 10 weeks, Rohrbach et al[26] obtained 12 weeks, and it lasted 8 weeks in this study. This may be due to the difference in the technique of application, dosage and the manufacturing material used. Injection method allows precise control of the dosage, while the topical one does not. The higher Botox-A dose used, the longer time of efficacy is obtained with a toxic dose if greater than 200 U.[29] Although we had no gross adverse side effects in this study, yet, our results were based on subjective satisfaction of a reasonable duration.

Conclusion

Botulinum toxin-A as anticholinergic agent has a significant

role in secret-motor rhinitis especially in the improvement of rhino rhea and nasal sneezing. We concluded that intra-turbinal nasal injection of Botox-A is quite effective, safe, and easily applied symptomatic treatment option. Botox-A is a good alternative to the standard therapeutic regimens for the treatment of secret-motor rhinitis. Further study to assess nasal symptoms objectively, may help to determine the optimal therapeutic dose of Botox-A needed to lengthen the duration of efficacy and to know how can repeat the application safely.

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ACOUSTIC PHARYNGOMETRY, COULD IT PREDICT OBSTRUCTIVE SITES IN SUBJECTS WITH SLEEP APNEA?

Mohamed A. M. Eltoukhy MD and Samer Attia MD

Department of ENT, Benha Teaching Hospital, Egypt

Abstract

Objective: *is to evaluate the role of acoustic pharygometry as a tool in assessing the narrowing sites of the upper airway in obstructive sleep apnea syndrome (OSAS) patients, to plot a predictor diagnostic curve if would be, and to choose the proper management technique.* ***Study design:*** *A prospective, clinical trial.* ***Methods:*** *Thirty adult patients of both sexes with OSA who had at least 2 of 3 major symptoms of snoring, daytime somnolence, and apnea with witness were recruited in this study. Another 30 healthy young adult individuals of both sexes without OSA (as control) were included. Assessment of the dynamic changes in the upper airway (UA) for the control group and the patients by acoustic pharyngometry in upright sitting and supine positions were done. The pharyngeal parameters for each patient were compared with the mean standard parameters of the control.* ***Setting:*** *Benha Teaching Hospital.* ***Results:*** *A significant difference in parameters were observed between sleep disordered patients and the control ones in the amplitude of the oral wave, the extension and amplitude of the Oro-Pharyngeal segment and the hypopharyngeal area. Patients with moderate and severe OSA had significantly narrower cross-sectional area at the level of Oro-pharyngeal junction especially in supine position; during expiration compared with patients with mild OSA ($p < 0.05$) and the control group ($p < 0.003$).* ***Conclusions:*** *Acoustic pharyngometry is a simple test both in predicting the oral and hypopharyngeal sites of obstructive sleep apnea, choosing and post-operative monitoring of palatal surgery in patients with sleep disorders.*

Introduction

Obstructive sleep apnea (OSA) is absence of airflow despite persistent ventilator effort. It is characterized by recurrent total or partial upper airway narrowing or collapsing that occurs at different sites during sleep with significant health, socioeconomic impact and high prevalence.[1] It is manifested clinically with snoring, restless sleep, apnea, daytime sleepiness, morning headache and can cause cardiovascular, metabolic, neuropsychological morbidity as well as death.[2] The exact pathophyiology of OSA has not been fully understood, but obstruction of the UA during sleep is a role.[3] Pharyngeal size, compliance, and the dynamic behavior of the upper airway has been considered important factors in the pathogenesis of OSA. Objective measurement of the pharyngeal cross sectional area using lateral cephalometry[4] and Muller's maneuver[5] has demonstrated reduction of this area in patients with OSA, compared with normal subjects. D'Urzo et al[6] compared acoustic pharyngometry with computed tomography in measuring the glottis area in patients with OSA with no statistically significant difference. Acoustic pharyngometry by using reflected acoustic signal emitted from device and sent into the oropharynx assesses the geometry of the oropharyngeal cavity.[7] Computer processing of the incident and reflected sound waves from the airways provides an area distance curve representing the lumen from which minimal cross-sectional area and volume can be derived.[8]

Bradley & Philipson[9] suggested that the upper airway size and functional dynamics are significant factors modulating airflow. Acoustic pharyngometry is considered valid, rapid, non invasive and easily repeatable test to study the compliance of upper airways.[10] The aim of this study was to evaluate the role of acoustic pharyngometry in assessing the precise narrowing site of the upper airway in obstructive sleep apnea (OSA) patients, to plot a predictable diagnostic curve if would be, and to choose the proper management technique.

Patients and Methods

Thirty patients (25men and 5 women) complaining of obstruc-

tive sleep apnea syndrome, age ranged between 18-60 years and mean (44±15yr) were recruited to the study. According to their clinical history and sleep questionnaire {snoring daytime sleepiness, sleep habits and apnea/ hypoapnea index (AHI)} and physical exam, they were subdivided into three stages according to the total number of apneas and hypoapneas divided by the total number of slept hours as evidenced by the partner witness, regardless the duration of apnea; into: mild OSA if AHI is between (5- 15); moderate OSA if AHI is between (15-30) and severe OSA if AHI is ≥30, where apnea is regarded a complete cessation of airflow for at least ten seconds and hypoapnea is a reduction in airflow of less than 50 percent (assessed by thoraco-abdominal muscles activities).[11]

Another thirty, healthy individuals were chosen as a control group (22 males and 8 females) age ranged between 16 and 24 years fulfilling the following criteria:

* Negative history for snoring or OSA symptoms.
* Negative history for upper airway problems.
* Absence of craniofacial skeletal anomalies or dental malocclusion.
* Negative signs suggestive of possible upper airway obstruction such as: palatine tonsil hypertrophy, redundant soft palate, posterior pharyngeal wall banding or large tongue.
* Body Mass Index (BMI) ≤ 27.
* Neck circumference < 42 cm.

All the patients and the control group underwent detailed medical and full ENT exams to identify causes of nasal, oral, Oropharyngeal obstruction. Craniofacial skeleton assessment of the mandible, maxilla and dental occlusion are performed in the Frankfort horizontal position with a line joining the superior external auditory canal and infra- orbital rim using occlusal pattern.12 Evaluation of the cervico-mental angle was done to assess the position of hyoid bone. Mental status and degree of alertness were noted. The body mass index (BMI) was calculated as weight in kg/ height in meter^2 and value over

30 is defined as obesity; the neck circumference at the level of cricothyroid membrane was measured.

Nasal endoscopy using 30° and 70° Hopkins telescope was done to uncover possible obstructive nasal and nasopharyngeal lesions and to examine anatomical and functional changes of the hypo-pharynx and larynx. Muller's maneuver: forced inspiration effort against occluded mouth and nose during fiber optic-nasopharyngoscopy was done in the sitting position to evaluate passavant sphincter and identify retropalatal and retroglossal obstructive sites.

Acoustic pharyngometer (Eccovision-Acoustic pharyngometer-Hood lab, Boston; USA) was used to assess the areas and volumes of the mouth, pharynx, and larynx. Informed consent was obtained before evaluation. Pharyngometry was done for both control group and patients in sitting and supine positions.[13] Patients are kept awake and seated during the exam with their backs in vertical position avoiding neck flexion or shoulder elevation. The sound wave generator tube is placed horizontally, and the subject closes his / her mouth over the mouthpiece and slowly breathes through their mouth. The nostrils should be compressed externally throughout the exam. During the exam the patient pronounces the silent "oooh", to keep the velum closed and to avoid measurements of the nasal volume. Saliva must be prevented to enter into the mouthpiece. The sound wave emitted, travels along the tube and the airways of the patient, partially reflected whenever there is a change in the airway cross-sectional area. These waves are recorded by a microphone and have an amplitude and frequency that depend on the area of the airway and the time taken for the reflected waves to return. Four trials were performed for each patient achieving a coefficient of variance of 10% or less before the curve is accepted. So, it is possible to assess cross-sectional area and volume from the oral cavity to hypo pharynx.

Fig. 1): shows the standard morphology of the pharyngogram of the control; plotted between the x-axis which is the airway

distance in cm and the y-axis which is the airway area calculated in cm^2. The pharyngogram is subdivided into three regions: oral, pharyngeal and laryngeal. On the chart (Fig.1) the distance zero is the end piece of the tube placed over the incisors. The first or oral wave (A) indicates the beginning of the mouth with its peak (B) at a distance of 2cm. The curve deflects reaching a minimal area at a distance of 8cm (C), which anatomically corresponds to Oro-pharyngeal (O-P) junction. The curve begins to move up reaching its peak at about 13 cm on the x-axis; which is the hypo-pharynx. The (F) point is placed at approximately 16 cm from the incisors; this is the glottis region, represented by gentle Valsalva, which represents the narrowest area of the curve. The (E-F) deflection is followed by an area of sub-glottic expansion (G). The principal pharyngometric parameters 8 are:

* The amplitude of the oral wave (A-B) identifies changes in the volume of the tongue;
* The minimum C curve (O-P) segment represents the Oro-pharyngeal junction and assesses the contact surface in the oropharynx;
* The extent of the O-P segment represents mean pharyngeal area (from Oro-pharyngeal junction to the glottis level) with maximum and minimum amplitudes;
* The area of the hypo pharynx is considered the area below the Oro pharynx; its peak is (E) point with amplitude lower than the oral one. The minimum (F) curve is the glottis area that follows the hypo pharynx. All the parameters are calculated by computer system.

Results

All the individuals of the control group were ENT clinically free; whom an averaged single curve was obtained for each one. Their mean pharyngometric parameters were considered the standard one for this study. The degree of apnea, snoring and associated clinical pathology, was assessed. There were 16 cases (53.3%) complained of mild OSA with associated snoring among twelve of them, 12 cases of moderate OSA representing 40%, and 2 patients had severe stage of OSA. All the pa-

tients with moderate and severe OSA were snorers. Daytime sleepiness with morning headache and intellectual changes were found in 10 cases representing 33.3% of all the patients.

ENT clinical and endoscopic exam had revealed: bilateral hidden nasal polypi in 2 cases (6%), 2 cases of nasal allergy with asthma (6%), marked deviated septum in 4 patients (12%), redundant soft palate in 18 cases (60%), hypertrophied uvula in 13 cases (43.3%), chronic tonsillar hypertrophy in 17 patients (56.6%), hypertrophied lingual tonsils in 5 cases (16.6%), macroglossia in 3 patients (10%), and one case of chronic edentulous mandible.

Associated clinical pathology was found in 56.6% of the cases; four patients reported chronic obstructive pulmonary diseases (COPD; 2 mild & 2 moderate OSA), 4 had diabetes mellitus (2 mild & 2 moderate OSA), 10 patients had high blood pressure with mild OSA, 2 patients had nasal allergy with bronchial asthma (one mild, one moderate OSA). We had 3 cases of obesity with hypercholesterolemia (all were moderate OSA). The 2 cases with severe OSA had metabolic syndrome (obesity, diabetes mellitus and hypertension) with co-morbidity of cardiovascular insult. Muller's maneuver showed the collapsibility of pharyngeal walls in all the cases; side to side collapse in 24 patients (80%), and antero-posterior collapse in 6 cases (20%). The mean BMI was 32.08±4.1 (Mean ±SD) and neck circumference values above 42 cm were seen in 13 patients with (46.7±3.7 cm) mean ±SD representing (43.4%) of the cases. Cervico-mental angle measurement showed low positioned hyoid bone in 63.3% of the cases.

The Pharyngometric parameters (amplitude of the oral wave, extension of the Oro-pharyngeal segment, its amplitude and area of the hypo pharynx) all were significantly decreased in OSA patients (males & females) compared with the control group, both in supine and sitting positions. In supine position, there were statistically significant decrease at O-P junction (0.78±0.18) cm2 and mean

pharyngeal area (1.38±0.33) cm^2; mean ±SD, among OSA group, compared with (1.22±0.19) cm^2 and (1.91±0.28) cm^2 respectively in the control one with p <0.05. In the sitting position, the cross sectional areas of the O-P junction was (1.42±02) cm^2 and the mean pharyngeal area was (2.33±040) cm^2 in OSA group, compared with (1.59±026) cm^2 and (2.50±041) cm^2 respectively in the control without statistical significance. There was no correlation between pharyngometric parameters and age. However male predominance in OSA was noticed in 83.3% of the cases.

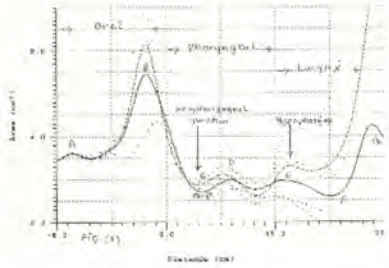

Fig. (1)

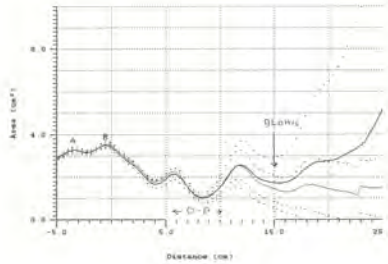

Fig. (2)

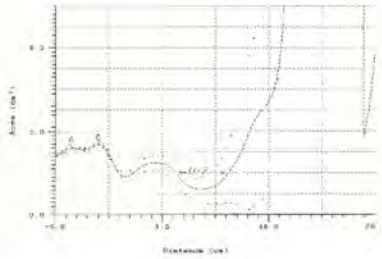

Fig. (3)

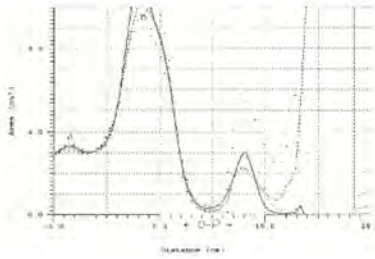

Fig. (4)

Fig. 2: represents a case of chronic hypertrophied palatine tonsils (kissing ones), lingual tonsils, redundant soft palate and hypertrophied uvula with low oral wave and low, elongated O-P segment. In **Fig. 3:** a case of associated macroglossia, hypertrophied lingual tonsils and redundant soft palate with marked reduction A & B oral waves and depressed, elongated O-P segment. **Fig. 4:** shows a clinical case of high arched palate, hypertrophied tonsils, and long uvula with high oral peak (B wave) and depressed O-P segment. An increased distance of O-P segment was seen in almost all of the cases with OSA (93.3%). The amplitude of O-P segment showed very low values in OSA patients compared with the standard value of the control group.

Discussion

Obstructive sleep apnea syndrome (OSAS) is a complex problem with multifactorial etiology mostly related to upper airway anatomical changes.[14] Obesity, upper airway soft tissue hypertrophy, craniofacial characteristics and decreased neuromuscular tone may contribute to the relaxation of pharyngeal dilator muscles during sleep.[15] Monahan et al[16] showed difference in the relation of oropharyngeal dimensions and sleep disordered breathing status according to gender and ethnicity. Pharyngometry, have been compared with other standard methods (Cephalomerty which measures landmarks from standardized lateral radiographs, Computed tomography which offers accurate determination of upper airway cross-sectional area and volume, MRI which is useful in evaluating the efficacy of soft tissue surgery, fiber optic visualization, fluoroscopy which permits dynamic evaluation of the upper airway, etc) in evaluating patients with OSA with no significant difference in the diagnostic accuracy.[17] Sherif et al[10] suggested an algorithm for leveling the site of obstruction in OSA patients using acoustic reflection. Jung et al[18] showed that the Oropharyngeal junction area in supine position is the most predictive parameter to discriminate between subjects with or without OSA. Our results confirmed that all OSA patients had smaller upper airway area and volume in supine position than in sitting one and this may be due to decreased muscle tone or effect of gravity.

Although, Young et al[19] measured objectively nasal obstruction in more than 24% of OSA cases and they stated that its role in the genesis of sleep disordered snoring is not clear, yet, we had variable nasal obstructive pathology in 26% of cases which may act as contributing factors to the severity. However, nasal surgery on OSA patients proved benefit effects.[20]

The most prevalent upper airway obstructive lesions showed in this study were: redundant soft palate (60%), tonsillar hypertrophy (56.6%) and uvular hypertrophy (43.3%). Posterior tongue or its enlarged base plays a major role in the pathogenesis of OSA more than snoring with occlusion

of the vallecula and posterior pushed epiglottis on Muller's maneuver. All UA obstructive lesions could cause apnea, or apnea with snoring. There were more than one upper airway anatomical lesions in the same patient. The most frequent association was that between tonsillar hypertrophy, redundant soft palate and uvular hypertrophy, the region that presents collapse on Muller's maneuver. The direction of pharyngeal collapsibility on Muller's maneuver may guide in surgical decision making when the collapse is retropalatal. We had no statistical correlation between OSA and loudness of snoring, apart from one can select patient who would benefit from palatal surgery to reduce snoring. These findings have a significant influence on OSA management by tailoring all of the clinical lesions into a single surgical procedure individually.

The high incidence of hypertension 40% in our patients is due to an increased of sympathetic nerve activity and blood vasoconstrictors caused by recurrent hypoxemia.[21] The 2 cases of severe OSA in this study were due to metabolic syndrome, which had bigger neck circumference than those in the control one that may add to the severity. Thus, the severity of OSAS may proportionate to Body Mass Index (BMI) and neck circumference suggesting that weight reduction prior to any surgical procedure is recommended. Nevertheless, Galerdi et al[13] reported that snoring did not correlate with anthropometric variables such as body mass index and neck circumference. Waist circumference; not neck one and body mass index are consistent, independent risk factors for all severity levels of sleep disordered breathing[22] Busetto et al[23] proved that both the mean pharyngeal and the glottis areas were lower in obese men than in non-obese ones, both in upright sitting and supine positions denoting to the obesity (upper body fat distribution) as a risk factor in OSA. The high incidence of OSA among males are due to longer airway, less genioglossus muscle activity, higher pharyngeal resistance and a larger volume of fat in pharyngeal wall.24However, Patel et al[25] suggested the genetic basis of sleep

apnea by finding that the minimal cross-sectional area of the oropharynx is a highly heritable trait. Conti et al[26] considered an increase OSA risk development among affected family members by fourfold greater if three relatives are affected.

Acoustic pharyngometry is non- invasive, easily repeatable and provides a real- time display; dynamic image with simultaneously assessment of the upper airway which allows precise plotting of oral, pharyngeal, hypopharyngeal and laryngeal cross-sections on a characterized pharyngogram.[27] Although acoustic pharyngometry cannot be performed during sleep, cannot assess the nasopharynx and it does not provide adequate information about the pharyngeal wall,[28] it allows free tidal breathing during the procedure.

In this study, the pharyngometric parameters especially Oropharyngeal junction; its amplitude; extension (mean pharyngeal area) and area of hypo pharynx, demonstrated significances in patients with OSA compared with the control ones. Kamal [29] could recognize 3 patterns of pharyngogram depending on the possible pathology of pharyngeal obstruction in snorers with OSA. However, we have significant pharyngeal parameters of OSA that could not be categorized into path-gnomonic curve due to the relatively low cases in the study. While, Gelardi etal[13] reported that the amplitude of the O-P segment that quantifies the oropharyngeal tract area was always proportional to the severity of the respiratory disorder, we had not this constant correlation between amplitude of O-P segment and the severity.

The hypo-pharynx was significantly reduced in patients with even mild OSA, reaching low values in metabolic syndrome patients with severe OSA and measurement of this area may correlate with the severity of the case.

Acoustic pharyngometry may localize the possible narrowing sites and offer a predictive value for potential cases of OSA. Although we thought that this study has the deficit in measuring the parameters during wakefulness

with subjective variability to practice the test accurately, Malhotra et al[30] suggested that UA collapsibility measured during wakefulness does provide useful physiologic information about pharyngeal mechanics during sleep. However, acoustic parameters should be interpreted with complete clinical and laboratory data such as drug- induced sleep videoendoscopy which reveals absent muscle tone loss in awaking state and nocturnal polysomnography which objectively and accurately assesses type and severity of OSA during sleep. Our results agreed with that Kamal[31] approved in pharyngometry: that the dependent variable (apnea-hypoapnea index) can be predicted from a linear relation with the independent variable (pharyngeal area).

Conclusion

Pharyngeal size, compliance and the dynamic behavior of upper airway have been considered important factors in the pathogenesis of OSA. Acoustic pharyngometry represents a simple, quick, non-invasive, reproducible method for evaluating upper airway structure and function. It is not only a screening method to predict for potential sites of sleep related upper-airway obstruction, but it also monitories medical or surgical regimen. Patients with OSA have significant differences in the parameters of the cross-sectional area of the Oro- and hypo pharynx than do non-OSA during expiration, especially in supine position. The degree of reduction hypo-pharyngeal area may correlate with the severity of the clinical condition. We concluded that acoustic pharyngometry is a valid tool for predicting patients with OSA; nevertheless, laboratory sleep study is essential for diagnosis the type and severity of sleep apnea in many cases, which one should not disclaim.

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ACOUSTIC PHARYNGOMETRY, COULD IT PREDICT OBSTRUCTIVE SITES IN SUBJECTS WITH SLEEP APNEA?

Mohamed A. M. Eltoukhy MD and Samer Attia MD

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COMBINED TRIAMCINOLONE ACETONIDE POSTERIOR SUBTENON INJECTION AND GRID PHOTOCOAGULATION FOR MANAGEMENT OF DIABETIC MACULAR EDEMA

Hussein T. Elhosseiny MD

Department of Ophthalmology, Banha Faculty of Medicine, Banha University, Egypt

Abstract

Objectives: *To evaluate the outcome of posterior subtenon triamcinolone acetonide (TAA) injection one-week prior to grid photocoagulation compared to photocoagulation alone for management of diffuse diabetic macular edema (DME) in patients with type-2 diabetes mellitus (DM-2).*

Patients & Methods: *The study included 40 DM-2 patients; 17 males and 23 females with mean age of 61.4±8.7 years. All patients underwent full history taking and complete general and ophthalmic examination including estimation of the best corrected visual acuity (BCVA) and foveal thickness using optical coherence tomography (OCT). Patients were randomly allocated into 2 group (n=20): Group A assigned to undergo subtenon injection of 4 mg/0.1 ml preservative free TAA and one week later patients underwent photocoagulation and Group B (Control group) assigned to undergo photocoagulation alone. Patients were monitored 1, 3 and 6 months after treatment for BCVA, macular thickness, intraocular pressure (IOP) and cataract development.*

Results: *All patients showed significant progressive improvement of BCVA reaching a peak at 3-postoperative (PO) months in both groups. The percentage of change in relation to baseline BCVA showed significant improvement in group A compared to group B at 1st and 6th month PO, with significantly better BCVA at the 6th PO month compared to baseline measures. In parallel, macular thickness showed significant decrease in all patients at the 1st PO month, however started to re-increase progressively despite being still significantly thinned compared to baseline thickness, but the percentage of change in relation to baseline thickness showed significant difference in favor of group A.*

Four eyes in group A developed elevated IOP that was temporary and responded to local anti-glaucomatous therapy, but no patient developed cataract or required cataract surgery.

Conclusion: *posterior subtenon TAA injection one-week prior to grid photocoagulation provided outcome superior to grid photocoagulation alone with minimal temporary IOP elevation.*

Keywords: *Subtenon injection, triamcinolone acetonide, diabetic macular edema, grid photocoagulation.*

Introduction

Progression of diabetic retinopathy, especially the development of proliferative diabetic retinopathy with retinal neovascularization at the disc or elsewhere, can lead to severe visual loss and new onset blindness from vitreous hemorrhage or traction detachment of the retina if left untreated [1].

Macular edema is a frequent manifestation of diabetic retinopathy and an important cause of impaired vision in individuals with diabetes. A population-based study estimated that after 20 years of known diabetes, the prevalence of diabetic macular edema (DME) was approximately 28% in both type-1 and type-2 diabetes. Intensive diabetic control and control of associated hypertension are the most widely accepted methods to reduce the risk of vision loss from DME [2].

Focal/grid photocoagulation of eyes with DME involving or threatening the fovea reduced the 3-year risk of losing three or more lines of visual acuity by 50%. A number of additional treatments for DME have been proposed including vitrectomy, pharmacologic therapy with oral protein kinase C beta inhibitors, intravitreal injection of aptamers or antibodies targeted at vascular endothelial growth factor and intravitreal injection of corticosteroids such as triamcinolone acetonide[3,4,5,6].

Multiple reports were published for the use of intravitreal injection (s) of triamcinolone acetonide in the treatment of DME suggesting that intravitreal triamcinolone was potentially an efficacious treatment for DME and this treatment gained widespread use. However, a multitude of case series presentations, case reports and clinical

experience suggested that intravitreal triamcinolone produced a short-term reduction in macular edema and concomitant improvement in visual acuity and others suggested requirement for repeat injections to sustain a reduction in edema, but steroid-related complications such as cataract and glaucoma have been reported in these case series [7,8,9].

Thereafter, combined corticosteroid therapy in conjunction of grid photocoagulation was tried and proved successful policy to provide the best chance for vision conservation and/or restoration; thus, the current study aimed to evaluate the outcome of posterior subtenon injection of triamcinolone acetonide followed one-week later by grid photocoagulation compared to photocoagulation alone for management of diffuse DME in patients with type-2 diabetes mellitus (DM-2).

Patients & Methods

The present prospective comparative study was conducted at Ophthalmology Department, Al-Salama Hospital, United Arab Emirates; after obtaining written fully informed patients' consent, 40 DM-2 patients assigned for grid photocoagulation for diffuse DME in one eye were included in the study since June 2008 till Jan 2010 so as to provide a minimum follow-up period of 6 months for the last operated case.

All patients underwent full history taking with special regard to duration of diabetes, and complete general and medical examination and control of other diabetes related manifestations. All patients gave blood samples for estimation of fasting blood glucose and determination of percentage of glycosylated hemoglobin (HbA_{1C}) as a marker for blood glucose control.

Ophthalmic examinations to evaluate macular edema were performed using +78 diopter non-contact lens slit lamp biomicroscopy. Fluorescein angiography, color fundus photography, and optical coherence tomography were also performed. The best corrected visual acuity (BCVA) was determined with the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. The ETDRS BCVAs were converted to the

logarithm of the minimum angle of resolution (logMAR) scale for analysis. Foveal thickness was measured by optical coherence tomography (OCT) using the Fast Macular Thickness scan. IOP was measured using a Goldman applanation tonometer.

Exclusion criteria included a prior history of vitrectomy, previous photocoagulation or other intraocular surgery within 6 months prior enrolment, presence of ischemic maculopathy documented on preoperative Fluorescein angiography, prior history of elevated IOP secondary to steroid treatment, history of glaucoma or ocular hypertension or the presence of comorbid ocular conditions that might affect VA.

Enrolled patients were randomized, using sealed envelopes, into 2 equal groups (n=20) : Study group (Group A) assigned to undergo posterior subtenon injection of 4 mg/0.1 ml preservative free triamcinolone acetonide (TAA) and one week later patients underwent photocoagulation. Control group (Group B) assigned to undergo photocoagulation alone.

In group A, local anesthetic drops were applied and then 0.1 ml TAA (4 mg) was injected into the superotemporal conjunctival fornix with a 25 gauge 3-cm long needle attached to a tuberculin syringe.

After eye dilatation using application of mydriatic eye drops (Mydriacyl) into the inferior conjunctival sac, Macular grid photocoagulation was performed on spots 100 µm in diameter, with width spacing of 1-2 burns, an exposure time of 0.1-0.2 sec, and a laser power of 100-150 mW. Grid laser photocoagulation was performed by placing medium white laser burns over the entire areas with thicknesses of 350 µm as documented on OCT Fast Macular Thickness scans. The laser was irradiated on the area 500-3000 µm from the macular center at the thickened retina showing capillary blood vessel occlusion and considered to be the origin of the leakage as shown by fundus fluorescein angiography. Laser treatment over papillomacular bundles was avoided.

Patients were monitored for

potential injection-related and laser-related complications. Estimation of BCVA and macular thickness were carried out at baseline and 1 month, 3 months, and 6 months after treatment. Intraocular pressure was also regularly checked and if elevated topical anti-glaucomatous therapy was prescribed. Regular slit lamp examination for cataract development and fundus examination were also conducted.

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using paired t-test for inter-group comparison and Wilcoxon (Z-test) for unrelated data for comparison between groups. Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

The study included 40 patients; 17 males and 23 females with mean age of 61.4±8.7; range: 41-78 years and all had preoperative controlled DM-2. There was non-significant difference between both study groups as regards the constitutional data, clinical and laboratory findings, (Table 1).

All patients showed significant progressive improvement of BCVA reaching a peak at 3-months PO in both groups, (Fig. 1). However, for proper result adjustment the percentage of change in relation to baseline BCVA was calculated and showed significant improvement in group A compared to group B at 1st and 6th month PO, with non-significant difference at the 3rd month measure but in favor of group A. In both groups, the percentage of BCVA improvement was significantly high at the 6th PO month when compared to baseline BCVA, (Table 2, Fig. 2).

In parallel, macular thickness showed significant decrease in all patients at the 1st PO month, however started to re-increase progressively despite being still significantly thinned compared to baseline thickness, (Fig. 3). The percentage of change in relation to baseline thickness showed significant difference between both study groups in favor of group A.

It is to be noted that group B showed heterogenecity of diminution response manifested as high standard deviation of measures, while in group A such heterogenecity was less evident, (Table 3, Fig. 4).

All patients passed smooth intraoperative and postoperative courses without complications; however, 4 eyes in group A developed elevated IOP after a mean duration of 18.5±3.9; range: 14-23 days, fortunately such IOP elevation was temporary and responded to topical anti-glaucomatous therapy. Throughout the follow-up period, no patient developed cataract or required cataract surgery.

Table (1): Time course changes of visual acuity reported in both groups.

		Group A	Group B	Total
Age (years)		62.3±9.3 (41-78)	60.4±8.2 (48-74)	61.4±8.7 (41-78)
Gender; M:F		8:12	9:11	17:23
Duration of DM (years)		15.5±4.9 (8-24)	16.1±5.5 (6-25)	15.9±5.3 (6-25)
Clinical findings	BCVA (logMAR)	0.7±0.21	0.67±0.19	0.68±0.20
	IOP (mmHg)	14.3±2.1 (11-18)	15.5±2.3 (12-19)	14.9±2.3 (11-19)
Laboratory findings	FBG (mg/dl)	160.3±10.8 (139-178)	164.7±9.7 (145-178)	163.7±10.2 (139-178)
	HbA_{1C}	10.12±1.8 (7.2-13.2)	9.81±1.5 (6.5-12.3)	10±1.6 (6.5-13.2)

Data are presented as mean±SD; ranges are in parenthesis DM: Diabetes mellitus
VA: visual acuity FBG: Fasting blood glucose HbA_{1C}: Glycosylated hemoglobin

Table (2): Time course changes of best corrected visual acuity reported in both groups.

Time	Variable	Group A		Group B	
		Finding	Statistical Analysis	Finding	Statistical Analysis
Baseline		0.7±0.21		0.67±0.19	$Z=1.458$, $p_4>0.05$
1st month	Measure (logMAR)	0.45±0.17	$t=10.176$, $p_1<0.001$	0.49±0.17	$t=10.052$, $p_1<0.001$ $Z=2.356$, $p_4=0.018$
	% of change	33.6±11.5		30±8.19	$Z=2.651$, $p_4=0.008$
3rd month	Measure (logMAR)	0.39±0.17	$t=16.998$, $p_1<0.001$ $t=2.942$, $p_2=0.008$	0.43±0.16	$t=10.411$, $p_1<0.001$ $t=3.269$, $p_2=0.004$ $Z=2.242$, $p_4=0.025$
	% of change	42.9±15.9	$t=2.547$, $p_1=0.020$	39.5±13.3	$t=3.222$, $p_1=0.004$ $Z=0.805$, $p_4>0.05$
6th month	Measure (logMAR)	0.51±0.19	$t=10.446$, $p_1<0.001$ $t=6.110$, $p_2<0.001$ $t=5.480$, $p_3<0.001$	0.56±0.17	$t=10.514$, $p_1<0.001$ $t=6.571$, $p_2<0.001$ $t=4.333$, $p_3<0.001$ $Z=2.135$, $p_4=0.033$
	% of change	23.9±9.6	$t=6.390$, $p_1<0.001$ $t=4.879$, $p_2<0.001$	20±7.5	$t=7.088$, $p_1<0.001$ $t=4.417$, $p_2<0.001$ $Z=2.427$, $p_4=0.015$

Data are presented as mean±SD
p_1: significance versus baseline measures p_2: significance versus 1st month measures
p_3: significance versus 3rd month measures p_4: significance versus group A measures

Table (3): Time course changes of macular thickness reported in both groups

Time	Variable	Group A		Group B	
		Finding	Statistical Analysis	Finding	Statistical Analysis
Baseline		385.8±126		323±132	Z=1.232, p_4>0.05
1st month	Measure	238±58	t=8.704, p_1<0.001	236±109	t=11.602, p_1<0.001 Z=0.112, p_4>0.05
	% of change	36.3±8.6		28.3±7.6	Z=2.539, p_4=0.011
3rd month	Measure	278±75.6	t=8.318, p_1<0.001 t=7.039, p_2<0.001	260±114	t=10.207, p_1<0.001 t=9.335, p_2<0.001 Z=0.448, p_4>0.05
	% of change	28.1±7.8	t=9.207, p_1<0.001	19.4±6.1	t=9.870, p_1<0.001 Z=2.464, p_4=0.014
6th month	Measure	312±82.5	t=6.307, p_1<0.001 t=10.461, p_2<0.001 t=7.280, p_3<0.001	283±119	t=9.314 p_1<0.001 t=12.350, p_2<0.001 t=8.588, p_3<0.001 Z=0.784, p_4>0.05
	% of change	19.2±8	t=16.156, p_1<0.001 t=8.853, p_2<0.001	12.2±3.7	t=12.237, p_1<0.001 t=8.026, p_2<0.001 Z=2.352, p_4=0.019

Data are presented as mean±SD
p_1: significance versus baseline measures
p_2: significance versus 1st month measures
p_3: significance versus 3rd month measures
p_4: significance versus group A measures

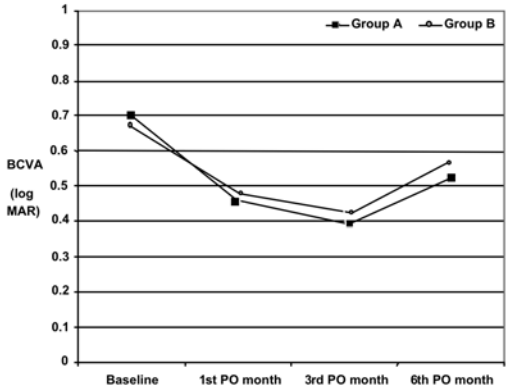

Fig. (1): Mean VA recorded throughout 6months PO in both study groups

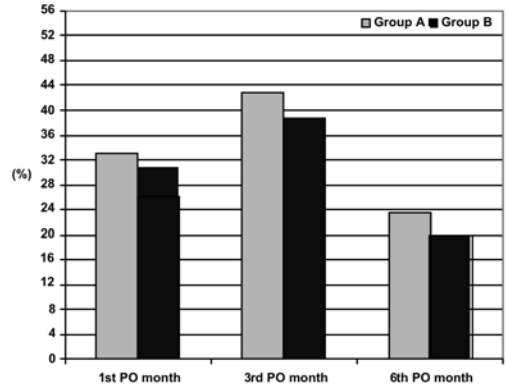

Fig. (2): Mean percentage of change of VA recorded in the study groups throughout follow up period

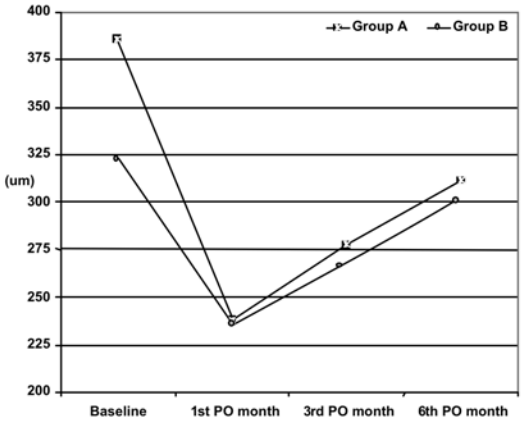

Fig. (3): Mean foveal thickness recorded throughout 6 months PO in both study

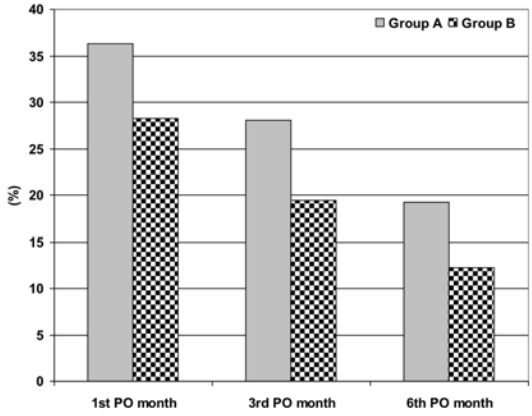

Fig. (4): Mean percentage of change of foveal thickness recorded in the study groups throughout follow-up period

Discussion

The present study reported significant improvement of visual acuity in both study groups peaking at the 3rd month PO and slightly declined thereafter but still significantly better compared to baseline measures. The present study reported also significant decrease in macular thickness in

both study groups peaking at the 1st month PO and slightly increased thereafter but still significantly better compared to baseline measures. These data indicated the applicability of both modalities as management lines for diabetic macular edema (DME) and go in hand with Lee et al.(10) who compared between the efficacy of macular laser grid photocoagulation alone and plus intravitreal triamcinolone acetonide (TAA) therapy in DME patients and found the combined therapy had a better therapeutic effect than photocoagulation alone for improving visual acuity and central macular thickness at the early follow-up time periods.

Diabetic retinopathy clinical research network (11) in 2010, conducted a prospective multi-center comparative study for intravitreal 0.5 mg ranibizumab or 4 mg TAA combined with focal/grid laser compared with focal/grid laser alone for treatment of DME and reported that the reduction in mean central subfield thickness in the TAA + prompt laser group was similar to both ranibizumab groups and greater than in the sham + prompt laser group, and in the subset of pseudophakic eyes at baseline visual acuity improvement in the TAA + prompt laser group appeared comparable to that in the ranibizumab groups.

Recently, Googe et al.(12) found the addition of intravitreal TAA injection in eyes receiving focal/grid laser for diabetic macular edema and panretinal photocoagulation is associated with better visual acuity and decreased macular edema by 14 weeks.

Moreover, patients received combined therapy showed significantly better outcome as regards both visual acuity and macular thickness compared to those received photocoagulation alone till the end of follow-up at 6 months with significantly higher percentages of change compared to baseline measures. These data go in hand with Shimura et al.(13) who prospectively evaluated the efficacy of subtenon injection of TAA before laser grid pattern photocoagulation for the treatment of DME and found subtenon injection of TAA prior to grid pattern photocoagulation allowed for

treatment with a lower intensity of laser spots and also prevented the decrease in central visual field sensitivity. Kuo et al.[14] retrospectively compared the postoperative data for 34 eyes with cystoid macular edema treated with photocoagulation alone or combined with subtenon TAA and a follow-up of 6 months, suggested that subtenon injection of TAA combined with macular photocoagulation provides a better chance of stabilizing vision loss in patients with diabetic cystoid macular edema than photocoagulation alone.

However, concerning the use of TAA, a point of discrepancy is the site of injection whether intravitreal or posterior subtenon; Tunc et al.[15] investigated the efficacy of posterior subtenon TAA injection combined with focal laser (FL) photocoagulation in treatment of diffuse clinically significant DME and reported that posterior subtenon capsule application of TAA may improve early visual outcome in diffuse diabetic macular edema when combined with focal laser photocoagulation. Ozdek et al.[16] retro-prospectively evaluated the clinical consequences of posterior subtenon and intravitreal TAA injections in DME refractory to conventional grid laser photocoagulation and found both injections caused a significant increase in visual acuity and a decrease in central foveal thickness, especially in the short term; however, posterior subtenon injection seemed to be a safe and effective technique for the treatment of DME. Chung et al.[17] compared the efficacy of posterior subtenon TAA injection combined with modified grid macular photocoagulation with intravitreal TAA injection in the treatment of DME and found combined subtenon TAA injection and photocoagulation provided significant improvement of vision in patients with diffuse DME over 3 months, and achieves outcomes comparable to those after intravitreal treatment, however, with fewer complications.

Posterior subtenon TAA injection resulted in temporary elevation of IOP in 4 eyes but responded to local therapy and no cataract development was reported till end of follow-up. These findings indicated safety of posterior subtenon TAA injection and are in

line with Chung et al.[17] who reported only increased IOP in little number of patients received posterior subtenon TAA injection combined with macular photocoagulation, while a significant increase in average cataract grading were observed with intravitreal TAA injection. Chew et al.[18] evaluated long-term effects of anterior and posterior peribulbar injections of TAA on IOP elevation and cataract development and found that over 2 years, anterior peribulbar TAA injections are associated with an increased incidence of IOP elevation and an increased risk of cataract development compared with laser or posterior peribulbar injections, but the association of posterior injections with IOP elevation is less certain.

In conclusion; posterior subtenon TAA injection one-week prior to grid photocoagulation provided outcome superior to grid photocoagulation alone with temporary IOP elevation in few number of eyes.

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COMBINED TRIAMCINOLONE ACETONIDE POSTERIOR SUBTENON INJECTION AND GRID PHOTOCOAGULATION FOR MANAGEMENT OF DIABETIC MACULAR EDEMA

Hussein T. Elhosseiny MD

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EFFECT OF PIOGLITAZONE AND SIMVASTATIN ON DIABETIC NEPHROPATHY IN TYPE 1 DIABETIC RATS, POSSIBLE MECHANISM OF ACTION

Amany N. Ibrahim MD and Amro El-Karef MD*

*Department of Pharmacology, Benha Faculty of Medicine, Benha University and*Department of Pathology, Mansoura Faculty of Medicine, Mansoura University*

Abstract

Diabetic nephropathy is one of the major complications of type 1 and type 2 diabetes and it is currently the leading cause of end-stage renal disease. The aim of this study was to investigate the effect of pioglitazone and/or simvastatin on diabetic nephropathy in type 1 diabetic rats and possible mechanism of action especially lipid peroxidation production as malondialdehyde in plasma. Streptozotocin-induced type-1 diabetes mellitus (DM) model was set up in adult male albino rats. Diabetic rats were treated for 8 weeks with pioglitazone (10 mg/kg/day) and/or simvastatin (10mg/kg/day). The serum urea, creatinine, urine albumin, renal histopathology, renal blood flow and lipid perioxidation production as malondialdehyde of the different groups were tested compared to control group. Relative to rats in the normal control group, rats in the diabetic group had significantly disrupted serum urea, creatinine, urinary albumin, renal blood flow, kidney histology and significantly enhanced expression of lipid peroxidation. Rats in the pioglitazone and/or simvastatin treatment group experienced significant amelioration of these effects.

Conclusion: *In type 1 diabetic rat model, pioglitazone and/or simvastatin ameliorated many of the physiological, cellular, lipid perioxidation associated with diabetic nephropathy.*

Key words: *pioglitazone, simvastatin, diabetic nephropathy*

Introduction

Diabetic nephropathy is one of the major complications of type 1 and type 2 diabetes and it is currently the leading cause of end-stage renal disease. Hyperglycemia is the driving force for the development of diabetic nephropathy. It is well known that hyperglycemia increases the production of free radicals resulting in oxidative stress [1]. Many reports have demonstrated that increased oxidative stress in diabetes plays an important role in the progression of diabetic complications, including nephropathy [2]. Oxidative stress is determined by the relationship between reactive oxygen species (ROS) and the antioxidant defense system including antioxidant enzymes.

Increased ROS leads to lipid peroxidation which increases oxidative injury [1] and causes damage to cellular protein and nucleic acid. Many mechanisms are involved in the production of ROS including glucose autoxidation, non-enzyme protein glycation, and generation of advanced glycation end products [3] activation of protein kinase C and NADPH oxidase [1]. It has demonstrated that glomeruli are especially sensitive to oxidative injury [4] animal that contributes to the progression of diabetic nephropathy. Studies have shown that primary antioxidants or genetic manipulation of antioxidant defenses can ameliorate this oxidative stress and consequentially, reduce severity of diabetic complications in models.

Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors that play a role in insulin sensitivity, lipid metabolism and inflammation[5]. However, the nephroprotective effects of PPAR gamma agonist pioglitazone have not been fully examined in type 1 diabetic nephropathy. A report suggested that medications to treat hyperglycemia and hyperinsulinemia are expected to inhibit the accumulation of advanced glycation end-products in the diabetic kidney and improve renal function by inhibiting oxidative reactions [6].

On the other hand, HMG-CoA reductase inhibitors (statins), including simvastatin, haven been shown to be effective lipid-

lowering agents. Many studies have demonstrated that statins have pleiotropic effects independent of their cholesterol-lowering effects [7]. A report by Jones et al. [8] suggested that statins have antioxidative effects that benefit cardiac myocytes. . Rikitake et al. [9] demonstrated the antioxidative properties of fluvastatin which intercept atherosclerosis in cholesterol-fed rabbits. Statins also have protective effects on glomeruli Rikitake et al. [10] but the precise mechanisms remain unclear. In this study, we examined the effect of pioglitazone, an insulin sensitizer, and/ or simvastatin on diabetic nephropathy, and their possible mechanism of action especially antioxidant effects in streptozotocin (STZ) - induced type 1diabetic rats.

Materials and methods

Drugs and chemicals

• Streptozotocin (STZ) powder creamy white: (Sigma Chemicals Co, U.S.A)

• Pioglitazone powder (Unipharma, Egypt)

• Carboxy-methyle cellulose (power) (El Nasr Pharmaceutical Chemicals Co.)

• Simvastatin powder (sigma chemical Co, U.S.A)

Animals

Fifty adult male albino rats, weighting (120-150gm). They were brought from Experimental Animal Breeding Farm, Helwan - Cairo. All animals were housed in controlled laboratory condition at 20 -25C in a 12h light/dark cycle and had free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They have acclimatized for one week and were caged (10/ cage) in fully ventilated room (at room temperature) in Pharmacology Department, Benha Faculty of Medicine. All experimental protocols were approved by the ethics committee of Benha University.

Study design:

After acclimatization for 1 week, rats were randomly divided into 5 experimental groups, 10 rats each and treated for 8 weeks as follow:

Group 1 (normal control

group): received physiological saline and served as normal control group (CN),

Group 2 (DM group): rats injected with single dose of streptozotocin (65mg/kg/day i.p) in 0.1 mmol/l Sodium citrate, pH 4.5 Crespo et al. [11]

Group 3 (Pioglitazone treated group): diabetic rats received pioglitazone (10mg/kg/day) by gavage Tanimoto et al. [12].

Group 4 (simvastatin treated group): diabetic rats received simvastatin (10mg/kg/day) by gavage Mohamadin et al. [13]

Group 5 (pioglitazone + simvastatin treated group): diabetic rats received pioglitazone and simvastatin (10mg/kg/day) by gavage

All experimental protocols were approved by the ethical committee of The Faculty of Medicine, Benha University.

Parameters used:

I- Biochemical parameters:

1-Fasting blood glucose levels (FBG):

One drop of blood of the adult rats was obtained by puncture of the retrobulbar sinus; the capillary end of the glass tube was inserted into the medial canthus of the eye. The sinus was punctured and blood entered the tube by its own pressure forming a free flow of blood. The blood was biochemically investigated for FBG according to Fossatip[14]. Diabetes was confirmed in rats by showing blood glucose levels above 300 mg/100 ml Ganda et al. [15].

2- Estimation of renal excretion of albumin:

All rats were housed individually in metabolic cages for 24 hours with free access to water and a normal chow. Albumin concentrations were measured in 24-hour urine (mg/24 hours) samples using a Minineph microalbumin kit (The Binding Site, Birmingham, UK) Showell et al. [16].

3-Measurement of serum urea, creatinine:

At the end of the 8th week rats were anaesthetized with urethane. Venous blood samples were collected by heparinized capillary tubes from the retro-orbital plexus

of rats Timm [17].

3.1. Serum levels of urea:

A serum level of urea was determined using a commercially available kit according to the manufacture instructions.

3.2. Serum creatinine:

The kit used for measuring creatinine levels (Randox Laboratories, Crumlin, County Antrim, UK) is based on the Jaffe' reaction. In an alkaline solution, creatinine combines with picric acid to form an orange-red complex (the creatinine-picric acid complex). The increase in absorbance using a spectrophotometer at 510 nm is proportional to creatinine concentration (mg/dl) Jaffe [18].

4- Measurement of malondialdehyde (Sunderman et al., 1985):

Malondialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals, is considered a reliable marker of oxidative stress and was determined by measurment of the chromogen obtained from the reaction of malondialdehyde with 2-thiobarbituric acid, according to Aruoma et al. [19]. The MDA values are expressed as (umol/ml)

II- Renal blood flow (RBF) (Haywood et al., 1981):

At 8th week, rats were anaesthetized with urethane and then were fixed on operating table, the abdominal cavity was opened and the kidneys were exposed. The flow probe was placed on top of the right renal artery for the measurement of renal blood flow by the flow meter (Hadeco ES 1000 SPM, Japan) at the end of the study Haywood et al. [20].

III- Histopathological parameter:

The rats in all groups were sacrificed, and their kidneys were cut; each kidney was divided into two halves. The specimens were preserved in 10% formalin, dehydrated in ascending grades of ethyl alcohol (50%, 70%, 90% and 100%) and cleared; the two halves were embedded in soft and hard paraffin, respectively. Paraffin blocks were generated, and sections (3-um thick) were cut on a microtome and subjected to haematoxyllin and eosin staining

Drury et al. [21].

Statistical analysis (Goldstone, 1983 [22]):

Data represented as mean ± SEM. Multiple comparisons were performed using one-way Anova analysis of variance (ANOVA) followed by tukey's test as a post-hoc test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using GraphPad Istat version software package.

Results

1-Effect of streptozotocin-induced changes in rats:

Single dose of streptozotocin (65mg/kg/day i.p) significantly increased ($p<0.05$) fasting blood glucose level (FBG), 24- hour urinary albumin excretion (table 1) , Also, STZ significantly increased plasma urea, creatinine concentration and lipid peroxidation (MDAp) in all diabetic groups compared with normal control group (table 2), On the other hand, streptozotocin significantly decreased renal blood flow (table 3).

2- Effects of pioglitazone and simvastatin administration on streptozotocin -induced changes:

1-Fasting blood glucose level and serm creatinine level:

Administration of pioglitazone (10mg/kg/day) and simvastatin (10mg/kg/day) significantly decrease fasting blood glucose level (table 1). While, simvastatin alone had no significant effect. On the other hand, administration of pioglitazone (10mg/kg/day) and simvastatin (10mg/kg/day) significantly decreased serum creatinine level but no significant differences were noted when compared with the normal control and between the all three treatment groups (table 1).

2- Renal excretion of albumin:

Administration of pioglitazone (10mg/kg/day) and simvastatin (10mg/kg/day) significantly reduced the effects of streptozotocin on renal albumin excretion but no significant differences were noted when compared with the normal control and between the all three treatment groups (table 1).

3- Lipid peroxidation (MADp):

Administration of pioglitazone (10mg/kg/day) and simvastatin (10mg/kg/day) significantly reduced lipid peroxidation (MDAp) but no significant differences were noted when compared with the normal control and between the all three treatment groups. (table 3).

4- Renal blood flow:

Administration of pioglitazone (10mg / kg / day) and simvastatin (10mg/kg/day) significantly improved the effect of streptozotocin on renal blood flow but no significant differences were noted when compared with the normal control and between the all three treatment groups (Figure 1, table 3).

5- Renal histopathological structure:

Histopathological examination of renal tissues of diabetic rats revealed diffuse diabetic glomerular sclerotic lesion which is characterized by diffuse thickening of glomerular capillary wall and generalized increase in mesangial matrix in all mesangial regions of all glomeruli, (Figure 2). While, histopathological examination of renal tissues in pioglitazone and/or simvastatin treated rats, showed mild thickening of glomerular capillary wall and mild changes in mesangial matrix (Figure 2).

Table (1): Effects of simvastatin, pioglitazone and their combinations on Fasting blood glucose level (FBG) and 24 hours urinary albumin excretion (UAE),serum urea and creatinine (mg/dl) in diabetic rats.

Parameters / Groups	FBG (mg/dl) Mean ± SE	Urine albumin (mg/24 h) Mean ± SE	Serum urea(mg/dl) Mean ± SE	Serum creatinine(mg/dl) Mean ± SE
Control	88 ± 2.9	3.89±2.3	36.4 ± 2.8	0.9±0.06
DM group	360±20.1*	14.5±1.1*	145±4.6*	3.7±0.17*
simvastatin treated	350±18.4#	10.4±0.54**	48.2±3.1**	2±0.11**
Pioglitazone treated	112± 5.2**	9.8±0.51**	45.4± 2.9**	1.6±0.11**
Pio+sim treated	105± 4.1**	7.28±0.53**	40.1± 4.2**	1.4±0.1**

Data are presented as Mean ± SEM .Statistical analysis by using One Way ANOVA and Tukey Kramer post-test at $P< 0.05$.

*: Significant difference from control group.
**: Significant difference from diabetic group.
#: No significant difference from diabetic group.

Table (3): Effects of simvastatin (Sim), pioglitazone (Pio) and their combinations on renal blood flow (RBF) and Lipid perioxidation (MDAp) in diabetic rats

Parameters / Groups	Renal blood flow (ml/min.) Mean ± SE	MDAp (µmol/ml) Mean ± SE
Control	11.4±0.9	3.2 ± 0.31
DM group	2.3±0.15 *	7.11 ± 0.31 *
simvastatin – treated	6.5±0.39 **	4.63 ± 0.19 **
Pioglitazone- treated	7.1±0.45 **	4.11 ± 0.41 **
Pio+sim treated	8.8±0.46 **	3.91± 0.23 **

Data are presented as Mean ± SEM .Statistical analysis by using One Way ANOVA and Tukey Kramer post-test at P< 0.05.

*: Significant difference from control group.

**: Significant difference from diabetic group.

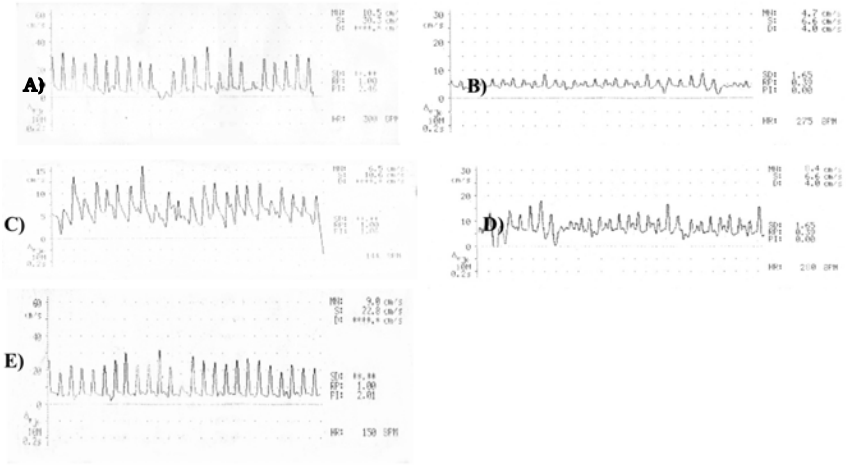

Figure (1): Effects of pioglitazone and/or simvastatin on renal blood flow

A) A trace showing renal blood flow in normal control group.
B) A trace showing significantly decreased renal blood flow in STZ induced diabetic nephropathy.
C) A trace showing significantly normalized renal blood flow in STZ induced diabetic nephropathy simvastatin treated group.
D) A trace showing significantly normalized renal blood flow in STZ induced diabetic nephropathy pioglitazone treated group.
E) A trace showing significantly normalized renal blood flow in STZ induced diabetic nephropathy pioglitazone and simvastatin treated group.

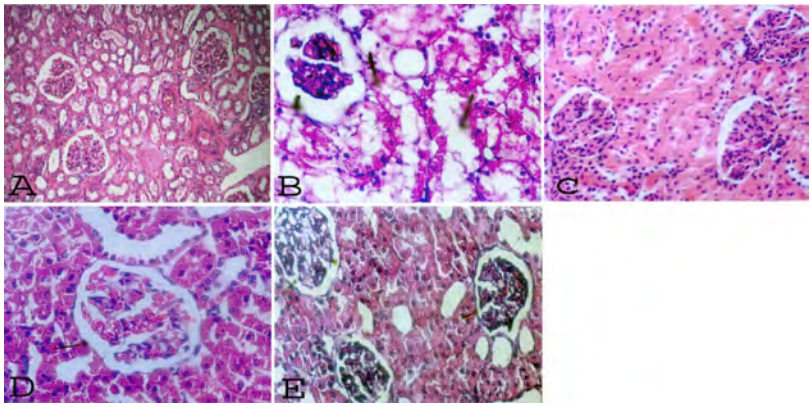

Figure (2): effects of pioglitazone and simvastatin on histopathological changes in rats with diabetic nephropathy.

A) Cut section of normal renal tissue of control group.
B) Cut section of renal diabetic nephropathy revealed diffuse diabetic glomerular sclerotic lesion which is characterized by diffuse thickening of glomerular capillary wall and generalized increase in mesangial matrix in all mesangial regions of all glomeruli.
C) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with pioglitazone showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E × 40).
D) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with simvastatin showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E × 40).
E) Cut section of renal tissues of STZ induced diabetic nephropathy in rats treated with pioglitazone + simvastatin showing mild thickening of glomerular capillary wall and mild changes in mesangial matrix (H & E × 40)

Discussion

The current study showed that injection of STZ (65mg/kg/day i.p) induced elevation of blood glucose level in all rat groups, increased 24-hour urinary albumin excretion, serum urea and creatinine and increased lipid peroxidation. Also, it decreased renal blood flow and histopathological changes demonstrated the occurrence of diabetic nephropathy in all diabetic groups.

Administration of pioglitazone (10mg/kg/day orally) for 8 weeks

significantly lowered blood glucose level and decreased 24-houre urinary albumin excretion. These results are in agreement with Tanimoto et al. [12] who reported that pioglitazone had preventive effects on impaired glucose tolerance and urinary albumin excretion in diabetic rats. Ko GJ et al. [23] added that pioglitazone not only improves insulin resistance, glycaemic control and lipid profile, but also ameliorates renal injury through an anti-inflammatory mechanism in type 2 diabetic rats.

n the present work administration of simvastatin (10mg/kg/ orally) daily for 8 weeks ameliorates 24-hour urinary albumin excretion but had no significant effect on blood glucose level in diabetic rats. These results are in line with Matsumoto et al. [24] who found that Pitavastatin improved urinary albumin/ creatinine ratio apparently because of suppression of eNOS uncoupling and its antioxidant effect in the kidneys of diabetic mice.

Regarding renal blood flow, our results showed significant decrease in renal blood flow in diabetic rats group compared with normal control group. Administration of pioglitazone and/or simvastatin significantly improved renal blood flow. These results are in agreement with Biscetti et al. [25] who found that pioglitazone restored the blood flow recovery and capillary density in ischemic muscle of diabetic mice and that this process was associated with increased expression of Vascular Endothelial Growth Factor. Also, Kataoka et al. [26] reported that pretreatment with pioglitazone in diabetic patients with acute myocardial infarction resulted in better myocardial perfusion with less reperfusion injury. Bellia et al. [27] stated that in type 2 diabetic individuals simvastatin improved endothelium-dependent vasodilation within one month, without affecting insulin-resistance, adiponectin levels and inflammation.

Histopathological examination of renal tissues of diabetic nephropathy rats revealed diffuse diabetic glomerular sclerotic lesion which is characterized by diffuse thickening of glomerular capillary wall and generalized increase in mesangial matrix in all mesangial

regions of all glomeruli compared with control group. While, histopathological examination of renal tissues in pioglitazone-treated rats, simvastatin treated rats and combination of pioglitazone and simvastatin showed mild thickening of glomerular capillary wall and mild changes in mesangial matrix compared with diabetic nephropathic rats with no treatment. These results are in agreement with Yoshimoto et al. [28] who found that treatment with pioglitazone significantly decreased proteinuria, and prevented glomerular injury and renal arteriolosclerosis in genetically obese diabetic rats. Also, Song et al. [29] stated that fluvastatin may be a potential candidate for developing a pharmaceutical approach to the prevention of diabetic nephropathy due to its both lipid-lowering and direct anti-renal extra cellular matrix accumulation actions.

Diabetic patients exhibit an oxidative stress status that is an imbalance between reactive oxygen species and antioxidant defenses, in favors of the first ones. This oxidative stress, together with formation of advanced glycation endproducts (AGEs), is involved in diabetic complications Bonnefont-Rousselot et al. [30].

Parameter of lipid peroxidation MDAp at week 8 was also measured at this study in all the diabetic groups which were significantly increased compared with the normal control group. But changes of MDAp in the DM+pio group, DM+sim group and DM+pio+sim group were less pronounced ($p<0.05$) compared with the DM group. This is results is in agreement with Mohamadin et al. [13] who observed that simvastatin has a protective effect against STZ-induced oxidative damage by scavenging the free radicals generation and restoring the enzymatic and nonenzymatic antioxidant systems

In conclusion :

Pioglitazone and/or simvastatin had protective effects in ameliorating diabetic nephropathy in STZ induced type 1 diabetic rats. And these protective effects may be through significant antioxidant effects.

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EFFECT OF PIOGLITAZONE AND SIMVASTATIN ON DIABETIC NEPHROPATHY IN TYPE 1 DIABETIC RATS, POSSIBLE MECHANISM OF ACTION

Amany N. Ibrahim MD and Amro El-Karef* MD

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EVALUATION OF STATIC LOCKED INTRAMEDULLARY NAILING FOR TREATMENT OF COMMINUTED FEMORAL SHAFT FRACTURES

Salah Youssef MD and Mohamed El-Menawy MD

Departments of Orthopedic, Faculty of Medicine,
AL-Azhar University, Egypt

Abstract

Background: *The treatment of femoral shaft fractures has always been a focus of interest, but till now remains a clinical problem, and a subject of controversy. The aim of this work is to evaluate the role of the static interlocked nailing in treatment of comminuted fracture shaft of the femur in our initial cases managed by this technique at the Azhar University Hospitals.*

Patinets and Methods: *The results of treatment of comminuted fractures of the femoral shaft with static interlocking nail were reviewed retrospectively in 60 patients. Ten patients of them had bilateral fractures, which allowed 70 fractures to be reviewed.*

Results: *All managed fractures (100%) had healed without conversion to dynamic intramedullary fixation. No broken nails have been detected. At the final postoperative follow-up evaluation according to Thoresen et al we obtained excellent and good results in 65 femoral fractures (92.85%), fair in 5 femoral fractures (7.15%) and no poor result (0%). We had 100% fracture union for all cases with a mean time 8 months postoperatively. Complications: Only 1 patient (1.4%) had deep infection. Eight patients (11.4%) had pulmonary embolism which was the major cause of morbidity associated with bed recumbence in our study.*

Conclusions: *We concluded that this method of treatment "static intramedullary nailing fixation" for comminuted shaft fractures of the*

femur is an effective method that maintains the length, alignment with low incidence of complications.

Introduction

Femur is the longest and strongest, largest and heaviest tubular human bone. Femoral shaft fractures are common in adult population due to vulnerability to road traffic accidents, falls from a height, industrial accident or firearm injuries so these fractures are commonly caused by high-energy forces mechanism and are often associated with multisystem trauma [1].

Femoral shaft fractures can lead to a major physical impairment, not because of disturbed fracture healing, but rather due to fracture shortening, fracture malalignment, or prolonged immobilization of the extremity by traction or casting in an attempt to maintain the fracture length and alignment during the early phases of healing. Even minor degrees of shortening and malalignment may result in a limp and posttraumatic arthritis. The art of femoral fracture care is a constant balancing of the often conflicting goals of anatomic alignment and early functional rehabilitation of the limb [2,3,4].

The treatment of femoral shaft fractures has always been a focus of interest, but till now remains a clinical problem, and a subject of controversy. Several techniques have been developed for the treatment. Operative treatment in the form of plating, nailing and external fixation is still being carried out for these fractures in many parts of the world.

Closed reduction and intramedullary nail fixation, as has been proposed by Kuntscher, is the most biological way of treating fractures of the shaft of femur [5]. Grosee-Kempf [6] introduced the locked intramedullary nail for the treatment of comminuted fractures of femur. They reported that this method gave axial, rotational and bending stability to the fracture, with low incidences of infection, non-union and malunion. The indications of intramedullary fixation of fractures of the femoral shaft have been greatly expanded

by techniques of interlocking nailing since then[7,8]. Many investigators have been concerned that static interlocking nailing might interfere with fracture-healing, due to the decreased loads across the site of a fracture that has been treated with this type of fixation.

However, postoperative fixation failure by dynamic intramedullary nailing in terms of maintenance of reduction of the fracture, shortening and rotation reported by Brumback et al [2] prompted us to use static interlocking nailing for the treatment of all fractures of the femoral shaft, regardless of the degree that the fracture was comminution or the location of the fracture.

Static interlocking nailing describes the interlocking construct in which both proximal and distal interlocking screws are been inserted. Shortening and malrotation are controlled by transferring the axial and rotational stresses through the nail rather than through the site of the fracture [9].

The aim of this work is to evaluate the role of the static interlocked nailing in treatment of comminuted fracture shaft of the femur in our initial cases managed by this technique at Azhar University Hospitals.

Patients and Method

We reviewed retrospectively the medical records and radiographs of all patients who had acute fresh metaphyseo-diaphyseal comminuted fractures of the femoral shaft that had been treated with static interlocking nailing and had been maintained until fracture united with a minimum duration of follow-up one year postoperatively between January 2006 and December 2009 at Azhar University Hospitals. There were 60 patients. Ten patients of them had bilateral fractures, which allowed 70 fractures to be reviewed. Any patient younger than 16 years old, or had pathological fracture, trochanteric fracture, old fracture, or re-fractures were excluded from this study.

The duration of follow up ranged from twelve to twenty four months with a mean 15 months. The age range was from 16 to 63 years with average age thirty three

years old (Table 1). The male patients were 53 (90%) and female were 7 (10%).

Technique :

All patients were operated by the same technique where the patients positioned laterally on fracture table. The mean time from admission to operation was 7 days (Table-3) Patients were given either spinal or general anaesthesia. The method of interlocking nails was close or open femoral nailing under image intensifier (Table 4). The type of nailing was ante-grade interlocking nails.

After reduction, the entry point was through pyriformis fossa by using awl then a guide wire was introduced into the medullary canal then across the fracture site under image control. This is followed by reaming with power drill using flexible intramedullary reamers which were then used to increase the diameter of the canal in 0.5 mm increments in a stepwise manner to 1 mm more than the nail size to be used (Table 5). With the appropriate nail inserted in its suggested place a special jig was used to introduce proximal locking screw. The distal screws were then placed percutaneously by free hand technique under C arm control.

The postoperative follow up policy was to allow partial weight bearing when tolerated within the second or third postoperative day for eight to twelve weeks when radiographs showed sufficient bridging callus then it was gradually increased to full weight bearing.

The patients were being followed at regular intervals every 2 weeks after discharge for the first two months, then monthly for one year and every six months in the second year. Minimum follow up was 12 months. Maximum was 24 months. Healing of the fracture, characterized by bridging callus seen on two radiographs made with different projections.

Hospital stay:

Mean hospital stay was 17 days, ranging from minimum 6 days to maximum 82 days. Mean preoperative period was six days.

Classification:

The incidence of fracture in

different parts of femur is shown in Table-6. Comminution of the fractures was graded according to Winquist-Hansen Classification[10,11] which is based on the percentage of intact circumferential cortical contact of the major fragments. According to Winquist classification (Table 7) type-I fracture was in 15 operated femurs (21.4%), type-II fracture was in 20 operated femurs (28.6%), type-III in 19 operated femurs (27.15%) and type-IV in 16 operated femurs (22.85%).

Result for fracture union was evaluated both clinically and radiologically, and graded at 12 months after treatment by Thoresen's criteria (Table 8) [12].

Treatment outcome was analyzed regarding fracture healing, joint movements, and implant failure. For this purpose, these patients were post-operatively followed at intervals of 2 weeks, 6 weeks, 3 months, and then monthly thereafter regularly for a total of 24 months. Patients were discharged as soon as their condition allowed. The criteria of classification which was applied as proposed by Thoresen et al[12], with the addition of non-union at 12 months or nail breakage as criteria for a poor result (Table-8). The outcome results regarding fracture union at 12th months were graded as Excellent, Good, Fair and Poor. Clinical criteria of union included absence of pain and local tenderness on stressing the fracture site or full bearing weight of the operated limb. Radiological healing of the fracture was defined as presence of callus around the fracture circumference with density similar to that of adjacent cortex, or obliteration of fracture line, whichever was earlier. Healing of the fracture was considered complete when both clinical and radiological criteria of union were fulfilled to the satisfaction of the authors and the independent observer. Delayed union was recorded when the fracture united between 4 and 6 months, while non-union was noted when union had not occurred after 8 months of treatment. All of our patients were followed-up for at least 12 months.

Clinical criteria of union included absence of pain and local tenderness on stressing the

fracture site or full bearing weight of the operated limb. Radiological healing of the fracture was defined as presence of callus around the fracture circumference with density similar to that of adjacent cortex, or obliteration of fracture line, whichever was earlier.

Healing of the fracture was considered complete when both clinical and radiological criteria of union were fulfilled to the satisfaction of the authors and the independent observer.

Results

The mode of injury found was road traffic accident (RTA) in 88%, fall from a height in 9% while pedestrian 3%. Associated injuries were occurred in 35 patients (50%).

The clinical outcome showed that all patients 100% had union at 8 months. The average time for union was 3 weeks. One patient had deep infection. This patient was 43 years old male admitted with fracture shaft of right femur, he was operated on the same day of admission. Two months later the patient had developed symptoms and signs of infection. The patient was then operated under general anesthesia where debridement and irrigation was done. Systemic antibiotic after culture and sensitivity test was given to the patient till all symptoms and signs of the infection were resolved. By follow-up evaluation we found that the fracture was united at 8 months postoperatively.

Abductor weakness resulting in trendelenberg gate was observed in one patient even after three months of his nail removal. There were four cases had limited range of motion in the knee joint. There were no cases of significant malrotation, axial shortening or lengthening.

No dynamization was done in any of the cases. Static interlocking nailing was performed in all patients. Closed nailing was accomplished in 80 per cent of the patients. In the remaining 20 per cent open reduction was necessary because of failure to pass the guide wire across the site of fracture under fluoroscopic control.

The mean time to union was

found to be 16 weeks {when adequate radiographic callus was visible}. All fractures united well.

Eight patients (13.3%) had developed pulmonary embolism which was major cause of morbidity associated with bed recumbence.

There was proximal screw breakage in two patients, distal screw breakage in four patients.

At the last follow up the average flexion of the hip was 100 degrees {range 80 to 135 degrees}. No patient had a fixed flexion contracture of the knee. The patients had less than 90 degrees of flexion of the knee were associated with fracture of the ipsilateral tibia (12 patients).

In three patients who had a comminuted fracture of the distal third of the femur, an angular malunion developed, meaning that there was more than 5 degrees of varus or valgus angulation at the site of the fracture. These fractures were treated with an interlocking nail, but the distal location permitted placement of only one locking screw. Loss of reduction occurred in all three patients. None of them needed an additional operation, but a supracondylar femoral osteotomy was planned for one patient of them. This case had a varus malunion.

Three patients had a limb length discrepancy of less than two centimeters but it was not associated with any functional impairment. No reconstructive operation was performed in any of these patients.

The clinical\radiological evaluation was done at the final follow-up evaluation with minimum of 12 months postoperatively and the final observations were made according to the criteria by Thoresen et al.12 We obtained excellent results in 58 patients (82.85%), good in 7 patients (10%), fair in 5 patients (7.14%) with no poor result (0%).

Majority of the patients complained of pain at the fracture site and knee this was treated with analgesia. Overall there were 22 complications detected and were

Classified into:

Table (1): Age distribution.

Age group	16-25 years	26 -35 years	36 -45 years	46 -55 years	56 plus
No. of patients	15	30	10	3	2
%	25 %	50 %	16.67 %	5 %	3.33 %

Table (2): Gender and side distribution.

Male	90%
Female	10%
Unilateral	Left 39% Right 44.4%
Bilateral	16.6%

Table (3) : Duration (Hospital stay) from date of admission to date of operation.

Within 24 hours	24-48 hours	48-72 hours	4-9 days	More than 10 days
2 femurs (2.9 %)	7 femurs (10%)	13 femurs (18.5%)	42 femurs (60%)	6 femurs (8.6%)

Table (4): Method of reduction used.

Closed reduction	Open reduction
56 Femurs (80 %)	14 Femurs (20 %)

Table (5): Nail dimensions used.

Diameter	9	10	11	12	-	-
%	15.7	37.1	44.3	2.9	-	-
Length	34	36	38	40	42	44
%	2.9	4.2	20	50	20	2.9

Table (6): Fracture site distribution.

Upper third	Mid third	Lower third	Segmental	Combination
12	41	7	5	5

Table 7: Fracture classification according to Winquist-Hansen.

Type:	0	1	2	3	4
No.	0	15	20	19	16

Table 8 : Criteria for classification of results (Modified from Thoresen)[1]

Criteria	Results			
	Excellent	Good	Fair	Poor
Malalignment of femur (degrees)				
Varus/Valgus	0	5	10	>10
Antecurvatum/Recurvatum	5	10	15	>15
Internal Rotation	5	10	15	>15
External rotation	10	15	20	>20
Shortening of femur (cm)	1	2	3	>3
Knee motion (degrees)				
Flexion	>120	120	90	>90
Extension deficit	5	10	15	>15
Pain/Swelling	None	Minor	Significant	Sever
Non-union/Nail breakage	Absent	Absent	Absent	Absent

Table (9): Mode of trauma.

RTA	Fall from height	Pedestrian
88.33 %	8.33 %	3.33 %

Table (10): Associated injury and /or chronic illness.

Head injury	Chest injury	Abdominal injury	Other bony injuries	Associated Medical Condition. -D.M, obesity-Ischemic heart diseases.
8	5	5	17	10

Table 11 : General Complications.

Complications	Number of Patients
Pulmonary Embolism	8 (13.33%)

Table 12: Local Complications.

Complications	Femur operated
Deep Infection	1 (1.4%)
Knee limitation of range of motion	4 (5.7%)
Malunion	3 (4.3%)

Table 13: Complications Related to the Implant.

Complications	Interlocking Nail
Proximal screw breakage	2 (2.85%)
Distal screw breakage	4 (5.75%)

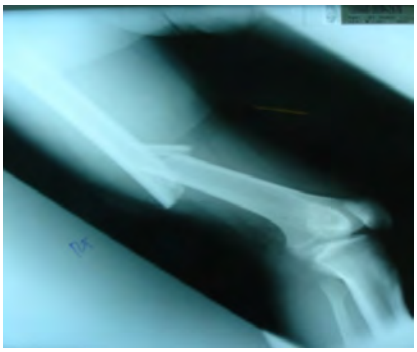

Fig (1) : Initial radiograph demonstrating comminution and shortening of the fracture.

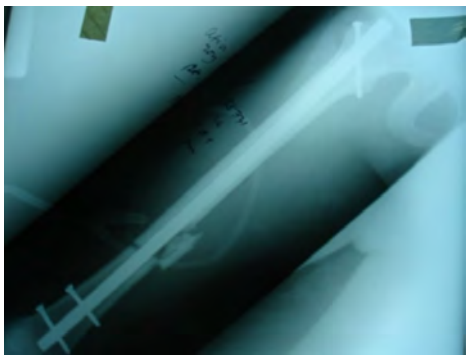

Fig (2) : Immediate postoperative x-ray showing static interlocking fixation.

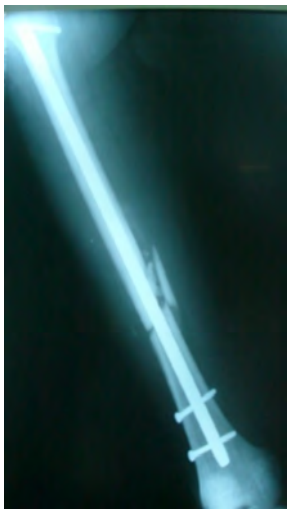

Fig (3) : Radiographic evaluation during follow-up 4 months postoperatively.

Discussion

Comminuted fractures of the femur are particularly difficult to treat. Usually a result of high energy trauma, this complex fracture is frequently accompanied by severe injuries to other organs and systems. In our study the mode of injury was road traffic accident in 88%, fall from a height in 9% while pedestrian 3%. Associated injuries were occurred in 35 patients (50%). This conduct a social message based on our study: None of the driver or passengers was wearing seat belts.

Conservative treatment of comminuted fracture femur yields unacceptable results and a high complication rate as malunion and non union[13]. There are many benefits of early fracture stabilization, the procedure facilitates patient mobility, improves pulmonary function, decreases pain and thus the need for non-steroidal anti-inflammatory and narcotics. Early fracture fixation also decreases inflamatory mediators response thus decreases thromboembolic phenomena. Over-all early stabilization of femoral fractures has been shown to decrease morbidity and mortality.

Early attempts at internal fixation with plate and screws of femoral fracture were fraught with serious complications especially infection and implant failure [14].

The use of a plate to achieve osteosynthesis of comminuted fractures of the femoral shaft necessitates a wide operative exposure and extensive stripping of the soft tissue, resulting in increased blood loss and operative time [15,16]. The risk of infection is increased. Failure of the plate is common and the need for primary bone graft adds additional morbidity to the procedure. Stress shielding by the plate has been blamed for residual weakness at the site of healed fracture with the resulting relatively high rate of refractures [17,18].

Due to these high rates of complications associated with this type of fixation most orthopedics advocated intramedullary nailing IM of comminuted fractures. Open intramedullary nailing with supplemental fixation does not always produce stable osteosynthesis [11,19,20] and involves an increased risk of infection.

Conventional closed nailing eliminates unsightly scaring of the thigh, minimizes disruption of the soft tissues at the site of the fracture, reduces the risk of infection and restores anatomical alignment[21-23]. Intramedullary interlocking nailing has become the treatment of choice for fractures of the femoral shaft fracture. Interlocking nails are weight sharing implants which allow immediate weight bearing after static locking even in unstable fractures. They have the advantage of providing greater fatigue strength, better stability in all planes especially if locking screws are used and providing reamed bone at the fracture site. The basic concept of interlocking nailing is to combine the advantages of closed interamadulary nailing with the added fixation of transfixing screws; this prevents axial sliding and rotation. Interlocking nailing has biological and biomechanical advantages in comparison with plate osteosynthesis [4,6,9,24].

Closed interlocking nailing required appropriate pre-operative management, preventive antibiotics together with excellent operative techniques and skills and is not without complications. Errors in the positioning of the patient, incorrect portals of entry and inadequate reduction can lead to

angulatory deformities, to inequalities in limb lengths and to implant failure. In our study all patients were positioned laterally on the operative table during surgery. This position facilitated assessment of rotational alignment and makes insertion of the distal locking screws easier and accurate intraoperatively (25). Sufficient cortical contact between the proximal and distal fragments was needed so that axial and rotational loading could be withstood without loss of length or rotational malalignment of the fracture. With the advent of static interlocking nailing, more complex fractures of the femoral shaft were treated with intramedullary techniques (26-28). It has been shown that static locking of comminuted fractures without dynamization does not result in an increased rate of non-union (1,29). Many of the malunion that have been reported in the literature were due to dynamic locking of unstable fractures (30). Therefore, in our study of comminuted fractures, all nails were statically locked. We agree with Winquist and other authors that static interlocking fixation is necessary for all femoral fractures that have type 3 or type 4 comminution. [7,24,26,27,31,32] .

Brumback et.al [3,4] concluded that immediate weight bearing after stabilization of a comminuted shaft of femur with astatically locked intramedullary nail is safe when contrast has high fatigue strength (two distal locking bolts). Early mobilization reduces postoperative complications, maintain joint motion and decrease the duration of hospitalization.

The mean union time for these femoral shaft fractures in our study was 8 months which is comparable to the result of other studies. [4,12,24].

The overall results obtained during evaluation at 12 months postoperatively according to Thoresen criteria (1) was comparable with to that of Razak (33) where they had (83.33%), good in 4 patients (8.50%), fair in 3 patients (6.38%) and poor in 1 (2.12%). The results were designated as excellent, good, fair, or poor according to the alignment of the fracture, the range of motion of the ipsilateral knee, the degree

of pain and swelling and implant breakage. An excellent result meant that the patient had full, pain-free function of the extremity. In two patients who would have had a good result, simultaneous malalignment caused it to be downgraded to fair. In three operated femurs good results was downgraded to fair because there was significant pain and edema.

On the basis of our results, we recommend static locking of all comminuted femoral fractures. We believe that static interlocking nailing is the treatment of choice.

Non compliance was a common problem. Patients started against medical advice to bear weight on the operated limb prematurely as a result of which we had proximal screw breakage in two patients, distal screw breakage in four patients.

Shortening and malrotation, which plagued earlier methods of treatment are well controlled with available interlocking fixation (5). However, fractures of the femoral shaft that are not treated by interlocking both the proximal and the distal fragment may be susceptible to postoperative loss of fixation. In our study three patients who had a comminuted fracture of the distal third of the femur, an angular malunion developed, meaning that there was more than 5 degrees of varus or valgus angulation at the site of the fracture. These fractures were treated with an interlocking nail, but the distal location permitted placement of only one locking screw. Loss of reduction occurred in all three patients. These same three patients had a limb length discrepancy of less than two centimeters which was not associated with any functional impairment. No reconstructive operation was performed in any of these patients.

We had low incidence of major complications related directly to the procedure. Only 1 operated femur (1.4%) had deep infection, but we had viewed eight patients (13.33%) with pulmonary embolism which was major cause of morbidity associated with bed recumbence in our study.

Advantages of interlocking nailing are pain free, early mobiliza-

tion is possible even with highly comminuted fracture and supplemental external support in the form of traction or cast bracing is rarely necessary.

Conclusions

Fracture shaft femur is commonest in most productive years of life, (92%) <45 years of age. 88% resulting from RTA. Most of the patients resumed their work in (approximately) 5-6 months postoperatively. This study is comparable to other international studies published in various journals, distinguishing in higher number of severely comminuted fractures, minimal complications but highest (13.33%) percentage of pulmonary embolism. Present protocol of treatment has given the most satisfying results. It is to conclude that locked intramedullary nailing has become the gold standard producing excellent results with acceptable rate of complications. We recommend static interlocking nailing for the treatment of all acute closed fractures of the femoral shaft because of the predictability of static interlocking fixation in terms of maintenance of length and alignment of the site of the fracture until union and because of the minimum risk of late re-fractures. Intra operative assessment of the stability of fixation of a fracture is of paramount importance.

Lastly a social message based on our study, 88% of patients in this study were victims of road traffic accidents. None of the driver or passengers was wearing seat belts.

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EVALUATION OF STATIC LOCKED INTRAMEDULLARY NAILING FOR TREATMENT OF COMMINUTED FEMORAL SHAFT FRACTURES

Salah Youssef MD and Mohamed El-Menawy MD

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ACUTE ACROMIOCLAVICULAR DISLOCATIONS : RESULTS OF CORACOCLAVICULAR SCREW FIXATION

Mohamed El-Menawy MD

Department of

Faculty of Medicine AL-Azhar University, Egypt

Abstract

Background: *The vast majority of traumatic dislocations of the acromiclavicular joint (ACJ) occur either during sports or other daily activities. Most frequently, the mechanism of injury is force acting on the shoulder from the lateral side with the arm is an adducted position.*

Patients and Methods: *Twenty patients with recent complete acromioclavicular dislocation were treated operatively by coracoclavicular screw fixation, repair of torn ligaments together with exploration and debridement of acromioclavicular joint. Sling immobilization for 2 weeks and early post operative rehabilitation were carried out.*

Results: *Patients were followed up for a period ranged from 6-30 months (average 18m). Accending to constant score, 14 patients had excellent score, 4 good and 2 fair. while no poor results were obtained.*

Complications: *Implant failure with backing out of the screw occurred in one patient 6 weeks post operatively with no influence on the final outcomes superficial wound infection occurred in 3 cases and needed no further surgical treatment, only dressing and oral antibiotics for two weeks. Shoulder stiffness was mild in two patients and did not impair in the patient daily activities.*

Conclusion: *Good results can be obtained in complete types III, IV and V (ACJ) separation by repair of both AC and CC ligaments, proper fixation of the screw, repair of deltotrapezius muscles and fascia and meticulous adherence to the post operative rehabilitation program.*

Introduction

The vast majority of traumatic dislocations of acromioclavicular joint occurs either during sports or other daily activities[1-3]. Most frequently the mechanism of injury is force acting on the shoulder from the lateral side with the arm in an adducted position [4]. On the basis of direction and amount of clavicular displacement, Rockwood introduced a classification of acromioclavicular dislocations from grade I to vI [5] (Fig 1) 8. It is mostly accepted that grade I and II lesions can be treated conservatively[6,7]. There is also wide consensus that type IV, V and VI injures should be treated operatively. For type III ACJ injuries the discussion concerning conservative or operative treatment is still controversial 2-4. There are well over 100 citation in the literature reviewing the operative treatment of AC dislocations, all of these methods can be categorized into the following 3 groups primary AC joint fixation, primary coracoclavicular (C.C) fixation on excision of the distal clavicle and dynamic muscle transfer. These reports offer many modification of previously reported techniques such as C.C screw fixation, primary ligamentous repair, transfer of surrounding soft tissues, use of synthetic materials, fascial grafts and the hook plate technique [8-26]. The different varieties of procedures indicates the interest exists among the orthopaedic surgions to develop a procedure offering minimal morbidity to surrounding tissues and a biomechanically sound solution to reconstruct the displaced AC joint. The aim of this study is to report the functional outcome of cases with complete ACJ dislocation managed by open reduction, C.C screw fixation with repair of ligaments and surrounding soft tissues.

Patients and Methods

In this study twenty patient with AC dislocations were treated in AL-AZHAR university hospital in new Damietta in the period from 2006-2010. Nineteen patients were males and only two were female. The mean age was 32 years with a range from 18 to 40 years (Table 1).

The cause of injury were motorcycle accidents in 11 patients, motor car accidents in 5 and sports

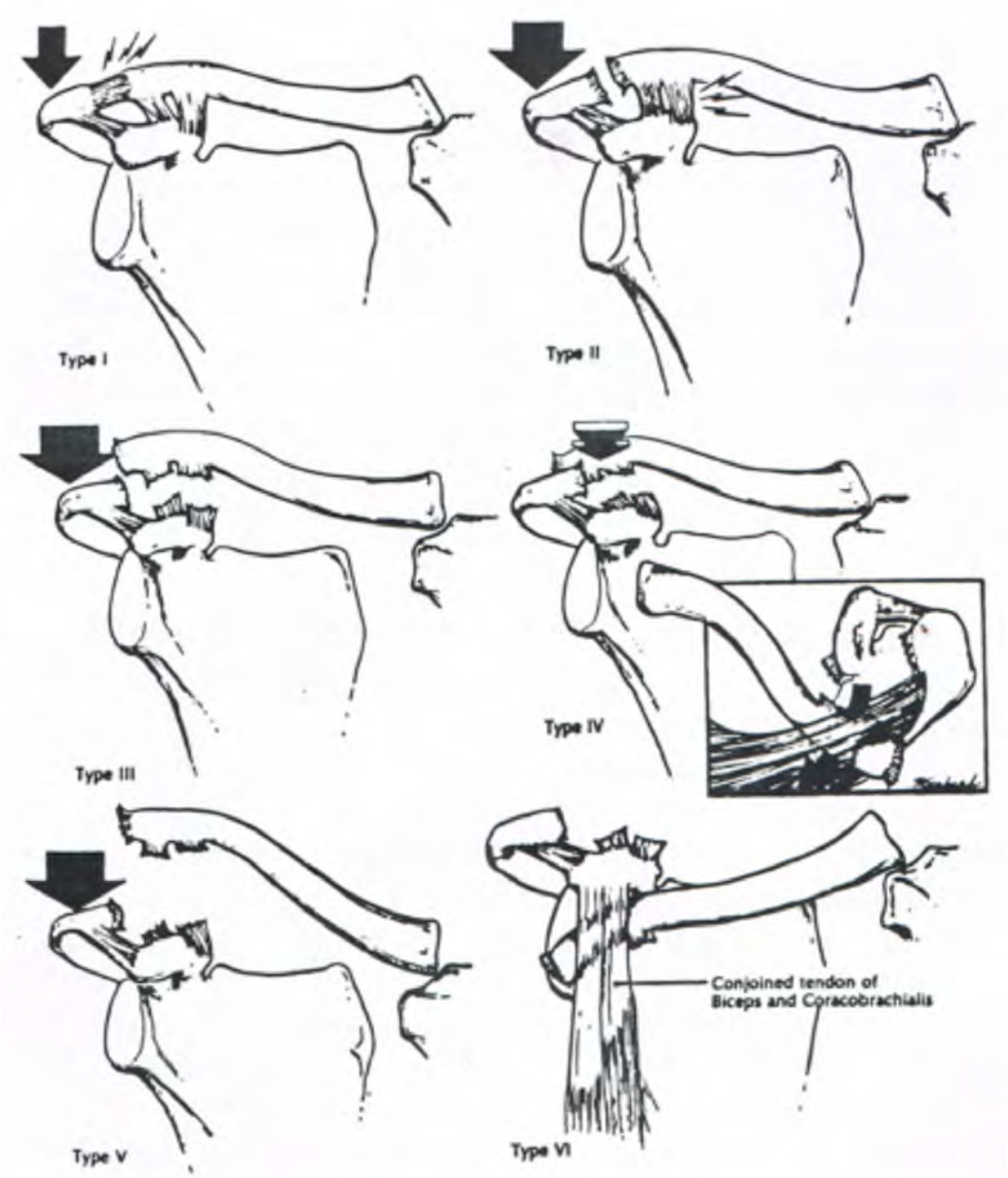


Fig. (1) : Classification of acromioclavicular injuries.

injury in 4 patients (Table 2).

Table (2) Causes of injury

The right AC joint was injured in 12 patients and the left in 8 patients (Table 3) there is no predilection for either the dominant or the non-dominant side.

Clinical picture:

Acute AC dislocation was diagnosed clinically by swelling with prominence of the lateral end of the clavicule. There is localized tenderness over the ACJ and painful shoulder movements specially abduction.

Radiography:

Two A.P standing radiographs for both shoulders were taken with the x-ray tube placed at the level of the ACJ and the rays perpendicular to the joint.

In the first view the patient was asked to stand relaxed with the arms adducted and the forearms pronated. In the second, 8 kilograms were suspended from the wrists [12].

Example, type III ACD was diagnosed by prominence of the lateral end of the clavicle by at least one full width of its lateral end above the acromion. The coracoclavicular inter space was 25% to 100% greater than normal shoulder, but in type V the inter space may reach up to 300% wider than the normal shoulder. Table (5) shows the number of patients in relation to the type of injury.

Surgical technique:

The top of the patient's shoulder should be completely free. The position is the semi setting.

Position under general anaesthesia.

A shoulder strap incision about 10 cm starting 2 cm posterior to the clavicle then across the clavicle about 3 cm medial to the ACJ and extends down wards to a point distal to the tip of the coracoids process.

A transverse incision is made in the fascia and periosteium over the lateral third of the clavicle reaching laterally to the ACJ splitting of the anterior fibers of the deltoid to visualize the coracoids process is carried out. The distal end of the clavicle is lifted upward

to expose the torn ends of the coracoclavicular ligaments and the base of the coracoids process. Debridment of the ACJ and excision of the disc is done if torn. Two prolene 2/0 sutures are inserted in the free ends of the C.C ligaments if they are torn in the middle. In 2 instances the ligaments were avulsed from the clavicle and curled over the coracoid. In these cases drill holes were made in the clavicle through which stay sutures were passed. These sutures were not tied. With ACJ reduced a vertical drill have with 2.7 drill bit is made passing from the clavicle to the base of coracoids process. Measuring the length of the suitable screw using AO depth gauge is done. Partially threaded 4 mm cancellous screw of a suitable length and a washer is then inserted. When the clavicle becomes leveled with the superior border of the acromion, the sutures in the CCLs are tied. Another half a turn is applied to the screw to take any tension off the sutures. Repair of all soft tissues including ACLs, deltoid and trapezius tears is then done. Also the fascia and the periosteum over the clavicle are repaired, followed by skin closure.

Postoperative care:

The arm was supported in a sling far 1-2 weeks. After 1-2 weeks, the use of the sling was discontinued. The patient could use the arm for most everyday living activities but was cautioned to avoid pushing, lifting and pulling for 6 weeks. The patient, ordinarily, had a good range of functional range of motion that allows the patient. to begin daily living activities. The screw was routinely removed 12 weeks after surgery usually under local anasthescia: After removal full active and passive ROM is encouraged. Once full ROM and strength were obtained return to atheletic competition was permitted.

Results

All patients were followed up for a period of 6-30 months with an average 18 months. Radiologically the standered x-ray films were done and CC distance was measured on either side. Clinically, the patients were assessed at the end of the follow up period. The functional outcome was evaluated by the constant Murley score [27]. This score represent a maximum of 100 points. Accord-

ing to the achieved points, the results were grouped into an excellent (>89 points), good (80-89 point), fair (70-79 points) and poor (<70 points) outcome. According to constant score, 14 patients had excellent score, 4 were good, 2 fair while no poor result were obtained. All patients returned to previous work after a maximum of one year especially in manual workness.

Complications:

One implant failure with backing out of the screw occurred six weeks, postoperatively and the screw was removed after detection without without affecting the final result. Superficial wound infection occurred in two cases without the need for further surgical treatment, only dressing and oral antibiotics for two weeks. Shoulder stiffness was mild in one case, and did not impair the patient daily activities. No deep infection or neurovascular injuries were found. Radiological evaluation by stress view films, to be sure about the reduction and healing of the coracoclavicular and acromioclavicular ligaments revealed good reduction. All patients returned to previous work after a maximum of one year especially in manual workness.

In all patients the AC and CC ligaments were torn, the tear in the CCLs was in the mid substance in 18 cases. The CCLs were avulsed from the clavicle in 2 cases. These 2 cases were graded as excellent. Debridment of the ACJ was carried out in all cases and the disc was found to be torn in 2 cases in whom excision of the disc was done. In these cases the result were accepted (Excellent and good). In one case excision of the lateral 2 cm of the clavicle was done after exploration of the ACJ and avulsion of articular cartilage from the lateral end of the clavicle The patient's age was 45 years and the outcome of the procedure was graded as fair.

Table (1): age distribution .

AGE IN YEARS	NUMBER	%
25	6	30
25-35	12	60
35-40	2	10
Total	20	100

Table (2) : Causes of injury.

CAUSE OF INJURY	NO	%
Motorcycle	11	55
Car accidents	5	25
Sport injury	4	20
Total	20	100

Table (3) Side of injury.

SIDE AFFECTED	NO	%
Right Side	12	60
Left side	8	40
Total	20	100

Table (4) Type of ACJ injuries.

TYPES OF INJURY	NO	%
III	4	20
IV	10	50
V	6	30

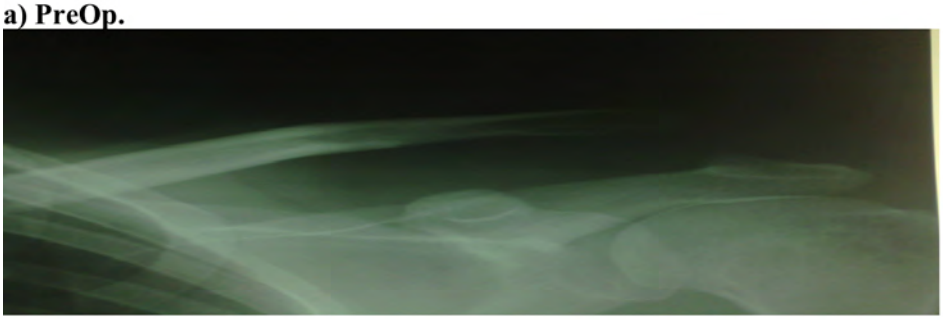
Table (5): Constant score for clinical shoulder evaluation.

Parameters	Degree	Points
Pain	None Mild Moderate Seven	15 10 5 0
Activities of daily living (Activity level)	Full work Full recreation / sport Unaffected sleep	4 4 2
Arm positioning	Up to waist Up to xiphoid Up to neck Up to top of head Above head	4 2 6 8 10
Range of motion		40
Power (1 point per pound of weight held in abduction by arm at 90 degree		25
	Total	100

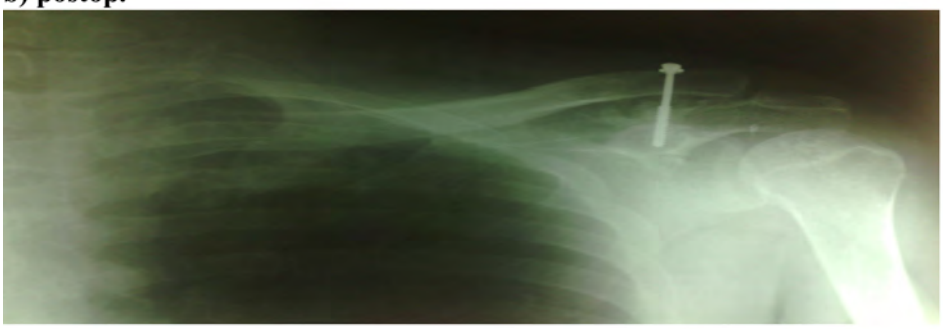
Table (6): Clinical and radiological result of the follow up period.

Parameter	Postoperative complications		Radiological results		Clinical constant score value	
	Superficial infection	2	Anatomical reduction	18	Excellent	14
	Impalnt failure	1	Subluxation	2	Good	4
	Limited range of motion	1	Redislocation	0	Fair	2
	No complication	16			Poor	0
Total		20		20		20

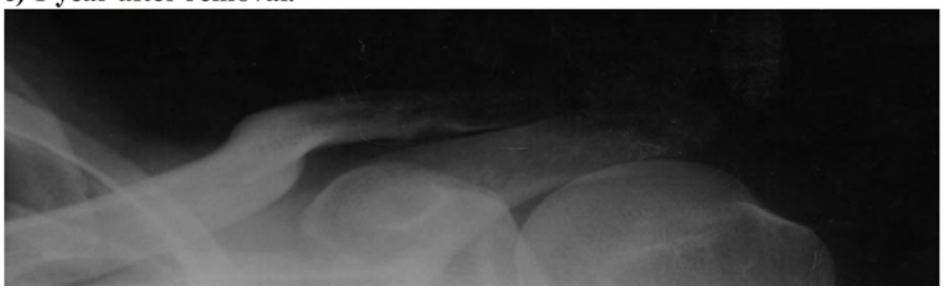
Fig. 1
a) PreOp.



b) postop.



c) 1 year after removal.



Discussion

Early repair of acute ACJ dislocations provides over all good clinical results independent of the surgical methods[1,4,7,28]. Whereas surgical treatment is recommended for type IV to VI ACJ injuries, the management of type III injuries is still controversial. Depending on the surgical method, one has to be aware of complications such as wound infection osteomyelities, nerve injuries, ossifications, osteoarthritis, stiffness and implant failure. For these reasons several authors recommend surgical reconstruction exclusively for young patients, in athletes or for heavy work[1,10,22] and there is still discussion whether ACJ injuries of type III should be treated by conservative or operative methods. In constant Mohsine et al[29] reported poor results (52%) of conservative treatment in grade I and II ACJ dislocations and they concluded that the severity of the consequences after grade I and II AC sprains is under estimated. The difficulties associated with surgery as reported in the literature are usually concomitant with either acromioclavicular fixation using either smooth or threaded k-wire, C.C fixation using stainless steel wire loops or soft tissue grafts, transfer of coracoacromial ligaments and transfer of coracoid process. Migration of pins, breakage of the wires, erosion of the bone and failure of fixation with subsequent recurrence of the deformity are the commonst complications[5,30]. In some cases the pins have been found in the heart, lungs and great vessels[31,32] Coracoclavicular screw fixation popularized by Boswarth[45]. The use of screws has been described alone and in combination with ligament reconstructions[33]. The use of CC screw fixation especially if accompanied by repair of torn ligaments will ensure enough protection of these ligaments till complete healing[5]. The most frequent complication associated with coracoclavicular screw fixation is the formation of bony bar in the coracoclavicular space. Most investigators reported that even in the presence of this calcification, patients had full range of shoulder motion[11]. Larsen and associates (1987)[32] found that this calcification had occurred in conservatively treated cases in nearly equal percentage as cases treated opera-

tively. However, in this series, none of the cases developed coracoclavicular calcification, this may be due to the relatively earlier operative interference and thorough washing of the bone debris resulting from drilling of the clavicle and coracoid.

Pulling out of the screw from the coracoid and subsequent loss of reduction was reported by some authors (Imatani et al., 1975). I believe that this complication can occur either in older age groups with osteoporotic bone or due to fixation of the screw to the tip or the waist of coracoid rather than its base This complication was not encountered in this series as all patients were young (18 - 47 years) and screw fixation to the base of the coracoid was always feasible.

Although, some authors do not recommend repair of the coraco clavicular ligament[3,33], others do strongly stress on the importance of their repair and reporting more favourable results[2]. It was found in this study that this repair is an easy job, restoring nearly normal anatomy and giving an extremely favourable results (24 of 25 were excellent and good).

Most studies comparing both conservative and operative treatment of complete acromioclavicular separation have not demonstrated superior results by surgical means [34], but, in heavy manual workers and young athletes one cannot feal satisfied to treat them conservatively. Even the most enthusiastic supporters of conservative treatment do recommend surgical methods in these subjects and consider these patients as an exceptional indication for surgery [16].

Five cases in this series had no visible deformity on clinical examination, the range of shoulder motion was nearly normal. The only clinical sign was tenderness over the acromioclavicular joint. All these patients had complete dislocation of the ACJ in both ordinary and stress radiographs. Therefore it is concluded that no clinical findings are completely reliable in diagnosis and that ordinary and stress radiographs should be resorted to in all cases of AC injuries to verify the type of disloca-

tion; these findings goes with the observations of other authors[35]. It was interesting to find no visible injury at operation of both trapezius and deltoid muscles in these 5 cases, this may explain the absence of deformity as the intact muscles were able to contract and fix the clavicle preventing its upward displacement [32].

I do not consider the need for a second operation to remove the screw as a disadvantage of the procedure. This screw can be removed under local anaesthesia guided by c. arm, the operation takes few minutes and a single stitch is needed.

Although, the number of cases is too small to draw definite conclusions, I believe that this procedure is easy, reliable and give extremely favourable results provided that the steps mentioned in the operative technique are strictly followed.

Conclusions

Good results can be obtained in complete types III, IV, and V acromioclavicular separation by repair of both the acromioclavicular and coracoclavicular ligaments, proper fixation of the screw, imbrication of the deltotrapezius muscles and fascia over the clavicle and meticulous adherence to the post-operative rehabilitation program.

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ACUTE ACROMIOCLAVICULAR DISLOCATIONS : RESULTS OF CORACOCLAVICULAR SCREW FIXATION

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HEPATOPROTECTIVE EFFECT OF L-CARNITINE AND ATORVASTATIN IN EXPERIMENTALLY INDUCED TYPE 2 DIABETIC RATS

Amany N. Ibrahim MD and Ayman M. Mousa MD*

Departments of Pharmacology and Histology,
Faculty of Medicine, Benha University, Egypt.

Abstract

Background: *non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes are associated with dyslipidaemia, inflammation and oxidative stress. However, the pathophysiology of NAFLD in type 2 diabetes with hyperlipidaemia is not fully known, as well as the utility of lipid-lowering drugs in ameliorating liver injury markers. The aim of this study was designed to study the protective effects of L-carnitine, co-administered with atorvastatin, on nonalcoholic fatty liver disease in streptozotocin induced type 2 diabetes and explore the possible mechanism (s) of action focusing on the role of the oxidative stress and inflammatory markers.* ***Methods:*** *hepatic complications of type 2 diabetes with hyperlipidaemia and the effects of atorvastatin and l-carnitine, singly and in combination, in hepatic inflammatory and oxidative stress markers were tested using STZ induced type 2 diabetes in rats. Results: type 2 diabetes aggravated the overall metabolic state and the hepatic markers of injury. Treatments with L-carnitine, or atorvastatin attenuated the development of NAFLD as evidenced by significant decrease in liver index, reduced liver and serum triglycerides, total cholesterol, and LDL cholesterol and confirmed by histopathological examination. Treatment with atorvastatin and l-carnitine further decreased liver tumor necrosis factor, hepatic oxidative stress (MDA) especially in carinitine (250mg/kg/day) +atorvatatin (30mg/kg/day) group.*

Conclusion: *L-carnitine and atorvastatin singly or in combination ameliorated fatty liver by attenuating liver inflammation and oxidative stress in type 2 diabetic rats.*

Keywords: *Type 2 Diabetes Mellitus, Carnitine, Atorvastatin, Nonalcoholic fatty liver (NAFLD).*

Introduction

Nonalcoholic fatty liver disease (NAFLD), one of the most common complications of type 2diabetes, is characterized by an increase in fatty acids, triglycerides, and cholesterol levels[1] and fat accumulation in the liver. Among type 2 diabetes patients, 50%-70% of individuals were diagnosed with NAFLD; in obese patients, that value increases to 95%[2]. Recent studies have shown that NAFLD and insulin resistance [3] were involved in metabolic syndrome (MS), especially in fatty acid metabolic disorder, which primarily occurred in obese and type 2 diabetes patients[4]. Prolonged exposure to free fatty acids damages pancreatic β-cells and hepatocytes [5]. Furthermore, excessive fat accumulation in the liver damages mitochondria, which are the primary cellular sites for fatty acid utilization[6,7].

It was initially found that NAFLD is a completely benign disorder; but histological follow up studies showed that progression to fibrosis occurred in about one third of patients[8]. However, the mechanisms that mediate the transition from steatosis to NASH and to fibrosis remain unknown. Several mediators and mechanisms have been suggested. The most recent prevailing concept is 'multiple-hit' hypothesis[9]. The starting point is insulin resistance that leads to a reversible accumulation of fat in hepatocytes[10], followed by oxidative stress and up-regulation of proinflammatory mediators that activate inflammatory pathway[11]. Several therapies, including diet and antioxidants have been tried to treat patients with NAFLD [12,13].

Atorvastatin, a HMG-CoA reductase inhibitor, is widely used in the treatment of dyslipidaemia. A transient rise in serum transaminases occurs in up to 3% of patients using atorvastatin but this is usually self-limiting and inconsequential[14]. Recently, statins have been reported to may improve hepatic steatosis; however, the effect on fibrosis is controversial[15].

L-Carnitine (L-β-hydroxy-γ-N-trimethylaminobutyric acid [16,17]) plays an important role in lipid metabolism; it acts an essential

cofactor for the β-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial membrane as acyl-carnitine esters. It can activate carnitine palmityl transferase-1 (CPT-1), the key enzyme in fatty acid oxidation[18].

The earliest study of the effect of L-carnitine on steatohepatitis was performed by Bowyer et al. in 1988[19]. They showed that L-carnitine supplements in patients with NASH (non-alcoholic steatohepatitis) greatly improved glucose plasma levels, lipid profiles and histological manifestations[20]. However, none of these studies focused on the effect of L carnitine and atorvastatin on NAFLD caused by type 2 diabetes. The aim of the present study was to explore the protective effect of L carnitine and atorvastatin on NAFLD in streptozotocin (STZ)- and diet-induced type 2 diabetic rats with regard to morphological and biochemical aspects. We also intended to determine their possible antioxidant mechanism of action.

Materials and Methods

Animals :

Adult male albino rats (n=50), weighting 150-200 g. They were brought from (Experimental Animal Breeding Farm, Helwan - Cairo). All animals were housed in controlled laboratory condition at 20 - 25C in a 12h light/ dark cycle and had free access to high caloric diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They have acclimatized for one week and were caged (10/cage) in fully ventilated room (at room temperature) in pharmacology department, Benha Faculty of Medicine. All experimental protocols were approved by the committee of Benha University.

Experimental protocol :

After acclimatization for 1 week, rats were randomly divided into 5 experimental groups, 10 rats each and treated for 8 weeks as follow:

Group 1 (normal control group): received physiological saline and served as normal control group (CN).

Group 2: (Diabetic group) Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ 60 mg/kg) dissolved in cold 0.1 mole citrate buffer (pH 4.5) after fed the rats with high fat diet for 2 weeks as a model for type 2 diabetes [21].

Group 3: (L-carnitine group) rats received l-carnitine (250mg/kg/day) by gavage. [22].

Group 4: (atorvastatin group) rats received atorvastatin (30mg/kg/day) by gavage [23] .

Group 5: (L-carnitine +atorvastatin group) rats received l-carnitine (250mg/kg/day) + atorvastatin (30mg/kg/day) by gavage

All groups injected with single intra-peritoneal injection of STZ (60 mg/kg, i.p., provided by Sigma) prepared in 0.1 N citrate buffer at pH 4.5 were given to rats and fed with high cholesterol diet except normal control group.

At the end of the experimental period, rats were anaesthetized by inhalation of ether and blood samples were collected from abdominal aorta and processed for biochemical measurements. Then rats were sacrificed and their livers were rapidly collected, blotted dry weighed and divided into 2 parts. One part was put in 10% formalin for histopathological examination. The second part was kept at -70^{o}C and used for biochemical measurements.

Parameters Measured :

1- Measur ment of liver index:

Liver index was calculated from the equation : (Liver weight / body weight) 100[23].

2- Biochemical measurements:

a- Serum levels of alanine amine transferase (ALT):

Serum level of ALT was measured using biochemistry automatic analyzer (Hitachi7600).

b- Serum lipid profile:

Serum levels of total cholesterol, LDL and triglyceride levels were measured using commercially available kits as described by (Siedel et al.,[24]; Noma et al.[25]; Mcgraw et al.[26])

3- Liver tissue content of triglycerides :

Triglyceride was assayed in

hepatic tissue using commercially available kits after lipid extraction as described by (Folch et al.[27]).

4- Estimation of malondialdehyde (MDA) content:

Malondialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals, is considered a reliable marker of oxidative stress and was determined by measurement of the chromogen obtained from the reaction of malondialdehyde with 2-thiobarbituric acid, according to Aruoma et al. [28]. The MDA values are expressed as micromole per gram of tissue protein (μM/g tissue).

5- TNF-α protein level in liver tissue:

This was taken as a marker of inflammation. TNF-α level was measured in liver tissue using commercially available kits (Genzyme Immunobiologicals, Cambridge, U.K.) following the protocol provided by manufacturer. Results are expressed as nanograms per milliliter of tissue protein (ng/ml).

6- Histopathological examination:

For the histological study, rat liver specimens were taken 5 mm away from the edge of the largest hepatic lobe, fixed with 10% formaldehyde; embedded in paraffin wax, stained with hematoxylin and eosin (HE) and then observed under the light microscope Drury et al. [29].

Statistical analysis of the data (Goldstone, 1983 [30]):

Data are shown as mean ± S.E.M. the results were analyzed by one way analysis of variant (ANOVA) followed by t-test or tuckey test with $p<0.05$ selected as the criterion for statistical significance.

Results

1-Effect of l-carnitine and atorvastatin on liver index:

Liver index was significantly increased in cholesterol-fed diabetic group compared with normal control group. In l-carnitine and atorvastatin treated groups liver index was significantly decreased compared with cholesterol fed diabetic group (table 1).

2- Effect of l-carnitine and atorvastatin on liver function tests:

There was significant elevation of serum ALT in non treated diabetic group compared with normal control group. ALT activity was decreased to normal values in l-carnitine and atorvastatin group (table 1).

3- Effect of l-carnitine and atorvastatin on liver triglycerides:

Liver contents of triglycerides were significantly increased in cholesterol fed diabetic group compared with the control value. Administration of l-carnitine and atorvastatin resulted in significant decrease in liver triglycerides compared with cholesterol-fed diabetic values (table 1).

4- Effect of l-carnitine and atorvastatin on serum lipid profile:

There were significant elevation of serum triglycerides, total cholesterol and LDL-C in diabetic group compared with normal control group. Treatment with l-carnitine and atorvastatin significantly decreased total cholesterol, triglycerides, and LDL-C to normal value (table 2).

5- Effect of l-carnitin and atorvastatin on liver content of MDA:

MDA in the livers of animals injected with STZ was significantly higher than normal control group. Treatment with l-carnitine and atorvastatin significantly decreased MDA to normal values (table 1).

6- Effect of l-carnitine and atorvastatin on liver inflammatory marker (TNF-α) protein level:

TNF-α protein level was significantly increased in liver after 60 days from STZ injection (43.27± 9.3 vs 6.12±1.6 ng/ml) when compared to control group, (table 2). L-carnitine and atorvastatin significantly reduced TNF-α level in liver tissue (27.57±8.18, 23.10± 3.31 and 18.32±2.54 vs 43.27±9.3 ng/ml, respectively) (table 2).

7- Effect of l-carnitine and atorvastatin on histopathological changes:

In the diabetic non treated group, there was significant in-

crease in microvesicular steatosis, inflammatory cells and fibrosis. The degree of hepatic fibrosis, inflammation were significantly decreased in l-carnitine and atorvastatin treated groups compared to diabetic non treated group (Figure 1).

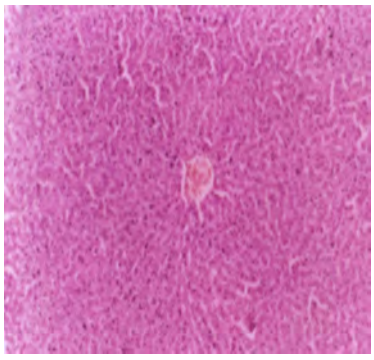
Table (1): Effects of l-carnitine and atorvastatin on liver index (g/g %), liver enzymes ALT (U/L), liver TG (mg/g tissue) and liver MDA (µM/g) :

	Liver index (g/g %)	ALT (U/L)	Liver TG (mg/g tissue)	Liver MDA (µM/g)
Control	2.3±0.1	20.4 ± 1.9	12.8±2.1	7.6±0.7
Diabetic	2.7 ± 0.1^{a}	70 ± 6.5^{a}	20.8 ± 3.6^{a}	16.5 ± 1.9^{a}
l- Carnitine treated	2.1 ± 0.1^{b}	38 ± 3.2^{b}	8.50 ± 1.09^{ab}	10.34 ± 1.04^{ab}
Atorvastatin treated group	2.0 ± 0.1^{b}	45 ± 2.1^{b}	9.40 ± 1.02^{ab}	9.51 ± 1.01^{ab}
l- Carnitine+Atorvastatin treated group	2.0 ± 0.1^{b}	30.1 ± 1.8^{b}	7.31 ± 0.8^{ab}	8.23 ± 0.7^{b}

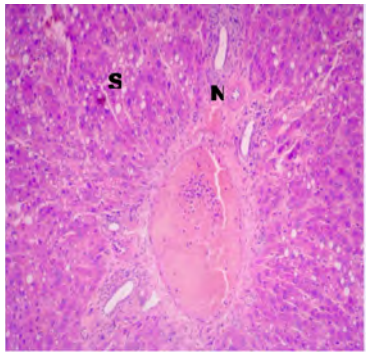
a : Significantly

Table (2): Effects of l-carnitine and atorvastatin on serum total cholesterol (mg/dl), LDL-C (mg/dl), triglycerides (mg/dl) and TNF-α (ng/ml):

	Total cholesterol (mg/dl)	serum TG (mg/dl)	LDL-C (mg/dl)	Serum TNF-α (ng/ml)
control	110±2.23	65.10±1.65	55.35±1.78	6.12±1.6
Diabetic	159 ± 2.70^{a}	149 ± 2.30^{a}	108.80 ± 1.39^{a}	43.27 ± 9.3^{a}
l-carnitine treated	131 ± 1.90^{b}	74.35 ± 2.10^{b}	88.83 ± 1.95^{b}	27.57 ± 8.18^{b}
Atorvastatin treated group	133 ± 3.20^{b}	69.35 ± 1.98^{b}	91.07 ± 1.86^{b}	23.10 ± 3.31^{b}
l-carnitine+Atorvastatin treated	121 ± 2.16^{b}	58.80 ± 1.62^{b}	73.40 ± 2.30^{bca}	20.32 ± 2.54^{b}



(A)



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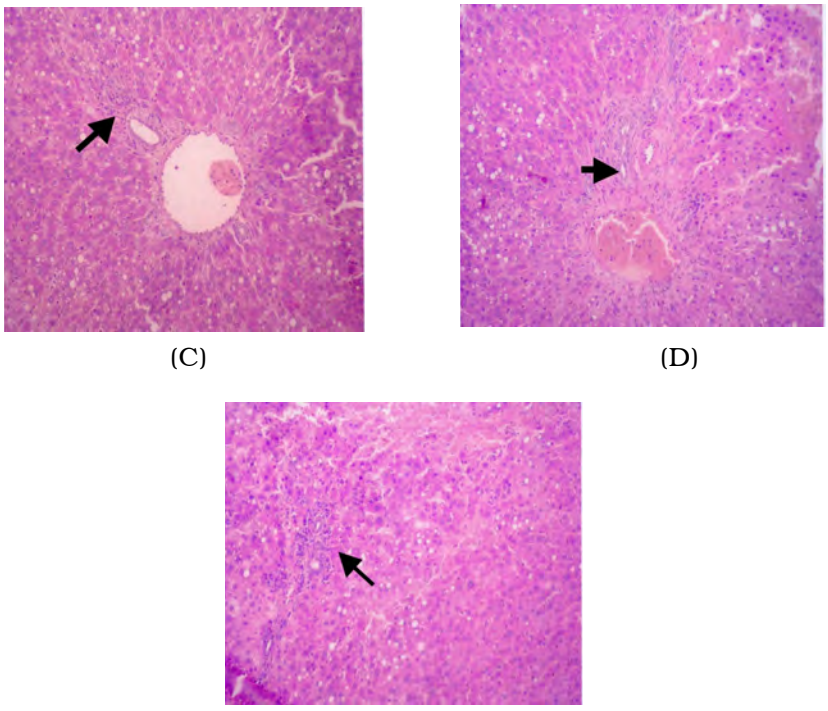

(C) (D)

(E)

Fig. (1): Effect of l-carnitine and atorvastatin on histopathological changes. Photomicrograph of the liver tissue (H&E 100x). A) represents a section from control group with normal liver tissue, B) a section from 2% cholesterol-fed diabetic group shows steatohepatitis (S) after 8 weeks of STZ injection with 2% cholesterol deit, hepatocytes showing vacuolated cytoplasm and eccentric nuclei, notice inflammatory cell infilteration, C) a section from l-carnitine-treated group shows liver tissue back to nearly normal aapearance in l-carnitine treatment rats, D)) a section from atorvastatin-treated group shows liver tissue back to nearly normal appearance in atorvastatin treatment rats, E) a section from l-carnitine + atorvastatin-treated group shows liver tissue back to nearly normal appearance in l-carnitine +atorvastatin treatment rats.

Discussion

The prevalence of NAFLD is high in obesity, diabetes, high-calorie diet and hyperlipidemia[31]. Our research confirmed the characteristics of NAFLD in diabetic rats. Excessive accumulation of triglycerides in hepatocytes, followed by lipid peroxidation and release of inflammatory mediators are potential players in the pathogenesis of liver injury in NAFLD[8,32] .

The present study investigated the effect of l-carnitine and

atorvastatin on the multiple steps involved in the pathogenesis of NAFLD. The study demonstrated that, high cholesterol diet in STZ type 2 diabetic rats successfully induced steatohepatitis as evidenced physically by significant increase in liver weight/body weight ratio (liver index) and confirmed histopathologically by increased deposition of fat droplets in hepatocytes, inflammatory cell infiltration and expressions of collagen. Administration of l-carnitine and atorvastatin significantly attenuated both physical and histopathological evidences of steatohepatitis. A similar finding has been reported by (Yunqiu Xia et al.,[22]; Matafome et al.,[33]; Matafome et al.,[34]).

ALT is a useful screening test for detecting liver injury [35]. When the hepatocyte is injured, plasma membrane is disturbed and the leakage through extracellular fluid of the enzyme occurs where they can be detected at abnormal levels in the serum. Our results showed that high cholesterol diet in STZ diabetic rats elevated serum ALT level. Similar finding has been reported by Miyaki et al.,[15]. Rats receiving l-carnitine and atorvastatin showed almost normalization of serum ALT.

In a trial to explore the mechanism of the protective effect of l-carnitine and atorvastatin, we measured hepatic tissue contents of triglycerides, lipid peroxidation product (MDA), and the proinflammtory enzymes TNF-α.

High cholesterol diet lead to significant increase in triglycerides in liver tissues compared with normal control group. It has been reported that the increase in hepatic triglycerides was due to failure of the liver to synthesize apolipoprotein required for packing and exporting fat from the liver thus accumulates triglycerides in the liver [36] (Thong-Ngam et al. 2007). In l-carnitine and atorvastatin-treated rats, there were significant decreases in liver triglycerides. Similar findings had been reported by (Yunqiu Xia et al. [22]; Roglans et al.[37]).

Previous studies showed that atorvastatin decreased hepatic triglyceride accumulation and circulating lipid concentrations

through its modulation of PPAR receptors. Hepatic PPAR-activation may provide an explanation for atorvastatin's antidyslipidemic actions observed in recent clinical trials. Also, l-carnitine decreased hepatic triglyceride through improves the insulin-resistance. Decreased availability of precursor substrates, including free fatty acid and glucose, diminishes hepatic synthesis and export of esterified lipids and caused a decrease in hepatic triglyceride accumulation and circulating lipid concentrations [37,38].

Lipid peroxidation serves as a marker of cellular oxidative stress and had long been recognized as a major causative factor of oxidative damage in chronic diseases [39] . Our results showed that l-carnitine and atorvastatin significantly reduced MAD in liver tissues. Previous studies suggested that l-carnitine and atorvastatin has a lipid peroxidation chain-breaking antioxidant effect [40,41].

Our results showed significant increase in the expression of TNF-α in liver tissues of cholesterol diet/STZ diabetic rats group. L-carnitine and atorvastatin administration significantly attenuated overexpression of TNF-α in hepatic tissue in NAFLD. In link with our finding, it has been reported that l-carnitine and atorvastatin reduced TNF-α expression in cardiac tissue in L-NAME induced hypertension and cardiomyopathy [42,43].

Conclusion

The present study demonstrate that L-carnitine, atorvastatin as monotherapy or in combination is effective in alleviation the progression of NAFLD in type 2 diabetic rats via decreasing lipid peroxidation and the expression of TNF-α in hepatic tissue. If these findings were to translate into actual clinical benefit, then L-carnitine might prove to be a suitable alternative to atorvastatin as therapeutic approach for management of NAFLD in type 2 diabetics or at least can be combined with atorvastatin to reduce its adverse effects and improve its efficacy. However, clinical studies are recommended to confirm its effect in human.

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HEPATOPROTECTIVE EFFECT OF L-CARNITINE AND ATORVASTATIN IN EXPERIMENTALLY INDUCED TYPE 2 DIABETIC RATS

Amany N. Ibrahim MD and Ayman M. Mousa MD

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SERUM LEPTIN AND ADIPONECTIN IN STEROID RESPONSIVE NEPHROTIC SYNDROME

Ahmad F. Rezk MD and Eman Eissa MD

Departments Of Pediatrics and Clinical Pathology

Banha Teaching Hospital

Abstract

Nephrotic syndrome is an albumin-losing nephropathy.Heavy proteinuria leads to hypoproteinemia,hyperlipidemia and hypercoagulable state, this makes nephrotic syndrome a high risk situation.

Aim of the study: *We aimed to evaluate the behavior of serum leptin and adiponectin (ADPN) in patients with steroid responsive nephrotic syndrome in relapse and remission.*

Subjects and methods: *This study was carried out on 35 children suffering from steroid responsive nephrotic syndrome had active disease (group 1) they were followed up for remission, 30 of them complete the study till remission (group 2) and 35 healthy children were selected as control (group 3). A-24 hours urine protein and 12 hours fasting blood samples were drown for measuring serum total protein, serum albumin and lipid profile as well as serum leptin and adiponectin.*

Results: *Our results revealed high significant increase in serum levels of adiponectin and high significant decrease in serum level of leptin in active state of nephrotic syndrome when compared with remission and control groups. A positive significant correlation was found between serum leptin and serum protein and albumin, while a significant negative correlation was found between serum adiponectin and serum total protein and albumin. Also a significant positive correlation was found between serum adiponectin and serum triglycerides, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and 24 hours urinary protein and a significant negative correlation was found between serum leptin and serum triglycerides, cholesterol, HDL, LDL and 24 hours urinary protein.*

Conclusion: *Raised serum adiponectin and decreased serum leptin*

can be used as markers for activity of nephrotic syndrome.

Keywords: *Nephrotic syndrome Adiponectin Leptin.*

Introduction

Nephrotic syndrome (NS) is an albumin- losing nephropathy. Heavy proteinuria leads to hypoproteinemia, hyperlipidemia and hypercoagulable state[1] this makes nephrotic syndrome a high risk situation[2].

Glucocorticoid responsive nephrotic syndrome (GRNS) remains the predominant type of nephrotic syndrome and after remission more than 50% of children will relapse and require further courses of steroid therapy[3]. GRNS is the most common NS type in children, displaying unique properties like minimal renal histological abnormalities and excellent response to steroids[4].

Adipocytes secrete a large number of biologically active proteins, which influence the function as well as the structural integrity of cardiovascular system (CVS)[2]. These cytokines include; tumor necrosis factor, angiotensin, plasminogen activator inhibitor -1, adiponectin (ADPN) and leptin which play important roles ranging from inflammation, coagulopathy and immunomodulation[5].

Adiponectin is the most abundant adipose tissue protein in human plasma and may have a protective influence on the CVS[6]. ADPN has antiatherognic and antiinflammatory properties[7], it suppresses the attachment of monocytes to endothalial cells and exerts an endothelial response to inflammatory stimuli[4]. Adiponectin has attached great attention due to its antiatherogenic and anti-inflammatory properties as well as its insulin sensitizing effect [7]. The strongest protective role of adiponectin is supported by observation of its low plasma level in patients with coronary artery disease and with type – 2 diabetes mellitus [8].

Leptin is a small peptide hormone secreted by a large number cell types; adipocytes, endothelial cells, T - lymphocyte, bone marrow cells and platelet[6]. Leptin

circulating levels directly correlate with the amount of adipose tissue mass and atherosclerosis [2].

Adiponectin and Leptin are markedly increased in patients with end stage renal disease[10]. NS is a high risk situation because of abundant urinary protein loss that trigger hypoalbuminemia, hyperfibrinogenemia and hypercholesterolemia that lead to hypercoagulable state and thromboembolism [2].

Aim of the study

We aimed to evaluate the behavior of adiponectin and leptin in patients with a steroid responsive nephrotic syndrom in relapse and remission.

Subjects & methods

This study was carried out on 35 children suffering from steroid responsive nephrotic syndrome (group1) at Benha Teaching Hospital during the period from Jan 2008 to December 2009; thirty of them (group 2) were followed up for relapse and 5 were dropped from the study. thirty five healthy children were selected as control (group 3) .

We excluded patients with hypertension, macroscopic hematuria, or abnormal renal function. Informed consent was taken from every caretaker.

After full history and clinical examination, a 24 hours urine protein and 12 hours fasting blood samples were drown from every child for measuring total protein, serum albumen, lipid profile as well as serum leptin and adiponctin.

Blood sampling

A 5ml of 12 hour fasting venous blood were drown under complete aseptic precautions from patients and controls, put into vacutainer serum tubes, allowed to clot followed by centrifugation with subsequent prompt separation of serum. An aliquet of serum was used for immediate assaying of total serum protein, albumin and lipid profile. The remaining amount of serum was stored at -70^{o}C for the subsequent assaying of leptin and adiponectin.

Principle of measurement of human leptin[11] and adiponec-

tin[12]. These assays employ the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for leptin or adeponection has been coated on to a micro plate sandards and samples are pipette into the wells and any leptin or adiponectin present is bound by the immobilized antibody. After washing away any unbound substances an enzyme linked monoclonal antibody specific for leptin or adiponectin was added to the wells. After washing a substrate solution is added and color developed in proportion to the amount of leptin or adiponectin.

Total protein in serum was measured colometrically by Biuret method[13]. Albumin was determined by Bromocresol Green colorometric method by a kit from Diamond Diagnostic[14]. HDL and cholesterol were measured by preceipitation method, LDL in samples precipitate with phosphotungstate and magnesium ions, after centrifugation,the cholesterol fraction which remains in the supernatant was determined[15]. LDL was estimated by using Friedwald formula (LDL = total cholesterol - HDL - triglyceride/5)[16].

Total protein in urine was measured by trichloroacetic acid precipitation method [17].

Statistical methods

The statistical study was done using the statistical program package the results are expressed as mean (X) ± SD. Statistical analysis was performed using student "t" test. A level of $p < 0.05$ was accepted as statistically significant. Spermans Rank correlation coefficient was used as a correlation test.

Results

Demographic data of the studied groups was shown in table (1): This study included (group 1) 35 patients with steroid responsive nephrotic syndrome 23 males and 12 females, with age ranged from 3.5-6.3 years (X ± SD : 4. 62 ± 0.99) and body mass index (BMI) ranged from 16.4 - 19.5 (X ± SD : 17.91 ± 0.88 years), 30 cases of them were followed up for remission (group 2) 19 males and 11 females, with age ranged from 3.6-6.1 years (X ± SD:4.73 ± 0.81 years) and BMI ranged from 15.3 - 19.5 (X ± SD 17.77 ± 1.15). Also

35 healthy children (group 3) 21 males and 14 females were chosen as a control, with age ranged from 3.6 - 6.2 years (X ±SD : 4.86 ± 0.85 years) and BMI ranged from 16.2-19.4 (mean ± SD : 18 ± 0.98).

Table (2) shows statistical comparison between studied groups versus control group as regard to serum biochemical parameters; total serum protein, albumin, triglycerides, cholesterol, HDL, LDL, and also 24 h proteinuria. Group 1 shows high significant decrease in total serum protein and serum albumin while serum triglyceride, cholesterol, HDL, LDL and 24 h urinary protein are highly significantly increased when compared with groups 1 and 2.

Table (3) and figures (1) and (2) show statistical comparison between studied groups versus control group as regard to serum leptin and adiponectin. A highly significant decrease in serum leptin is evident in group 1 in comparison to groups 2 and 3. However serum adiponectin is highly significant increased in group 1 compared to groups 2 and 3.

Table (4) shows statistical correlation between leptin and adiponectin with the biochemical parameters. Serum leptin is positively correlated with total serum protein and serum albumin only, while serum adiponectin is positively correlated with triglycerides, cholesterol, HDL, LDL and proteinuria and negatively correlate with total serum protein and serum albumin.

Discussion

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

Groups / Parameter	Group 1 (n = 35)	Group 2 (n = 30)	Group 3 (n = 35)
Age(years) range X ± SD	3.5- 6.3 4- 62 ± 0.99	3.6- 6.1 4.73 ± 0.81	3.6- 6.2 4.86 ± 0.85
Gender Males Females	 23(69.23 %) 12 (30.77%)	 19 (63.3%) 11 (41.67)	 21 (60%) 14(40 %)
BMI Range X ± SD	16.4 -19.5 17.91 ± 0.88	15.3 -19.5 17.77 ± 1.15	16.2 – 19.4 18 ± 0.98

X : mean SD : standard deviation BMI : body mass index

Table (2): statistical comparison between the studied groups versus control group as regards to biochemical parameters.

Groups / Parameters	Group 1 N=35			Group 2 N=30			Group 3 N=35
	X ± SD	(t) value	P	X ± SD	(t) value	P	X ± SD
Serum protein (gm/dl)	4.50± 0.68	- 12.028	< 0.001 **	6.64 ± 0.45	- 2.341	< 0.05 *	7.01± 0.29
Serum albumin (gm/dl)	2.38± 0.39	- 20.88	< 0.001 **	4.60 ± 0.38	- 6.087	< 0.001 **	5.53± 0.34
Seurm triglyceride (mg/dl)	354.26 ± 57.18	11.142	< 0.001 **	122.33±15.85	7.733	<0.001 **	85± 4.88
Serum cholesterol (mg/dl)	328.770± 59.60	11.95	< 0.001 **	153.793±13.79	5.364	< 0.001 **	129.2 ± 7.19
(HDL mg/dl)	47.93 ± 6.19	5.871	< 0.001 **	51.81 ± 7.26	3.520	< 0.001 **	60.4± 3.95
LDL(mg/dl)	229.06 ± 54.25	11.689	<0.001 **	77.51 ± 14.70	5.423	< 0.001 **	50.73 ± 7.99
24h proteinuria (mg/dl)	252.85 ± 66.63	13.660	<0.001 **	1.41 ± 0.92	3.719	< 0.001 **	0.405 ± 0.14

X: mean
SD: standard deviation
HD: high density lipoprotein
LD: low density lipoprotein

* *Significant* $p < 0.05$
** *Highly significant* $p < 0.005$

Table (3) Statistical comparison between the studied groups versus control group as regards serum leptin and adiponectin :

Groups / Parameter	Group 1 N = 35			Group 2 N = 30			Group 3 N = 35
	X ± SD	t value	P	X ± SD	t value	P	X ± SD
Serum leptin (ng/ml)	10.88 ± 4.38	- 37.74	<0.001*	15.63± 3.28	- 38.1	<0.001*	47.37 ±3.68
Serum adiponectin (ng / ml)	69.54± 16.08	19.77	<0.001*	34.57±7.66	14.42	<0.001*	15.54 ± 1.65

□ **Highly significant* $p < 0.005$
X : mean
SD : standard deviation

Table (4) Statistical correlation between leptin and adiponectin with Biochemical parameters.

	Total protein g/dl		Albumin g/dl		Triglycerides mg /dl		Cholesterol mg/dl		HDL mg/dl		LDL mg/dl		24-proteinurea mg/ dl	
	r	P	R	P	r	P	R	P	r	P	r	P	r	P
Leptin ng/ml	0.388	<0.05**	0.427	<0.05**	-0.32	>0.05	-0.35	>0.05	0.161	>0.05	-0.358	>0.05	0.395	>0.05
Adiponectin ng/ml	-0.928	<0.001*	-0.873	<0.001*	0.928	<0.001*	0.923	<0.001*	0.629	<0.001*	0.927	<0.001*	0.892	<0.001

** *Significant* *P<0.05*
* *Highly significant* *P < 0.005*
Non significant *P > 0.05*

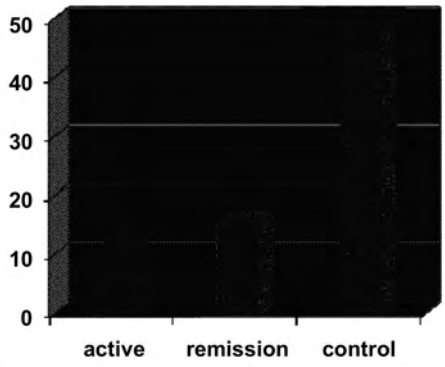

Fig (1) Comparison of serum levels of leptin in active and remission groups versus control group.

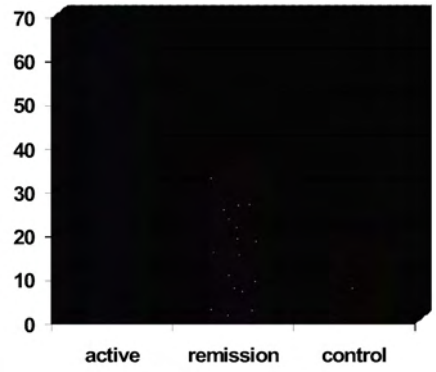

Fig (2) Comparison of serum levels of adiponectin in active and remission groups versus control group.

Steroid responsive nephrotic syndrome, the most common subtype of NS in childhood, is unique due to its great steroid response rate and excellent long term prognosis[18]. Serious complications can occur during the course of active disease including thrombotic events due to hypercoagulable state and vasculotoxic effect of biochemical alterations resulting from the nephrotic state[19].

In this context, we investigated serum ADPN and leptin levels and its potential correlates in patients with SRNS in relapse as well as in remission and comparing this with healthy controls.

In our study we found highly significant decrease in total serum protein and serum albumin, while there were highly significant increase in serum triglycerides, cholesterol, HDL, LDL and urinary protein in active disease when

compared with remission and control groups as expected, there were significant differences in serum protein, lipid parameters and 24- hour proteinuria level in SRNS, consistent with their disease activity[7].

In our study we found that serum leptin level was highly significantly decreased in patients with active disease and patients in remission incomparison with control group and this consistent with study done by kayo etal. who found that serum leptin levels were significantly lower in SRNS patients with massive proteinuria [20]. Ozata etal. however found no significant difference in plasma leptin concentrations between NS patient and healthy subjects [21]. Schroth etal. said that as leptin is a small peptide, it is principally cleared by the kidney and urinary excretion of leptin in proteinuric NS patients is significantly higher, on other hand serum leptin concentration levels have reported to be similar between healthy subjects and those with NS[22] .

Regarding to serum ADPN level we found that it is highly significantly increased in patients with active nephrotic syndrome than children in remission and control groups. Sevean etal. found that SRNS relapse patients has substantially higher serum ADPN levels compared to those in SRNS remission and control groups[7]. Increasing ADPN an antinflammatory and antisclerotic protein, is a counter regulatory response and an appropriate adaptation to reduce deleterious metabolic effect of neprotic syndrome [2]. In study done by Sevean et al. they found that SRNS relapse patients had substantially high ADPN serum level compared to those in remission and control groups. They added that this might be a reflection of compensatory response to nephrotic state characterized by massive proteinuria, hypoalbuminemia and hyperlipidemia [7].

In our study we found strong and inverse correlation between serum ADPN when compared to plasma protein and serum albumin, while there was direct correlation between serum ADPN level compared to serum TG, cholesterol, HDL and LDL and 24-h protienuria in patients with nephrotic

syndrome when compared to group 2 and 3.

As regard to serum leptin, there was direct correlation between it and total serum protein and albumin while there was no correlation between serum leptin and serum levels of TG, cholesterol and LDL in patients with nephrotic syndrome versus remission and control groups.

Kazuyoshi et al. found that plasma ADPN was high in patients with NS, also proteinuria and lipoproteinemia was strongly and directly correlate with plasma ADPN level [2]. Zoccali et al. also found that ADPN serum level correlate with degree of proteinuria, hypercholesterolemia and hypoalbuminemia[10].

Concolusion

We suggest that raised serum ADPN level and decreased serum leptin can be used as markers for activity of nephrotic syndrome.

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SERUM LEPTIN AND ADIPONECTIN IN STEROID RESPONSIVE NEPHROTIC SYNDROME

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RAPID DEATH IN A SELF-INFLICTED BURN DUE TO DUODENAL ULCER: A CASE REPORT

Ahmed R. Ragab MD and Rania H. Abdel Rahman MD

Department of Forensic Medicine & Clinical Toxicology,
Faculty of Medicine, Mansoura University, Egypt.

Abstract

Burns are a major health problem and constitute a significant source of high morbidity and mortality. Curling's ulcer is an infrequent burn-related complication which is rarely mentioned in forensic literatures. This brief report represents a scarce case of an adolescent female who committed suicide by burning using kerosene. The patient was admitted to Mansoura Emergency Hospital with 1^{st}-4^{th} degree burns all over the body and approximately 63% total body surface area burned. Despite adequate 1^{st} aid management and stabilization, she complained of sudden localized severe epigastric abdominal pain on the fourth day of admission. She became severely deteriorated after two attacks of massive haematemesis and rapidly died on the 10^{th} day as a result of perforated duodenal ulcer determined after performance of medicolegal autopsy. So, the forensic practitioner should have a comprehensive knowledge of different complications occurring in severe burns which may aid in identifying the cause of death.

Keywords: *Self-inflicted Burn, rupture duodenal ulcer.*

Introduction

Burns represent a major health problem contributing to high mortality, morbidity and economic loss. Globally, fire-related burns were responsible for approximately 300,000 deaths, and ranked among the 15 leading causes of deaths and burden of disease in children and young adults aged 5-29 years. Over 95% of these deaths occurred in low-and-middle-income-countries due to

poverty, substandard living condition, overcrowding, illiteracy and limited access to burn care[1,2].

Deliberate self-harm by burning carries a significant risk of death, with an overall mortality rate of 65% worldwide[3]. Markell et al. [4] stated that the overall incidence of abdominal complications in severe burns was 5% with curling's ulcer the most common malady (54%), followed by esophageal lesions (17%), hemorrhagic gastritis (11%) and acute necrotizing enterocolitis (10%). These complications are dramatic and often associated with poor outcomes.

On the other hand, curling's ulcer is a mysterious lesion with cursory description as it is seldom recognized during life because of its low incidence. Besides, it occurs most frequently in children and early adolescence whose symptoms are vague and in whom it is easy to overlook the diagnosis of more usual conditions. Curling's ulcer has always a serious prognosis and death happens most often due to haemorrhage, shock or perforation[5]. The forensic practitioner should have a comprehensive knowledge of different complications occurring in severe burns which may aid in identifying the cause of death. Curling's ulcer is an infrequent complication rarely mentioned in forensic literatures. In this respect, we discuss a rare case of an adolescent female who committed suicide by burning herself with kerosene and rapidly died within 10 days due to rupture duodenal ulcer.

Case Report

A fifteen years old female was admitted to Mansoura Emergency Hospital- Mansoura University on 26th October, 2010 due to self-inflicted burn using kerosene as a result of family troubles. She had first to fourth degree burns involving the front, back and sides of thighs, the whole front and back of the trunk, the whole hands, forearms and back of both arms. The total body surface area (TBSA) burned was about 63% as follows: 33% were deep burns while superficial and partial-thickness skin loss constituted 30% (Figures 1 & 2).

On admission, clinical examination revealed that her Glasgow-coma score was 13; temperature: 38.1˚C; pulse: 96 beats/ minute; blood pressure: 110/65 mmHg. One and quarter hours later, she developed shock and treated with 1000 c.c. saline infusion (0.9 %) and 800 mg hydrocortisone over two hours; and the condition of the patient was stabilized. Prophylactic antibiotic therapy, antacids with local and systemic management of the burns were continued until the 4th of November when the patient complained of sudden localized severe epigastric abdominal pain and she became severely deteriorated, with two attacks of massive haematemesis. The pulse was very weak; 130 beats/ minute, blood pressure dropped to 70/40 mmHg and despite intensive resuscitation, death rapidly occurred within one hour.

Medicolegal Autopsy

It was performed to the case after lapse of 13 hours. Naked eye examination revealed mild splenomegally, tiny haemorrhages and oedematous swelling of both kidneys. Both jejunum and ileum were distended, and the ileum was full of blood (Figures 3 & 4).

A small purplish-brown area about 0.75 centimeter in diameter was discovered at the junction of the first and second portions of the duodenum, and examination of the mucosal surface showed a small ulcerated area, about 1.5 centimeter in diameter at the outer margin of the descending portion. Another larger superficial area about 2 cm involving only the mucosa was evident at the same level in the posterior wall, and was considered to be a contact or "kissing" ulcer. The dissecting ulcer was funnel-shaped, due to loss of more mucous membrane than muscle tissue, and it was irregular. The edges were sharply and cleanly cut, and the base was clean and grayish in colour. Perforation and haemorrhage were observed and there were no signs of healing (Figures 5 & 6).

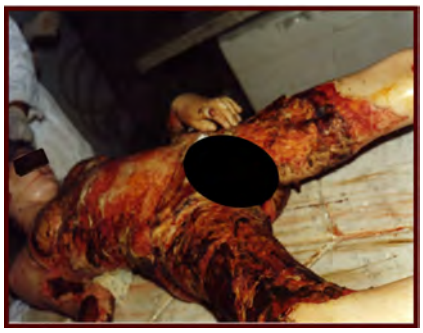

Fig. (1): A female adolescent showing dry thermal burns in the front of the trunk, thighs, forearms and arms.

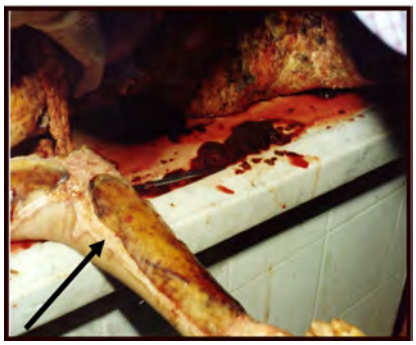

Fig. (3): Sloughing changes in the burnt area of the front of right forearm.

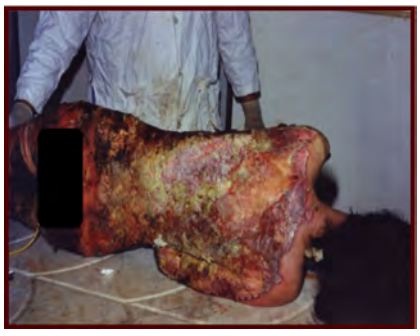

Fig. (2): Healing process and septic changes in the back of the studied case.

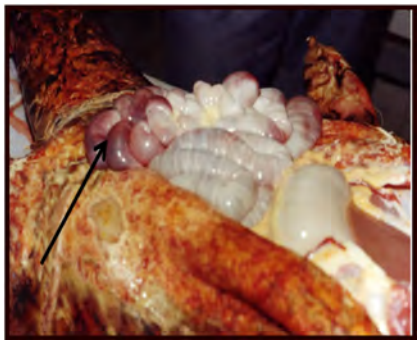

Fig. (4): Both jejunum and ileum were distended and the ileum was full of blood.

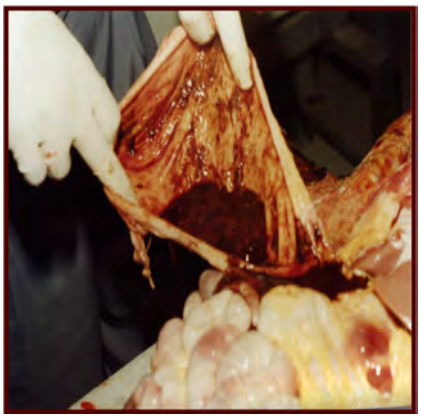

Fig. (5) : A longitudinal incision in the gastric wall showing a large amount of bloody content inside the gastric lumen.

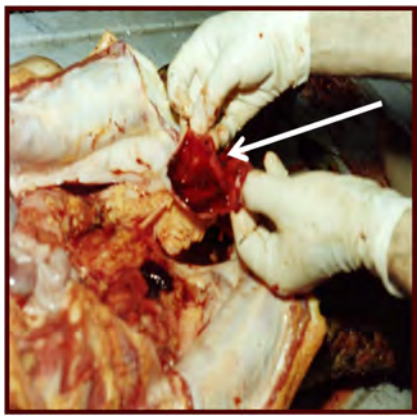

Fig. (6): A transverse incision in the duodenal wall showing curling's ulcer and bloody content inside the lumen.

Discussion

Burn is claimed to represent the widest spectrum of any form of trauma[6]. Self-inflicted burns were considered more serious than unintentional injuries and associated with a much poorer prognosis[7]. The present case is a female aged 15 years had 63% TBSA burned and developed shock within two hours of admission. Stabilization was done, however, massive haematemesis developed on the 10th day followed by death.

Markell et al.[4] reported that primary abdominal complications could occur in about 1 in 20 of the severely burned patients. Associated mortality reaches 45% and increases linearly with burn size where it is 100% for those who suffered from 61% - 80% TBSA burned.

In fact, TBSA affected was found to be the best predictor of survival, with an inverse relationship between TBSA burned and chance of survival. Mortality rate goes up slowly between 40% and

60% TBSA burned. The peak incidence of mortality is found to be in adolescent and young age groups "11-40 years" [8,9].

Only 0.5% of severely burned patients had ischaemic bowel complications diagnosed before death. In addition, about 2% of children and 7% of adults who died were identified with necrotic bowel after death as stated by Markell et al.[4]. Burn-complicated haemorrhagic ulcer and digestive tract haemorrhage occur more frequently in the stomach and duodenum [10].

The present case had shock about one hour after admission and she was stabilized. However, she developed severe epigastric pain on the 10^{th} day with two attacks of haematemesis and despite resuscitative measures, death occurs rapidly. Autopsy revealed an ulcer which had just perforated. Curling's ulcer usually manifests during the second week, when infection and toxic absorption from the separating sloughs are predominant. Unfortunately, there may be no symptoms at all when it could be diagnosed only in case of haemorrhage, perforation, or necropsy [5,11].

This may be also attributed due to the fact that the burn injury increases the risk of gastrointestinal (GI) mucosal damage and ulceration. Burn shock leads to splanchnic hypoperfusion and thus gastric mucosal ischemia, leading to mucosal atrophy, decreased capacity to neutralize hydrogen ions and impaired mucosal healing. In addition, pro-inflammatory cytokines released during the acute shock and post reperfusion cause direct cellular damage and cell death. These alterations lead to feeding intolerance, mucosal ulceration and bowel necrosis [12,13].

In summary, burn-complicated digestive tract haemorrhage is a sign of exacerbation of the general condition with poor prognosis. Clinical management is usually difficult because of extensive involvement, a large amount of blood loss and difficulty with enteroscopic haemostasis. It is necessary to consider the high possibility of duodenal ulcer in severe burns in

children and adolescents. Regardless of the cause, development of ischemic bowel remains a largely fatal complication in the severely burned patients, with a mortality rate approaching 100%.

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RAPID DEATH IN A SELF-INFLICTED BURN DUE TO DUODENAL ULCER: A CASE REPORT

Ahmed R. Ragab MD and Rania H. Abdel Rahman MD

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TENSION FREE PRIMARY CLOSURE COMPARED WITH MODIFIED LIMBERG FLAP FOR PILONIDAL SINUS DISEASE : (A PROSPECTIVE BALANCED RANDOMIZED STUDY)

Saleh El-Awady MD, Waleed Thabet MD and Mohamed Farid MD

Departments of General Surgery, Mansoura University, Egypt

Abstract

Aim: *Pilonidal sinus disease (PSD) is a common disease that affects the patient's quality of life. We analyzed the outcome of the tension free primary closure "TF 1ry" in comparison with the modified Limberg Flap technique 'MLF'.*

Methods: *120 patients were assigned to one of two equal groups by closed envelope balanced randomization. Group I represents' T F 1ry 'method and group II represents 'MLF'.*

Results: *No significant differences between group I and group II in terms of preoperative data (age, sex, disease presentation, preoperative disease duration and risky factors), immediate postoperative complications and disease recurrences.*

The operative time, blood loss, hospital stay, surgeon's performance scale, time to drain removal, frequency of seroma, wound hypothesia, wound cosmoses score, patient satisfaction score, patient quality of life (bodily pain & social functioning) were better in 'TF 1ry' group. The 'MLF' had better clinical results regarding postoperative wound maceration, times to sit on toilet & walk pain free.

Conclusion: *Both procedures are effective and must be tailored to the PSD type (simple - complex),PSD cleft site, gender (♂ - ♀) and surgeon (junior & senior).*

Key words: *Pilonidal disease. Tension free closure. Modified Limberg Flap.*

Introduction

Pilonidal sinus disease (PSD) is a common and well-recognized entity. Hodge in 1880 suggested the term pilonidal disease[1]. It typically affects young male patients after puberty[2].

Absence of the exact etiology explains the diversity of treatment lines[3] and failure of treatment options[4]. Radical treatment has been wide excision of the chronic tracts with or without closure. Higher morbidity rate had been reported with primary closure due to tissue tension [5,6].

The main problem is the high rate of recurrence which can diminish the patient quality of life[7], So the ideal operation should minimize PSD recurrence and financial cost (patient-community), should have short hospital stay, should cause minimal pain, should be associated with a low patient and procedure morbidity rates, and should be simple for surgeons [8].

Flap techniques reduced PSD recurrences and wound morbidity due to "Tension Free" healing site. Modified Limberg Flap technicque "MLF" is a simple modification of classic Limberg flap to eliminate midline maceration and reduce recurrence rate[9,10]. Tension free primary closure "TF 1ry "has been suggested to avoid wound dehiscence, wound infection and recurrence [11,4,12].

In this study sufficient sample size was enrolled, objective scoring systems were done for procedures, patients and surgeons.

The aim of study was to compare tension free primary closure versus modified Limberg flap technique regarding recurrence as a primary outcome measure.

Secondary outcome measures were:

1- Patient related factors: Post-operative pain score (patient inconvenience), time to sit on toilet and walk pain free (patient financial cost), quality of life and satisfaction.
2- Procedure related factors: Operative time, blood loss, immediate postoperative complication e.g. (urine retention, bleeding and constipation), hospital stay (com-

munity financial cost), and wound morbidity (maceration-infection- gaping - drain removal-seroma-wound hypothesia-wound cosmoses).

3- Surgeon related factor: Performance.

Patients and Methods

This study was a prospective randomized single blinded clinical trial.

Local ethics committee approval was obtained.

165 patients with PSD were referred to Mansoura Colorectal Unit between November 2006 and February 2011. 45 patients were excluded from the study, 15 were medically unfit and 30 had pilonidal abscess. The remainder was divided into two equal groups.

Patients were assessed by documentation of clinical symptoms and their duration, full discussion of pain, satisfaction and quality of life scores.

Patients were prepared by over night evacuation enema and operative site shaving on the day of surgery. Patient randomization was done at operative room using sealed envelope. Perioperative antibiotics "ampicillin -sulbuctam" were used and all patients received spinal anesthesia then put in prone Jack Knife position.

In group 1: excision of the sinus tract with elliptical skin incision was done followed by 3 cms lateral subcutaneous tissue release. Suction drain was inserted. Fascia closure was achieved by 3/0 vicryl sutures and subcutaneous tissue by 0 vicryl sutures. Skin closure was done with 3/0 polypropylene sutures or skin staples [10,13]. Fig. 5.

In group 2: excision and reconstruction with MLF technique was performed. We marked and placed the inferior apex of the rhomboid excision asymmetrically 1 to 2 cm lateral to the midline on the side opposite to the donor area. A right-sided or left -sided Limberg transposition flap, incorporating the gluteal fascia, was fully mobilizid on its inferior edge and transposed medially to fill the rhomboid defect. The defect on the gluteal region was closed primarily. The

subcutaneous layers were approximated with 2-0 vicryl interrupted sutures over a vacuum drain and the skin was closed with 2-0 proline interrupted sutures[14].Fig. 6.

Drains were removed when out coming serous fluid volume was below 20 cc per day. Sutures or staples were removed on 10th postoperative day.

Follow up visits were done every week for one month, monthly for the first year then every 3 months.

Operative time, blood loss, pain score according to the "VAS", hospital stay, immediate postoperative complications, wound morbidity in the form of maceration (soft, white and wet skin), Infection (cellulitis or purulent discharge from wound edge or drain), gaping (separation of all wound layers), seroma, time of drain removal, sit on toilet and walk pain free (walk comfortably without pain or tension) were noticed. Surgeon performance was noticed using VAS at end of surgery in terms of anatomy, tissue planes, and patient characters [15].

At the 3rd month : quality of life (Qol) assessment was done[16]. "SF-36 short form" 36 items are coded, summed, and scored on to a scale from 0 - 100[17]. It is a generic form for Qol study, all entity and usage rules are available at www.qualitymetric.com home page. Wound cosmoses scale, VAS was used[18] and patient satisfaction score using the same VAS; 0 maximal dissatisfied and 10 maximal satisfaction[19]. Later follow up aimed at detection of recurrence.

Patients completed 2 visual analog scales, the first evaluated postoperative pain and the second measured patient satisfaction at the 3rd postoperative month.

Surgeons similarly recorded 2 VASs, first was for performance and the second for wound cosmoses.

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

Results

This study was a prospective

randomized single blinded clinical trial. Local ethics committee approval was obtained.

The mean follow up was 43.5 ± 3.4 months with a range (12 - 60 months), with neither mortality nor major morbidity.

There were no statistically significant differences between the two groups with respect to preoperative demographic data "table 1", immediate postoperative complications "table 2" and recurrence "figure 1". Recurrence was detected in 2(3.3%) patients in group I (time of recurrence was 5^{th} and 10^{th} month respectively), detected in the inferior part of suture line and one patient (1.6%) of group II (time of recurrence was 6th postoperative month).

The mean operative time and blood loss "figures 2 & 3 respectively" were significantly lower in group I, but patients in group II reported more pain score at the first postoperative day and thereafter the differences were insignificant "table 3". Meanwhile surgeons' performance scores were better for group l "figure 4".

Pair wise comparisons between studied groups are outlined in tables 4 and 6. Significant differences regarding shorter times of drain removal, less seroma formation and more hypothesia were noticed in group II.

The mean satisfaction score and wound cosmoses score were higher for group I patients with insignificant difference regarding QOL for both groups apart from reduced bodily pain and better social functioning"table 5".

Table 1 : Preoperative demographic data.

	GI (60)		GII (60)	
Age (Y)(means ± SD)	27 ± (6.8)		28 ± (7.6)	
Sex	number	(%)	number	(%)
♂	52	86.6	50	83.3
♀	13.4	8	16.7	10
Presentation	number	(%)	number	(%)
Discharge	52	86.6	83.3	50
Pain	50	30	53.3	32
Pruritis	20	33.3	31.6	19
Bleeding	4	6.6	5	3
Preoperative duration (Y) (means ± SD)	1.8 ± 1.1		1.6 ± 1.2	

Table 2 : Immediate postoperative complications.

	G I		G II		P value
	N	(%)	N	(%)	
Urine retention	3	5%	4	6.6%	>0.05
Bleeding	0	0%	1	1.6%	>0.05
Constipation	3	5%	2	3.3%	>0.05

Table 3 : Pain score.

	G I	G II	P value
1st day	2.8 ± 1.2	4.2 ± 1.4	< 0.001
7th day	2.1± 1.05	2.5 ± 1.01	> o.o5
2nd week	1.0 ± 0.4	0.9 ± 0.47	> o.o5
4th week	0.10 ± 0.307	0.00 ± 0.00	> o.o5

Table 4 : Wound morbidity.

	G I	GII	P value
Hospital stay (day)	1.85 ± 0.7	3.8 ± 1.6	< 0.05
Time of drain removal (day)	10.2 ± 2.1	4.5 ± 2.4	< 0.01
Seroma formation	5 ± 8.4	1 ± 1.6	< 0.05
Time to sit on toilet (day)	8.1± 0.17	7.8 ± 0.3	> 0.05
Time to walk pain free (day)	6.9 ± 0.016	5 .9 ± 0.21	> 0.05

Table 5 : Quality of life estimation.

	GI	GII	P value
Physical function	71.1 ± 11.7	73.4 ± 12.5	> 0.05
Role of limitation of physical function	43.5 ± 15.1	41.9 ± 14.2	> 0.05
Bodily pain	54.3 ± 6.3	61 ± 4.6	0.001
Vitality and energy	74.5 ± 16.4	73.1± 17.4	> 0.05
General health	73.1 ± 10.7	75.3 ± 14.3	> 0.05
Emotional function	63.4 ± 5.3	66.2 ± 4.3	> 0.05
Social functioning	72 ± 8.7	59.6 ± 5.4	0.001
Role of limitation of emotional function	56.5 ± 13.7	55.4 ± 14.6	> 0.05
Physical health perception	75.6 ± 11.7	77.3 ± 12.6	> 0.05
Mental health perception	69.5 ± 6.4	57.5 ± 7.31	> 0.05

Table 6 : Wound complication.

	GI		G II		
	number	%	number	%	P value
Infection	3	5.0%	2	3.3%	> 0.05
Dehiscence	1	1.6%	1	1.6%	> 0.05
Oedema	2	1.6%	1	1.6%	> 0.05
Maceration	1	1.6%	0	0.0%	> 0.05
Hypothesia	2	1.6%	6	10%	< 0.05

Figure 1 : Recurrence.

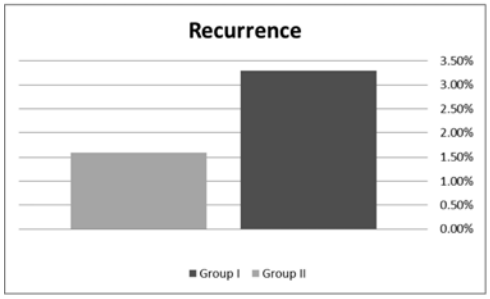


Figure 2 : Operative time.

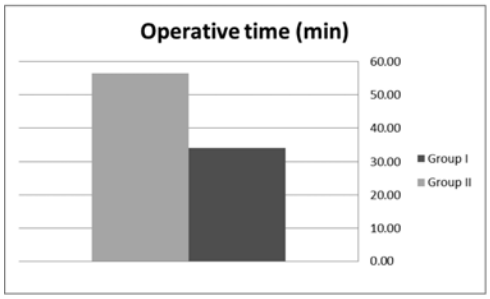


Figure 3 : Operative blood loss.

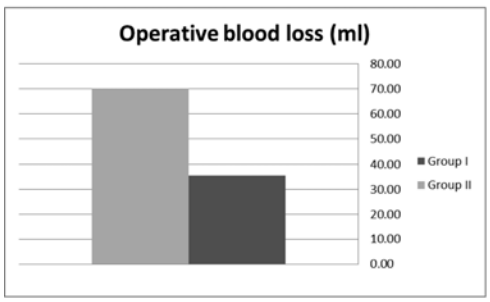
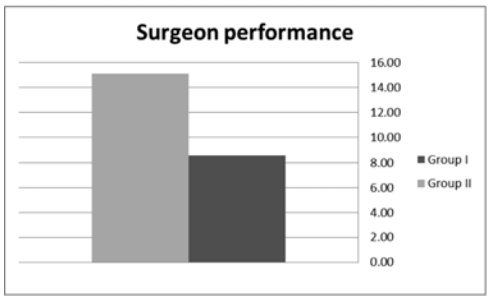


Figure 4 : Surgeon performance (competence).



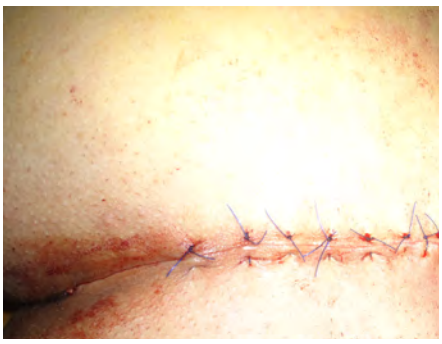


Fig. 5 : T F 1 ry method.

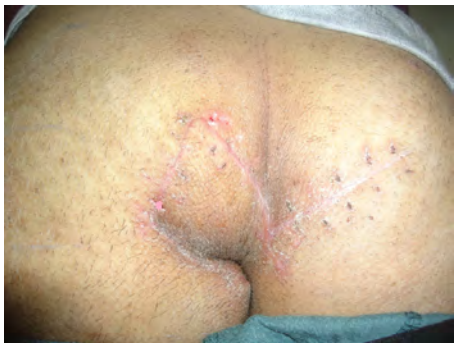


Fig. 6 : MLF.

Discussion

PSD and its recurrence are caused by forces focused on the midline (tension = force/surface area) mainly where the coccyx turns anteriorly[20], vacuum effect created between the buttocks attracts the anaerobic bacteria, hair and debris[21], friction movement of buttocks[22] in the presence of other risk factors as adiposity, hirsuteness and bad hygiene[23,26]. Thus effective procedure will eliminate shearing forces, vacuum effect and friction movement[25].

Both procedures had low recurrence rates with insignificant difference going with Tavassoli et al (26). But tension free primary closure had shorter operative time and less blood loss as Muzi et al reported[27] due to small defect and minimal dissection.

Flap techniques achieved lower VAS compared with direct 1ry closure due to tension free construc-

tion[28], so in the current study (1st day VAS of pain) was low in both groups compared to direct 1ry closure as found by Elshazly and Said[29]. But the relatively higher pain score in the MLF group is related mostly to more dissection and tension with net force.

As pain (mild to moderate in this study) is the most critical point that exacerbates postoperative urine retention and constipation, their frequency were infrequent in this study with minimal patient inconvenience.

The less postoperative pain and infrequent immediate postoperative complications in TF 1ry closure patients facilitated their early discharge as Muzi et al [27] minimizing the financial cost to the community.

Hence regarding operative time, patient inconvenience (immediate postoperative pain, immediate postoperative complications) and financial cost to the community (hospital stay) TF 1ry closure is more advantageous than MLF.

The case difficulty scale of surgeon was higher in MLF technique, being reserved for a senior surgeon, but the performance was better for TF1ry closure technique as it is simple to design and construct being reserved for junior surgeons.

The MLF procedure keeps system under net force, as the recipient side is heavier (more dangling) than donor side[30]. But the TF 1ry closure creates a TF midline healing site and keeps system in equilibrium as summation of force is zero[12]. Consequently, TF 1ry closure achieved less wound morbidity rate that didn't reach statistical significance.

Nearly the flat natal cleft for TF1ry closure group and lateralized midline for MLF group resulted in significant decrease of maceration rate going with Akca et al[11], compared to high incidence with classic Limberg flap technique (45.7%)[4], so both TF Iry closure and MLF are efficient to reduce maceration.

The reduced maceration rate resulted in less wound infection

as Akin et al and Muzi et al (10 & 27 respectively), compared to the higher incidence of wound infection for direct primary closure (21.8%) recorded by Zimmerman [31].

Generally flap procedures achieve proper wound healing with less wound dehiscence going with Mahdy[32] so both TF Iry closure and MLF techniques were equivalent regarding wound healing but MLF technique changed the anatomy of the gluteal region.

The MLF had shorter time till drain removal and less incidence of seroma formation, going with Erderm et al[33] that is related to more muscle exposure that deserve good absorptive power, so use of drains for MLF is controversial. The more dissection in MLF patients resulted in higher rate of hypothesia similar to Akin et al to (8.9%) and Soendenna et al (9.5%) (10 & 34 respectively)).

Patients after PSD surgery suffer wound tenderness (sitting on hard chairs and time off work) that negatively affects the patient financial cost[35]. Holm found 18% of patients suffered pain during sitting on hard chairs[36]. Moreover time off work reported by Cihan et al was 28.6 ± 3.11 days for direct 1ry closure (4). The current study confirmed significantly shorter times to walk pain free and to sit on toilet for flap surgery. Thus both improved financial cost.

The patient satisfaction score was better for TF 1ry closure group as Akin et al and Tavassoli et al[10,26] with positive community and patient costs, less disturbed anatomy and minimal patient inconvenience being associated with better satisfaction.

In PSD the main problem is the high rate of morbidity and recurrence, which can greatly diminish the patient's quality of life[3]. This study found TF1ry closure advantageous in terms of bodily pain and social functioning going with Ertan et al[35]. Quality of life drive is an important factor in decision making regarding PSD surgery modality.

Conclusion

Flap techniques are effective

and efficient for PSD. TF1ry closure can be tailored for PDS patients with deeper cleft, sinus tracts in the upper part of natal cleft, female gender and a junior surgeon. MLF can be tailored for PDS patients with shallow cleft, sinus tracts in the lower part of natal cleft, complex lateral disease, male gender and a senior surgeon.

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TENSION FREE PRIMARY CLOSURE COMPARED WITH MODIFIED LIMBERG FLAP FOR PILONIDAL SINUS DISEASE :

(A PROSPECTIVE BALANCED RANDOMIZED STUDY)

Saleh El-Awady MD, Waleed Thabet MD and Mohamed Farid MD

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IMPACT OF CIGARETTE SMOKING ON PENILE AXIAL RIGIDITY DETERMINANTS IN EGYPTIAN PATIENTS WITH ERECTILE DYSFUNCTION

Moheiddin Alghobary MD, Youssef El-Bayoumi MD, Talal Amer MD* and Mohammed Abol -Ata MD*

Andrology Unit, Mansoura Faculty of Medicine &
**Department of Radiology, Mansoura Faculty of Medicine, Egypt.*

Abstract

Background: *Smoking may cause erectile dysfunction (ED) through different mechanisms. Available studies addressed penile hemodynamic changes associated with smoking but did not explore the changes in axial penile rigidity & its other components-other than hemodynamics.*

Aim: *To determine the changes in axial penile rigidity & its various components in current smoker versus non-smokers with ED.*

Methods: *Forty six ED cases were enrolled in the study including 31 current smokers versus 15 non-smokers (never-smoked). Detailed history & examination with recording of pack-years of smoking, sexual health inventory for men [SHIM] score, penile length circumference (L/C) ratio & penile volume during flaccidity was done. Induction of erection by ICI of trimix followed by simultaneous Digital Inflexion Rigidiometer (DIR) & pharmaco-penile Duplex ultrasonography (PPDU) assessment was performed with recording of axial penile rigidity, hemodynamic changes, erect penile volume & calculation of tunical distensibility.*

Results: *Current smokers with ED had statistically significant lower SHIM score, lower axial rigidity values, lower PSV and RI versus non-smokers with ED ($P<0.05$), while results of L/C ratio, EDV, arterial dilatation & tunical distensibility were statistically insignificant. In current smokers, statistically significant negative correlations were recorded between the pack-years of smoking & SHIM score, axial rigidity, arterial dilatation, RI and PSV, while EDV showed significant positive correla-*

tion (P < 0.05). No significant correlation were found between the pack-years of smoking and both L/C ratio & tunical distensibility.

Conclusions: *Compared to non-smokers, current smokers have more severe ED & more impaired axial penile rigidity due to more impaired penile hemodynamics rather than impaired penile geometry or penile tissues mechanical properties. Smoking has a dose-dependent effect on the severity of ED.*

Key words: *Smoking- Erectile dysfunction - Penile rigidity.*

Introduction

Erectile potency is the product of an erection with sufficient and maintained penile axial rigidity that can resist buckling during sexual intercourse[1]. The penile axial rigidity is dependent on three components namely: penile hemodynamics, penile geometry and penile tissues mechanical properties[1-2]. Many risk factors are incriminated in the pathogenesis of ED, including smoking [3-5]. Smoking was also reported to be associated with progression of the severity of ED[6]. Moreover, cessation of smoking was reported to improve erectile functions[7-8].

Review of literature revealed that most of the work trying to explore the mechanism by which ED develops in smokers addressed mainly the penile hemodynamic changes[9-14]. ED in smoking was attributed by some investigators to damage of endothelial cells and development of arteriogenic ED[9-13]. Other investigators however referred to development of veno-occlusive dysfunction as the main cause of ED in smokers [14]. No previous work tried to explore the changes in penile axial rigidity, penile geometry and penile tissues mechanical properties associated with ED in smokers. So, studying the different components of penile axial rigidity in smokers with ED is thus needed. This will help in better understanding of the exact pathogenesis of ED in smokers and might help to determine the proper line of treatment of ED in this particular group of patients.

The aim of this work is to determine the changes in axial penile rigidity & its various components in current cigarette smokers versus non smokers with ED.

Patients and Methods

This study was conducted on patients with ED attending the andrology out- patient clinic of Mansoura University Hospital from July 2010 to December 2010. An informed consent was taken from every patient and the study was approved by the local medical ethics committee.

The inclusion criteria used in this study were:

1- Patient with ED duration of more than 6 months.
2- Presence of regular heterosexual relation with one partner (at least once per week).
3- Patients were either current smokers or non-smokers. Current smoker refers to those who had smoked at least 100 cigarettes during their life time & they are currently smokers. Non-smokers (never-smoked) refers to those who had smoked less than 100 cigarettes during their life time & they are currently non smokers. Former-smokers (those who had smoked more than 100 cigarettes during their life time and currently non-smoker) were excluded[3]. For every patient, the pack-years of smoking were recorded. Pack-years of smoking were calculated by multiplying the average number of smoked packs per day (one pack contains 20 cigarettes) by the number of smoked years[3].
4- Presence of normal total testosterone and prolactin levels.

Exclusion criteria used in this study were: cases of Peyronie's disease & those with decompensated medical disorders (e.g.cardiac, real or hepatic).

All patients were subjected to thorough history taking and clinical examination with recording of post-prandial blood glucose level & SHIM score (the abridged International Index of Erectile Functions or IIEF-5 score). Patients with SHIM score of 22-25 were considered as not having ED and were excluded [15]. During flaccidity, we measured both the penile length from the tip of the glans penis to the symphysis pubis & the penile circumference at the mid-shaft of the non-stretched penis. Penile measures were taken by one observer for all cases, with the patients in supine position, using a graduat-

ed plastic-tape. We then recorded the penile length circumference (L/C) ratio.

Pharmaco-Penile Duplex Ultrasonography (PPDU):

PPDU was conducted using color duplex ultrasound device (General Electric LOGIQ S6, Milwaukee WI, USA), and a 7.5 MHz superficial probe.

Examination of the patients was done in supine position. During flaccidity, we recorded of both the flaccid cavernosal arterial diameter and the flaccid penile volume. For penile volume, measurement was made based on the assumption that the penis represents a cylinder[16], and its flaccid volume (V_F) could be calculated according to the formula: $V_F = (\prod r^2) F \times h_F$.

Where: $(\prod r^2) F$ is the "area" of the circle "or base" of the cylinder, represented in the penis by the area of the flaccid penile mid-shaft. $(\prod r^2) F$ can be easily obtained by the LOGIQ duplex machine using the B mode & manual planimetry of the transverse - section (TS) sonographic image taken at the region of mid-shaft. On the other hand, h_F represents the "height" of the cylinder (or penile length from the symphysis pubis to the tip of glans penis during flaccidity), that was previously recorded with the graduated plastic-tape.

Induction of erection by ICI of 1/2 cc trimix was then performed (1 cc contains 15 mg. papaverine, 5µg. prostaglandin E1 and 0.1 mg phentolamine). Patients were asked to perform manual self-stimulation (MSS) to enhance erection if the response to ICI alone was not satisfactory[17]. The patients were imaged at 5-minutes interval. The cavernosal arterial diameter and peak systolic velocity (PSV) were carefully traced over the 1st 10 minutes and then throughout the test. The end diastolic velocity (EDV), resistance index (RI) and the erect penile volume were recorded during maximal clinical response. Erect penile volume was calculated according to the equation:

$V_E = (\prod r^2)_E \times h_E$.

Where V_E = erect penile volume.

($\prod r^2$) E = area of the erect penile mid-shaft, measured by LOGIQ duplex machine using the B mode & manual planimetry of the TS sonographic image at the region of mid-shaft. Finally, h_E = erect penile length (also from the symphysis pubis to tip of glans) was measured by the graduated plastic-tape. The tunical distensibility was then calculated according to the equation:

Tunical distensibility = erect penile volume/ flaccid penile volume [1].

$$= V_E / V_F$$

Penile axial rigidity recording:

This was achieved utilizing the Digital Inflexion Rigidiometer (DIR, Uroan XXI, Electromedicina, Palma de Mallorka, Baleares, Spain). The test was done while the patients were supine simultaneously with PPDU.

After induction of erection by ICI, repeated recording of axial penile rigidity was done regularly at 5 minutes intervals. For each reading, the measuring cone of DIR was pressed on the erect penis so that the longitudinal axis of the cone was perpendicular to the longitudinal axis of the penis. Vertical downward pressure was then applied by the examining andrologist through the cone over the glans penis with a force that was just sufficient to produce buckling of penis. The measure of axial rigidity was displayed after 15 seconds. This process was repeated at 5 minutes interval till the end of test. Data used for analysis were obtained during the maximum erectile response.

Statistical analysis:

Collected data were subjected first to test of normal distribution (Kolmogorov-Smirnov test). Parametrically distributed variables were analyzed by student t test and Pearson's correlation. Non-parametrically distributed variables were analyzed by Mann-Whitney test. Categorical variants were analyzed by Fisher's exact test.

Results

A total of 46 patients fulfilled the inclusion/exclusion criteria and completed the study, including 31 current smokers and 15 non-smokers. All patients were

Egyptians with mean age of 49.83 ± 8.28 years.

Co-morbidity is demonstrated in table 1 & no statistically significant differences were recorded between smokers versus non-smokers. Table 1 also demonstrates the differences between current smokers versus non-smokers in terms of age, SHIM scores, duration of ED, hormonal profile and blood sugar levels. Statistically significant lower SHIM score in current smokers versus non-smokers was recorded ($P < 0.05$) while other variants showed statistically insignificant results.

Table 2 demonstrates the differences in axial penile rigidity determinants in smokers versus non-smokers. Current smokers had statistically significant lower axial rigidity by DIR, significant lower PSV and RI compared to non-smokers ($P < 0.05$). On the other hand the penile length/ circumference ratio, changes in cavernosal arterial diameter, EDV & tunical distensibility showed statistically insignificant results.

Table 3 demonstrates the correlation between the pack-years of smoking & both SHIM score & axial penile rigidity determinants in current smokers (no = 31). The table showed that statistically significant negative correlations were recorded between pack- years of smoking and SHIM score, axial rigidity values, PSV, & RI while EDV showed statistically significant positive correlation ($P < 0.05$). On the other hand, the penile length / circumference ratio, % increase of arterial diameter & tunical distensibility were not significantly correlated with pack-years of smoking.

Table 1: Differences in age, co-morbidity, ED duration, SHIM score, hormonal and blood sugar levels in non-smokers versus current smokers:

	Non- smokers N=15	Current Smokers N= 31	Test	P value
Age in years (mean±SD)	51.55±8.89	47.33±6.88	-1.771*	0.0835
Co-morbidity				
a- *Diabetes (no , %)*	2 (13.33%)	4 (12.9%)	**	1.00
b- Hypertension (no , %)	1 (6.67%)	3 (9.68%)	**	1.00
c-Ischemic heart disease	1 (6.67%)	3 (9.68%)	**	1.00
d- Lumbar disc prolapse (no , %)	1 (6.67%)	1 (3.23%)	**	0.5507
e- TURP (no , %)	1 (6.67%)	1 (3.23%)	**	0.5507
ED duration in years (mean±SD)	2.82±1.18	2.22±1.45	-1.393*	0.1707
SHIM score (mean±SD)	13.80±4.69	10.52±4.75	2.205*	0.0328
Post-prandial blood glucose (mg/dl) (median-range)	107(79-485)	109(68-462)	0.574***	0.5659
Total testosterone ng/ml (mean+SD)	4.20±1.61	4.54±1.04	-0.865*	0.3918
Prolactin ng/ml (mean±SD)	8.45±2.9	9.24±1.8	1.251***	0.2160

* Student t test ** Fisher's exact test *** Mann-Whitney test

Table 2: Differences in axial rigidity determinants in non-smokers versus current smokers:

	Non-smokers N= 15	Current Smokers N= 31	Test	P value
Axial penile rigidity gm (mean±SD)	404.53±149.33	309.61±138.66	2.191*	0.0338
***Length* /circumference ratio (mean±SD)**	1.019±0.17	1.03±0.19	0.190*	0.850
% increase of arterial diameter (mean±SD)	0.853±0.289	0.760±0.245	-1.138*	0.2612
PSV cm /sec (mean±SD)	32.23±6.64	28.04±4.12	-2.635*	0.0116
EDV cm / sec (mean±SD)	7.633±5.118	10.016±3.923	-1.746*	0.0878
RI (mean±SD)	0.7807±0.1513	0.6432±0.2122	-2.243*	0.030
Cavernosal distensibility (mean±SD)	3.408±0.1455	3.4342±0.1626	0.539*	0.5942

*student t test

Table 3: Correlation between the pack-years of smoking and both the IIEF-5 score and the axial penile rigidity determinants in current smokers (no=31).

	Duration of smoking in years	
	r*	P
SHIM score	-0.5443	0.0015
Axial rigidity gm.	-0.4349	0.0145
% increase of arterial diameter	-0.0854	0.6478
PSV cm /sec	-0.3853	0.0323
EDV cm/sec	0.4148	0.0203
RI	-0.4266	0.0167
Legth/Circumference ratio	-0.2832	0.1226
Tunical distensibility	-0.0594	0.7510

*Pearson's correlation

Discussion

In this study, current smokers had more severe ED compared to non-smokers as indicated by a significant lower SHIM score. This finding was confirmed by the presence of a significant lower mean axial rigidity value in current smokers of about 300 gm compared to a mean value of about 400 gm in non-smokers.

Among the three determinant of axial penile rigidity, significant differences were recorded only in penile hemodynamics, namely the PSV and the RI, while both penile geometry (represented by penile L/C ratio) & penile tissues mechanical properties (represented by tunical distensibility) did not exhibit statistically significant differences in current smokers versus non-smokers. So, the lower axial rigidity values in current smokers versus non-smokers could be attributed to impaired penile hemodynamics rather than changes in the penile geometry or penile tissues mechanical properties. It has to be noticed that both groups did not exhibit statistically significant differences in terms of cavernosal arterial diameter or EDV ($P > 0.05$, table 2). However, as the PSV is more representative of the arterial supply and RI is more representative of veno-occlusive mechanism compared to cavernosal arterial dilatation and EDV respectively[18-19], we believe that significant changes in penile hemodynamics were responsible for the significant differ-

ence in penile axial rigidity recorded between current smokers versus non-smokers.

Our finding in terms of impaired hemodynamics add a support to previous reports that addressed changes in penile hemodynamic associated with smoking[4,19-20]. This could be possible despite variations in different studies in terms of population & the different methods used for assessment of hemodynaminc changes e.g, angiography[9], Rigiscan[10,14], colour duplex[11-14] and pharmaco-cavernosometry[14].

The molecular basis of impaired penile hemodynamic in current smokers could be related to either reduced nitric oxide (NO) bioavailability, which is the main mediator of vascular stimulation, or increased level of reactive oxygen species (ROS) that will damage the penile vascular endothelium[4,21-22]. This will initiate a cascade of pathological events, including diminished endothelial vaso-relaxation, increased expression of cell adhesion molecules (CAM) with trans-endothelial migration of monocyte-like cells, reduced response to vascular endothelial growth factors (vEGF) and disturbed regulation of thrombotic factors that lead to deterioration of the penile hemodynamic [23-27].

Review of literature demonstrated an evidence of increased plasma level of transforming growth factor beta 1 (TGF-beta 1) in smokers, a factor that could play a role in the development of ED in such group of patients[28]. This high level of TGF-beta 1 was reported to cause not only increased penile fibrosis involving the vascular endothelium, but it can also lead to reduction of the quality of the penile tissues mechanical properties by reducing the cavernosal tissues compliance[29-31]. Moreover, this fibrotic process may direct the choice of treatment option, where an anti-fibrotic agent may be beneficial in treatment of ED[32-33].

It would be rational then to address the changes of penile geometry & penile tissues mechanical properties that could be theoretically altered by smoking. To our knowledge, our study is the first trial to address these changes in

penile geometry and penile tissues mechanical properties associated with smoking. However, we did not found statistically significant changes in these two items in current smokers versus non-smokers with ED. These insignificant results in terms of penile geometry & penile tissues mechanical properties could be due to our relatively small sample size, or simply, a longer time was required to develop these changes in a clinically florid and measurable state, compared to a relatively shorter time needed for development of evident hemodynamic changes. If we take into consideration that the mean duration of ED in smokers in our study was around two years (table 1), this suggestion may sound reasonable. In other words, patients with ED caused by smoking could manifest early by measurable hemodynamic changes but in the same time, they may have sub-clinical changes in the geometry and/or penile tissue mechanical properties that would be clinically evident and measurable only late in the course of ED. Another point of view is that our study did not include a third group of potent healthy volunteers as this was actually difficult to recruit. The presence of such group would facilitate the demonstration of the changes in penile geometry and penile tissues mechanical properties that could be developed in current smokers in a rather clear and possibly significant way.

It has to be taken into consideration that there is no standard method that could be used for assessment of penile axial rigidity and penile volumetric changes. In our study we tried to use simple tools as much as we can for these purposes. A previous study tried to measure the axial rigidity during pharmaco-cavernosometry by using a graduated weight scale attached to a plastic cap fitting the glans penis [34] while an electronic bucklometer was used also for this purpose by another group[35]. In our study, we used DIR for axial rigidity detection & we did not perform pharmaco-cavernosometry because of its relative invasiveness. In terms of penile geometric assessment, we used the penile L/C ratio that was simply measured by a graduated plastic tape. Penile volumetric assessment during flaccidity and

erection actually represent a real challenge and generally lacks standardization. Some investigators performed these measures depending on pharmaco-cavernosometry and complicated mathematical models[2], while others used a 3-diminsional duplex device and tried to measure real- time volumetric changes during erection with the penis embedded in an artificial vagina in a water-filled basin[36]. Compared to these methods, we think that we presented a very simple technique for assessment of penile volumetric changes with the help of duplex machine and a very simple mathematical equation. However, we believe that the technique we presented in the current study for assessment of volumetric changes is better to be reassessed in future large studies, particularly for its sensitivity, specificity and reproducibility before being claimed to be generally accepted.

Our study also demonstrated the presence of statistically significant negative correlations between the pack-years of smoking & the SHIM score, axial penile rigidity values, PSV & RI ($P < 0.05$, table 3). Also, the EDV showed a significant positive correlation with pack years of smoking ($P = 0.02$, table 3). On the other hand the penile L/C ratio and tunical distensibility were not significantly correlated with duration of smoking ($P > 0.05\%$, table 4). These findings point to the presence of a dose-dependent effect of smoking on the severity of ED. Although our sample size is relatively small, our findings in terms of the presence of dose - dependent effect of smoking on the severity of ED may add a further support to previous reports in this context [10,13,37].

The main limitation of this study was its relatively small sample size. Adding a control group of potent healthy non-smoker volunteers would be also beneficial, but unfortunately it was not possible as mentioned above.

Conclusions

Current smokers have a more severe ED and lower axial penile rigidity values compared to non-smokers. This more severe ED is possibly caused by more impairment of penile hemodynamic. On

the other hand, results of the penile geometry and penile tissues mechanical properties did not exhibit statistically significant differences between current smokers versus non-smokers with ED. Smoking has a dose-dependent effect on the severity of ED. Larger scale studies exploring the changes in various components of axial rigidity associated with smoking, perhaps with the addition of sensitive methods than can detect ultra-structural & subclinical changes, is recommended.

Conflict of interest: None

Corresponding author : Moheiddin Alghobary MD, Andrology Unit, Mansoura Faculty of Medicine, El-Gomhouria street Mansoura 35111 Egypt, tel (+2) 0502373700, fax (+2)0502231191, Email; moheiddin_alghobary@yahoo.com.

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IMPACT OF CIGARETTE SMOKING ON PENILE AXIAL RIGIDITY DETERMINANTS IN EGYPTIAN PATIENTS WITH ERECTILE DYSFUNCTION

Moheiddin Alghobary MD, Youssef El-Bayoumi MD, Talal Amer MD and Mohammed Abol -Ata MD

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DUPLEX ULTRASOUND STUDY OF PORTAL AND SPLENIC VEINS AS A NON-INVASIVE MODALITY FOR PREDICTION OF HIGH-RISK ESOPHAGEAL VARICES IN CIRRHOTIC PATIENTS

Amr M. Helmy MD*, Sabry M. Al Azhary MD*, Khaled N. El-Fayoumy MD*, El Sayed E. El-Sayed MD* and Helmy A. Shalaby MD**

Departments of Internal Medicine, Faculty of medicine, Al- Azhar University, Damietta, Cairo**, Egypt.*

Abstract

Introduction: *Upper gastrointestinal bleeding is among the leading causes of death in patients with liver cirrhosis and portal hypertension[1]. It is recommended, that all patients with established cirrhosis should be screened by upper gastrointestinal endoscopy for the presence of varices at the time of diagnosis[2]. Abdominal ultrasound is a rapid, safe and non-invasive technique for the investigation of patients with suspected chronic liver disease. Pulsed and color Doppler ultrasound (US) has the advantage of allowing real-time observation of the portal hemodynamics in patients with portal hypertension repeatedly and non-invasively compared with other imaging modalities3. Ultrasound with duplex study of the portal venous system could predict both the presence of varices and the risk of variceal bleeding in cirrhotic patients, so could be used as a non-invasive method to detect patients with high risk for upper gastrointestinal bleeding[1].*

Aim: *The aim of this study is to evaluate the efficacy of duplex ultrasound study of portal and splenic veins hemodynamics for prediction of high-risk esophageal varices in cirrhotic patients.*

Methods: *the study was conducted on 30 patients with liver cirrhosis and 10 healthy volunteers as a control group. All subjects were submitted to medical examination (including complete history and clinical*

examination), laboratory investigations, radiological examination (including, abdominal ultrasound with duplex study on the portal venous system) and upper gastrointestinal endoscopy.

Results: *In cirrhotic patient; the presence of porto-systemic collaterals especially splenic collaterals show significant increase in cases with esophageal varices, especially large varices. Splenic size is increased in patients with varices but not correlated with the variceal size. Portal and splenic vein diameters show significant increase in cirrhotic patient and progressive increase in cases with esophageal varices. The degree of the varices is correlated with splenic vein diameter, while portal vein diameter is not correlated. Dupplex study of the splenic vein (flow velocity), portal vein (flow velocity, portal vein congestion index, portal hypertension index, liver vascular index), hepatic and splenic artery resistive index (HARI&SARI) show significant change in cirrhotic patient in comparison to normal subject and can predict presence of esophageal varices.*

Conclusion: Upper gastrointestinal endoscopy remains the gold standard method for diagnosis of esophageal and gastric varices. Although the use of duplex study of portal venous system with highly experienced operator can be used to predict the presence of esophageal varices but this is needed to be furtherly studied.

Introduction

Portal system is the unique circulatory system, which connects two systems of capillary beds; one in the wall of the small, large intestine and spleen and the second in sinusoidal area of the liver. The portal venous system includes all veins, which carry blood from subphrenic part of digestive tract: from pancreas, gallbladder, and spleen to the liver[4].

Portal hypertension is defined as an increase in portal pressure above the normal range of 6-10 mm Hg, or considering the gradient between portal and hepatic veins, as assessed by hepatic vein catheterization above 5 mm Hg. Portal hypertension is considered clinically relevant, i.e. capable of causing the development and rupture of esophageal varices when the portal-hepatic gradient is

above 10-12 mm Hg. The increase in resistance to outflow from the portal system, with the subsequent increase in portal pressure, causes the opening of portal-systemic collaterals[5]. Gastro-oesophageal varices are the most frequent and clinically relevant collaterals. Clinical-haemodynamic correlations have shown that varices may appear when Hepatic Venous Pressure Gradient (HVPG) increases to above 10mmHg6. HVPG is calculated by the following formula: HVPG = WHVP - FHVP. The wedged hepatic vein pressure (WHVP), a marker of sinusoidal pressure, and the free hepatic vein pressure (FHVP) are measured with radiological assessment[7].

Varices are most superficial at the gastro esophageal junction and thus have the thinnest wall in that region: consequently, esophageal variceal hemorrhage invariably occurs in this region[8].

Although several non invasive tests, in particularly platelet count, the presence of splenomegaly or data obtained from abdominal ultrasonography (such as increased portal vein diameter >13 mm), and more recently transient elastography have been suggested to be useful in selecting patients with a high risk of having large esophageal varices, none of these tests, alone or in combination is accurate enough to completely discard the presence of esophageal varices when non-invasive indicators are negative[9]. Thus, current recommendation is that all patients at the time of initial diagnosis of cirrhosis should undergo an upper gastrointestinal endoscopy for the screening of esophageal varices (for assessment of appearance, number, size and presence of red color signs)[10]. A potentially more acceptable way of screening for varices is the use of capsule endoscopy, although more information is required and its cost still is too high for wide scale use[11].

Zwieble (2000) stated that duplex ultrasonography provides an accurate assessment of the portal and hepatic venous system that is both non - invasive and convenient because the examination can be conducted at the bedside, further more it is portable

and relatively non-expensive[12]. The size of the portal vein may be increased in portal hypertension, with a portal vein diameter of >13 mm being 100% specific for portal hypertension, through this finding is present in only 75% of cases. The lower sensitivity is likely due to decreased portal vein size as portosystemic collaterals increase. The velocity of portal venous flow tends to decrease in portal hypertension[13]. In portal hypertension, the flow may be reversed in direction (hepatofugal) or it may remain orthograde (hepatopetal). Occasionally, it may show partial reversal e.g orthograde flow on the left branch and retrograde on the right branch and the portal trunk[14]. In portal hypertension the splenic vein becomes dilated greater than 1cm in diameter with loss of significant variation in caliber during deep inspiration & expiration[15]. Flow velocity in splenic vein is clearly lowered in patients with cirrhosis and portal hypertension than normal subject[16]. As splenic pressure increases, portal vein flow may become to and fro (biphasic) or the flow direction may reverse (hepatofugal flow), concomitant flow reversal may occur on the splenic vein is a variable finding in portal hypertension, because the flow direction in these vessels influenced by collateral development[17]. Mohammad et al (2008) found that Doppler ultrasound for portal haemodynamics is a non-invasive tool for estimating portal hypertension index and predicting the presence of large oesophageal varices with high accuracy[18].

Subjects and Methods

Subjects: This study included 40 subjects (after consent), attended to internal medicine department, Al-Azhar University hospitals (New Damietta, Al-Hussein and Sayed Galal), in the period from October 2009, to June 2010. They were divided into two groups:

A) Patients group: included 30 patients with liver cirrhosis. They were subdivided according to upper GIT endoscopy findings, into three subgroups:

Subgroup A: patients with no esophageal varices.

Subgroup B: patients with mild degree esophageal varices (grade I or II).

Subgroup C: with high risk

esophageal varices (grades III or IV).

B) Control group: included 10 healthy volunteers.

Patients with previous attacks of variceal hemorrhage, hepatorenal syndrome and patients with clear signs of portal hypertension (ascites, poto-systemic shunt and hepatic encephalopathy) all were excluded from the study.

Methods: All subjects were submitted to the following: clinical examination including detailed medical history, general and abdominal examinations with special emphasis on signs of portal hypertension as ascites and splenomegaly. Laboratory investigations including, CBC, liver function tests (Bilirubin total and direct, prothrombin time, serum albumin, AST, ALT, alkaline phosphatase, GGT), hepatitis viral markers, renal function tests. Radiological examination including abdominal ultrasound with Duplex ultrasound examination of the portal and hepatic venous systems with measurement of the following: a-Portal and splenic veins diameter by mm, flow velocity as time average, maximal flow velocity in cm/sec. b-Hepatic artery resistance index (RI) measured in intrahepatic main branch (RI=systolic velocity-end diastolic velocity/systolic velocity). c- Splenic artery RI measured intraparenchymally near to the hilum d- Congestion index of the portal vein e- Liver vascular index f-Portal hypertension index. Upper GIT endoscopy: for detection of esophageal varices and its degree; gastric varices and congestive gastroduodenopathy. Statistical analysis of data: The collected data were organized, tabulated and statistically analyzed using SPSS software computer package. For qualitative data, frequency and percent distribution were calculated and for comparison between groups, the chi square test was used. For quantitative data, mean and standard deviation were calculated and for comparison between two groups, the student (t) test was used. $P \leq 0.05$ was considered signification for interpretation of results.

Results

This study included 40 subjects, 10 healthy subject with mean age of 52.70±4.16 years as a

control group and 30 liver cirrhosis patients with mean age of 53.23±6.12 years as a patient group. There is no statistical significant difference between study and control groups as regard to gender distribution. Data were collected from the two groups.

Analysis of the data obtained showed the followings: There is statistically significant increase in portal vein and splenic vein diameters in patients with esophageal varices in comparison to those without esophageal varices. The diameter of splenic vein (not of portal vein) was increased in relation to increasing degree of esophageal varices, as shown in the following table and figure.

Portal vein velocity (not splenic vein velocity) was decreased in patients with esophageal varices compared to patients without, but there was no correlation between portal vein velocity and the degree of esophageal varices, as shown in the following table and figure:

There was statistically significant increase in HARI and SARI in cases with esophageal varices (0.78±0.075 and 0.73±0.063 respectively) in comparison to cases without esophageal varices (0.65±0.055 and 0.62±0.033 respectively). There was statistically significant progressive increase in hepatic artery resistance index and splenic artery resistance index in relation to increasing degree of esophageal varices, as shown in the following table and figure:

Table (3): Relation between cases with esophageal varices and cases without as regard hepatic resistance index and splenic artery resistance index

There was statistically significant increase in portal vein congestion index and portal hypertension index in cases with varices in comparison to cases without varices; while there was statistically significant decrease in liver vascular index in cases with varices in comparison to cases without varices. There was statistically significant progressive increase of congestion index and portal hypertension index in relation to increasing the degree of esophageal

varices, while there was statistically significant progressive decrease in liver vascular index in relation to increasing esophageal varices.

There was statistically significant increase in portal vein congestion index and portal hypertension index in cases with varices in comparison to cases without varices; while there was statistically significant decrease in liver vascular index in cases with varices in comparison to cases without varices. There was progressive statistically significant increase of congestion index and portal hypertension index in relation to increasing the decree of esophageal varices, while there was progressive, statistically significant decrease in liver vascular index in relation to increasing esophageal varices, as shown in the following table and figure:

Splenic size was increased in patients with esophageal varice, but it was not correlated with the size of varices as shown in the following table and figure:

Portal hypertension index was increased in patients with large varices in comparison to cases with small varices.

There was statistically significant difference between cases with large varices and those with no large varices as regard splenic vein diameter (increase), HARI (increase), SARI (increase), congestion index (increase) and liver vascular index (decrease), while this difference was statistically non-significant as regard portal vein diameter, portal vein velocity and splenic vein velocity.

Table (1): Relation between degree of esophageal varices with portal vein diameter and splenic vein diameter.

		Mean	SD	Minimum	Maximum	P value
Portal Vein diameter	No varices	12.51	2.72	11.00	18.00	0.20(NS)
	Small varices	13.91	1.26	12.00	16.00	
	Large varices	14.35	2.18	11.60	19.00	
	Total	13.83	2.09	11.00	19.00	
Splenic vein diameter	No varices	9.00	0.63	8.00	10.00	0.015(S)
	Small varices	11.80	2.13	8.00	16.00	
	Large varices	12.68	2.96	8.00	20.00	
	Total	11.65	2.72	8.00	20.00	

Table (2) : Relation between degree of esophageal varices with portal vein velocity and splenic vein velocity

		Mean	SD	Minimum	Maximum	P value
PV velocity	No varices	13.33	2.72	10.00	16.80	0.54(NS)
	Small varices	13.15	1.90	10.60	16.20	
	Large varices	12.45	1.49	9.60	14.50	
	Total	12.86	1.891	9.60	16.80	
SV velocity	No varices	14.61	1.58	12.50	16.00	0.42(NS)
	Small varices	12.57	1.51	10.50	15.10	
	Large varices	13.11	4.01	8.59	26.00	
	Total	13.23	2.98	8.59	26.00	

Table (3) :

		Mean	SD	Minimum	Maximum	P value
HA RI	No varices	0.69	0.064	0.65	0.79	0.006(S)
	Small varices	0.74	0.067	0.60	0.85	
	Large varices	0.81	0.070	0.73	1.00	
	Total	0.76	0.079	0.60	1.00	
SA RI	No varices	0.63	0.049	0.55	0.68	0.002(S)
	Small varices	0.71	0.039	0.65	0.79	
	Large varices	0.74	0.074	0.62	0.86	
	Total	0.71	0.072	0.55	0.86	

Table (4): Statistical study of congestion index, portal hypertension index and liver vascular index in the patients group in comparison to control group .

		Mean	SD	Minimum	Maximum	P- value
PV Congestion index	Control	0.055	0.0125	0.038	0.074	0.001(S)
	Study	0.119	0.0409	0.058	0.250	
	Total	0.103	0.0454	0.038	0.250	
Portal hypertension Index	Control	1.51	0.19	1.21	1.83	0.001(S)
	Study	2.52	0.64	1.44	3.75	
	Total	2.27	0.71	1.21	3.75	
Liver vascular index	Control	15.51	1.84	11.70	18.62	0.001(S)
	Study	9.26	3.20	2.70	16.53	
	Total	10.82	3.98	2.70	18.62	

Table (5): Relation between cases with esophageal varices and cases without as regard congestion index, portal hypertension index and liver vascular index in the study group:

		Mean	SD	Minimum	Maximum	P value
PV Congestion index	No varices	0.0761	0.0201	0.058	0.101	0.004(S)
	Small varices	0.1194	0.0314	0.082	0.174	
	Large varices	0.1383	0.0406	0.082	0.250	
	Total	0.1196	0.0409	0.58	0.250	
Portal Hypertension Index	No varices	1.638	0.147	1.53	1.93	0.003(S)
	Small varices	2.454	0.487	1.44	3.01	
	Large varices	2.747	0.759	0.53	3.75	
	Total	2.427	0.717	0.53	3.75	
Liver vascular Index	No varices	12.841	2.754	7.96	14.82	0.001(S)
	Small varices	9.879	2.634	7.06	16.53	
	Large varices	7.301	2.203	2.70	10.38	
	Total	9.268	3.205	2.70	16.53	

Table (6): Relation between splenic size & portal hypertension index with endoscopic findings in the patient group.

	Splenic size (>15.05) & portal hypertension index (>2.08)				Total	
	Yes		No			
	n.	%	n.	%	n.	%
No varices	0	0.0%	6	46.2%	6	20.0%
Small varices	6	35.3%	4	30.8%	10	33.3%
Large varices	11	64.7%	3	23.1%	14	46.7%
Total	17	100.0%	13	100.0%	30	100.0%

Table (7): Relation between splenic size and portal hypertension index with large varices

	Large varices		No large varices		P value
	Mean	SD	Mean	SD	
Splenic size	17.15	2.47	16.26	2.51	0.34(NS)
Portal hypertension Index	2.74	0.75	2.14	0.56	0.020(S)

Table (8): relation between different parameters with large varices

		Mean	S. D	Minimum	Maximum	P value
PV Diameter	Large	14.35	2.18	11.60	19.00	0.21(NS)
	No large	13.38	1.98	11.00	18.00	
	Total	13.83	2.09	11.00	19.00	
SV Diameter	Large	12.68	2.96	8.00	20.00	0.05(S)
	No large	10.75	2.19	8.00	16.00	
	Total	11.65	2.72	8.00	20.00	
PV Velocity	Large	12.45	1.49	9.60	14.50	0.27(NS)
	No large	13.21	2.16	10.00	16.80	
	Total	12.86	1.89	9.60	16.80	
SV velocity	Large	13.11	4.01	8.59	26.00	0.84(NS)
	No large	13.33	1.80	10.50	16.00	
	Total	13.23	2.98	8.59	26.00	
HARI	Large	0.81	0.07	0.73	1.00	0.003(S)
	No large	0.72	0.068	0.60	.85	
	Total	0.76	0.079	0.60	1.00	
SA RI	Large	0.74	0.074	0.62	0.86	0.013(S)
	No large	0.68	0.058	0.55	0.79	
	Total	0.71	0.072	0.55	0.86	
PV Congestion index	Large	0.138	0.040	0.082	0.250	0.016(S)
	No large	0.103	0.034	0.058	0.174	
	Total	0.119	0.040	0.058	0.250	
Liver vascular Index	Large	7.30	2.20	2.70	10.38	0.001(S)

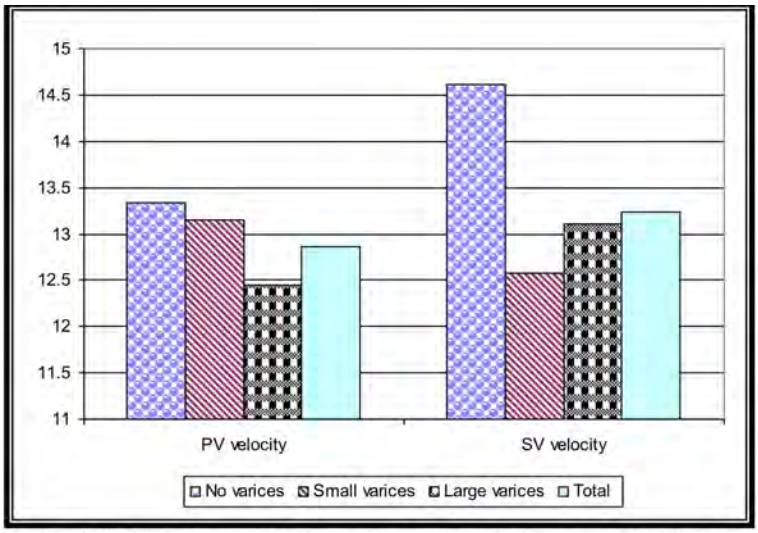

Figure (2): Relation between degree of esophageal varices with portal vein velocity and splenic vein velocity.

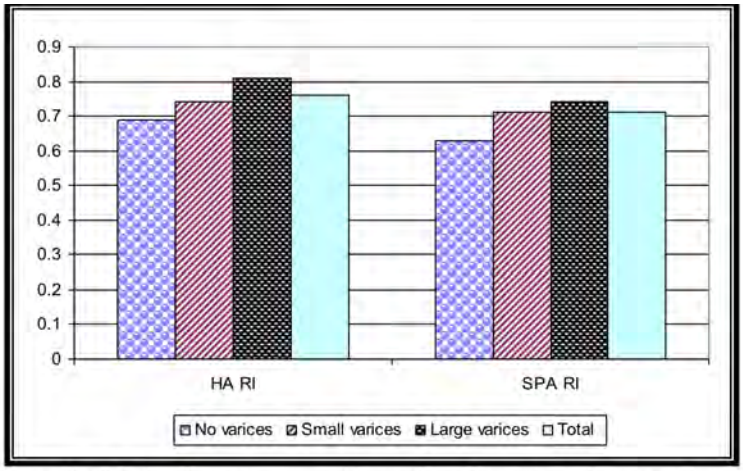

Figure (3): Relation between cases with esophageal varices and cases without as regard Hepatic resistance index and splenic artery resistance index.

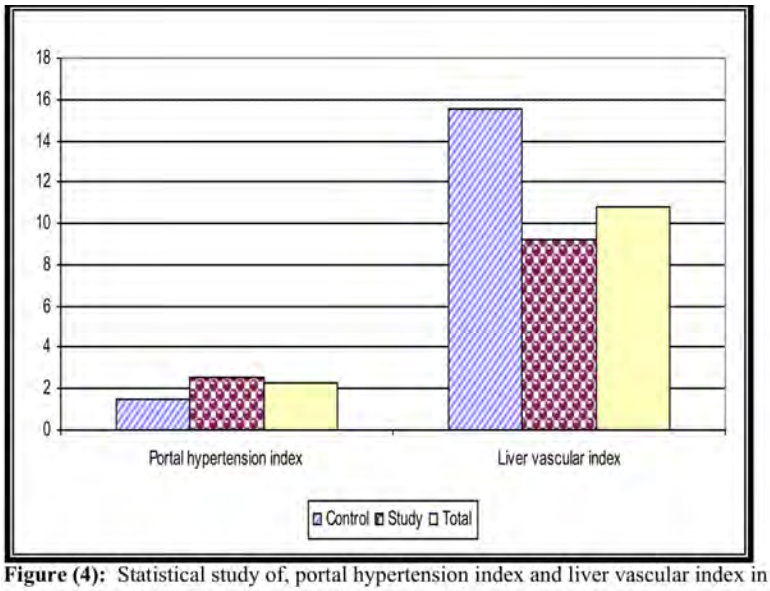

Figure (4): Statistical study of, portal hypertension index and liver vascular index in patients group in comparison to control group .

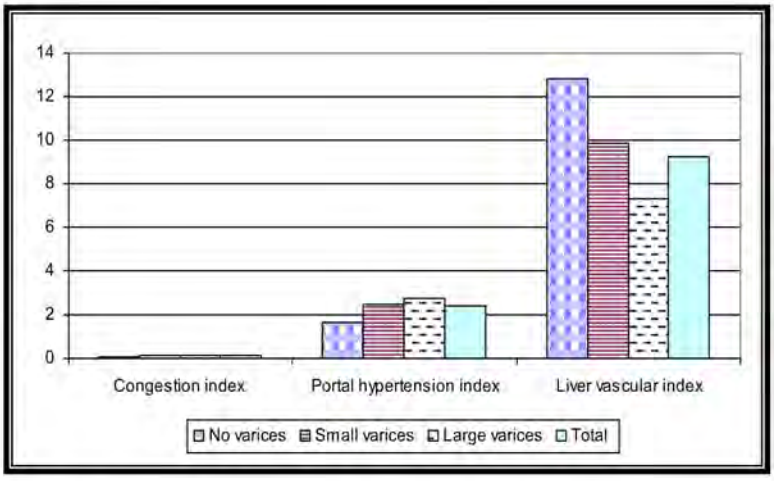

Figure (5): Relation between cases with esophageal varices and cases without as regard congestion index, portal hypertension index and liver vascular index in the study group

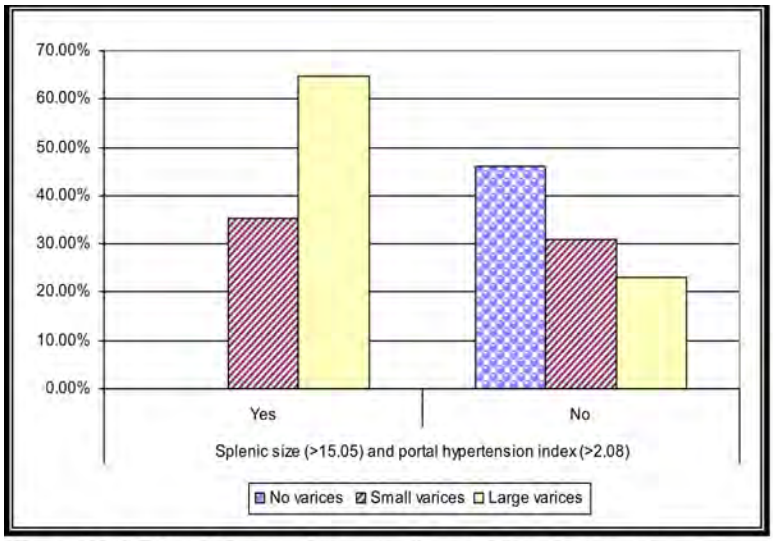

Figure (6): Relation between splenic size & portal hypertension index with endoscopic findings.

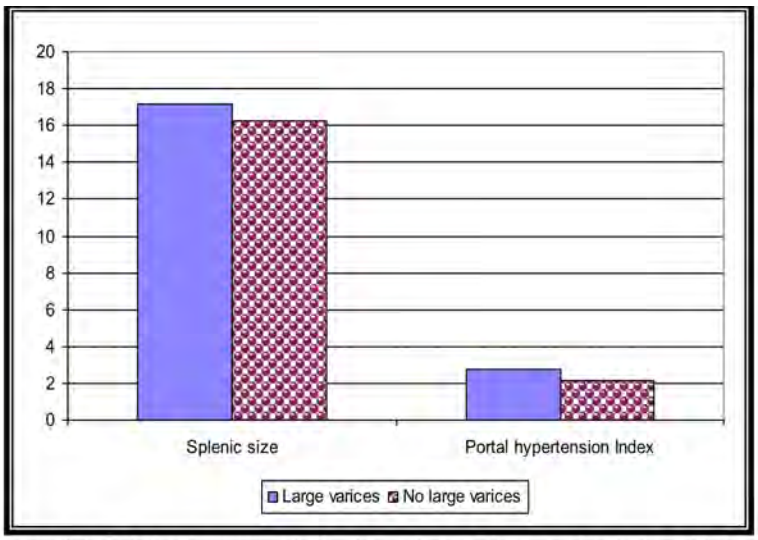

Figure (7): Relation between splenic size and portal hypertension index with large varices

Discussion

The prevalence of esophageal varices in patients with liver cirrhosis ranges between 32 and 66%[10]. Variceal formation results from interaction of haemodynamic, anatomical and angiogenic factors. Increased portal pressure is the initial and most important factor leading to the opening of preexisting vascular embryonic channels that connect the portal and the systemic circulation at several locations[11]. Screening every year in patients with small varices and every 2-3 years in patients without varices was recommended to allow initiation of primary preventive treatment[19]. The present study was designed to evaluate the efficacy of duplex ultrasound study of portal and splenic veins hemodynamics as a non-invasive modality for prediction of high-risk esophageal varices in cirrhotic patients.

The results of this study showed that the presence of splenomegaly either clinically or by ultrasongography is a predictor for the presence of esophageal varices. This result was supported by those done by[20,21,22,23]. We showed also that portal vein diameter is correlated with the presence but not the degree of esophageal varices. In agreement with the results of the present study, it was reported that, the size of the portal vein may be increased in portal hypertension, with a portal vein diameter of >13 mm being 100% specific for portal hypertension, through this finding is present in only 75% of cases[24]. In addition, they reported that, portal vein diameter was of statistical significance with the presence of cirrhosis and varices. But some cases with normal portal vein diameter show varices of small and large size[13]. In addition, Schepis et al., (2001) reported that, as the normal caliber of the portal vein cannot exclude portal hypertension because the portal vein diameter may be affected by the development of portosystemic collaterals[25]. On the other hand, portal vein dilatation may occur in the absence of portal hypertension e.g. in response to huge splenomegaly or acute portal vein thrombosis26, and this agreed with the present study as there are 4cases with PVD <13 mm and have esophageal varices and one case

with PVD= 18 mm without esophageal varices.

In the present study, there was statistically significant increase in splenic vein diameter in the patient group in comparison to control group (11.65±2.72 vs 6.70±0.94 respectively respectively). Furthermore, there was statistically significant increase of splenic vein diameter in cases with esophageal varices in comparison to cases without esophageal varices (12.31±2.63 vs 9.00±0.63). In addition, there was statistically significant increase in splenic vein diameter in relation to increasing degree of esophageal varices. The results of the present study coincide with those reported by Kayacetin et al (2004) who reported that, there is an increase in splenic vein diameter with the presence of varices and its degree with P value < 0.001[27].

In the present work, there was statistically significant decrease in portal vein velocity in the patient group in comparison to control group (12.86±1.89 vs 15.43±1.53 respectively). In addition, there was statistically significant decrease in PV velocity in cases with esophageal varices (12.74±1.67) in comparison to cases without esophageal varices (14.64±2.23). Furthermore, there was no statistically significant relation between portal vein velocities in relation to degree of esophageal varices. These results are in agreement with those reported by Dauzat et al. (1990) who reported that, the range of portal vein velocity is ranged from 10- 20 cm/ sec with an average of 16cm/ sec[28].

Abu-El Dahab (1997) found significant reduction in portal vein velocity in patients with portal hypertension due to schistosomal hepatic fibrosis but he didn't find any significant difference between the subgroups of portal hypertension that were classified according to the Doppler sonoscore[29]. However, in the study of Mohr et al. (1996), portal vein flow fell significantly with increasing degree of cirrhosis (according to Child-Pugh classification)[30]. There was no correlation between portal vein flow, splenic vein flow, or degree of splenomegaly and extent of esophageal varices. Furthermore, the results of the present study agreed

with those reported by Zimmerman et al. (2003) and Zironi et al. (1992) who reported that, the velocity of portal venous flow tends to decrease in portal hypertension and esophageal varices in comparison to normal controls[13,31].

A significantly lower mean portal venous velocity was noted in cirrhotic patients (13±3.2 cm/s versus 19.6±2.6 cm/s in controls)[31].

There was statistically significant increase in hepatic artery resistant index (RI) and hepatic artery pulsatility index (PI) in the patient group in comparison to control group. Also, there was statistically significant increase in HARI in cases with esophageal varices (0.78±0.075) in comparison to cases without esophageal varices (0.65±0.055). Furthermore, there was statistically significant progressive increase in hepatic artery resistance index in relation to increasing degree of esophageal varices.

Several indices have been suggested as useful in the prediction of liver disease and its severity. These include hepatic and splenic arteries RIs, modified liver vascular index (portal flow velocity/ hepatic artery RI), and portal hypertension index[32].

The results of the present study agreed with those reported by Gorka et al. (1996) who reported that, hepatic artery resistance index was significantly higher in cases with cirrhosis more than normal subjects. It was explained by the following: in portal hypertension, hepatic artery flow may increase substantially compensating for the diminished portal vein flow. The arterio-portal velocity ratio was significantly higher in patients with cirrhosis than in controls ($p < 0.0005$)[33].

In addition, Mouhamed et al. (2008) reported that, HARI in cirrhotic patient with esophageal varices are higher than those without esophageal varices, and these results are in agreement with those of the present study[18].

In the present study, there was statistically significant increase in congestion index and portal hypertension index in study group in

comparison to control group, while there was statistically significant decrease in liver vascular index in study group in comparison to control group. In addition, there was progressive statistically significant increase of congestion index and portal hypertension index in relation to presence and increase in the degree of esophageal varices, while there was progressive, statistically significant decrease in liver vascular index in relation to presence and increase in the degree of esophageal varices.

Hendy (1993) assessed the hemodynamic change in portal hypertensive patient with color Doppler. She stated that, the increased portal vein CI was proved to be most sensitive and specific parameter in the diagnosis of portal hypertension and she found that, the higher CI (0.12 cm/sec) the higher liability of bleeding from esophageal varices. They also reported that, there is statistically significant increase in HARI and SARI in case with esophageal varices with p value 0.001and this increase related significantly to the degree of these varices[34].

Furthermore, the resistive index and pulsatility index (PI= peak systolic velocity e mean velocity/ peak systolic velocity) have been shown to be higher in cirrhotic patients[35].

Moriyasu et al. (1986) reported that, CI in the patients with cirrhosis and IPH were 2.5 times higher than that in the normal subjects (average of 0.07 cm/sec in normal and average of 0.17 and 0.18 in cirrhosis and IPH respectively)[24].

Cirrhotic patients with a low congestion index < 0.05 and mean portal vein velocity of > 9 cm/ sec at a lower risk of variceal bleeding. The congestion index was higher in patients with bleeding and with mean portal velocity lower in these patients[36]. This was in accordance with this study of (Iwao et al. 1997) who reported that, CI of the portal vein was significantly higher (0.09±0.03)in cirrhotic patient than (0.05±0.01) in control ($p<0.01$)[35].

Iwao et al. (1997) show that the best cut off value of liver vascular index was 12 cm/sec with a sensi-

tivity and specificity of 97 and 93% respectively in diagnosis of cirrhosis and portal hypertension35. In addition, Mouhamed et al. (2008) found that liver vascular index in cirrhotic patient with and without varices was (08.31±2.72vs 17.8±6.28 respectively and p<0.0005)[18].

Portal hypertensive index, congestion index and modified liver vascular index and have been suggested as useful in prediction of liver disease and its severity, and these results agreed with[32].

This study show that; There is statistically significant increase of splenic vein diameter, HARI, SARI, congestion index, portal hypertensive index and decrease of liver vascular index, in patient with large varices in comparison to those without large varices, while this difference was statistically non-significant as regard increase of the splenic size, portal vein diameter and decrease of portal and splenic vein velocity.

A logistic regression model showed that , splenic size and portal hyper-tensive index were reported as the only good predictors of LEV, threshold values for these independent predictors of LEV for achieving a sensitivity> 75%. Portal hypertensive index > 2.08 and spleen size> 15.05 cm reached a sensitivity of 79% for detecting LEV in their study, but this not agree with This study which splenic size (>15.05) & portal hypertension index (>2.08) have 64.7% sensitivity in the diagnosis of large esophageal varices[18].

Conclusion

Upper GIT endoscopy is the gold standard in the diagnosis of esophageal varices. Duplex study of the portal venous system by highly experienced radiologist with the use of different parameters outlined could predict the presence of esophageal varices but its sensitivity in predicting its degree is needed to be further evaluated.

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DUPLEX ULTRASOUND STUDY OF PORTAL AND SPLENIC VEINS AS A NON-INVASIVE MODALITY FOR PREDICTION OF HIGH-RISK ESOPHAGEAL VARICES IN CIRRHOTIC PATIENTS

Amr M. Helmy MD, Sabry M. Al Azhary MD, Khaled N. El-Fayoumy MD, El Sayed E. El-Sayed MD and Helmy A. Shalaby MD

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DEXAMETHASONE AND LENGTH OF HOSPITAL STAY IN PATIENT WITH MUMPS ORCHITIS

Mohammad Saraya MD, Mariam Al-Fadhli MD* and Abd Elaziz Al-Fadhli MD**

**Department of Medicine, Infectious Disease Hospital, Kuwait*
Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt
***Department of Surgery, Amiri Hospital, Kuwait*

Abstract

Background: *Mumps orchitis is now rarely seen in children less than 10 years old since the introduction of the childhood vaccination programmed. Orchitis is the most common complication of mumps in post-pubertal males, occurring either unilaterally or bilaterally after 10 days of illness in up to 40% of this demographic group with mumps.*

Objectives: *To assess the effect of dexamethasone as adjunctive therapy for treatment of mumps orchitis on the length of hospital stay, which might cause earlier resolution of orchitis.*

Patients and Methods: *30 patients were diagnosed as mumps orchitis and divided into two groups, the first one involved 15 patients who received dexamethasone and supportive treatment, and the second one involved also 15 patients a supportive treatment only. The following laboratory test were done liver function test, kidney profile, complete blood count, blood glucose, C-reactive protein and the levels of interleukin-6 on the day of presentation, after 3 days of admission and on the day of discharge from the hospital.*

Result: *The mean length of hospital stay in the dexamethasone group was 4.5 days compared with 5.3 days in the placebo group which was of significantly difference. The mean time of reliving symptoms at presentation was 2.4 days in the dexamethasone group and 3.6 days in the placebo group. The mean time of reliving symptoms was significantly lower in dexamethasone group than in placebo group.*

Conclusion: *adding of dexamethasone to supportive treatment in pa-*

tients with mumps orchitis can reduce the length of hospital stay

Key words: *dexamethasone - mumps - orchitis.*

Introduction

Mumps is an acute infectious disease caused by Mumps virus, typically causing painful swelling of the parotid gland. The virus is highly contagious and weak resistance, heat intolerance, fear of the sun; commonly used disinfectants such as Lysol 2%, 75% alcohol can be within a few minutes to kill. The virus is an RNA virus of the genus paramyxovirus which is spread from human reservoir by direct contact, airborne droplets, fomites contaminated by saliva and possibly by urine[1].

Mumps classically presents with a prodrome of fever, malaise and myalgia, and is followed by parotitis, which is usually bilateral[2]. However, 10% to 20% of symptomatic cases of mumps have no parotid symptoms[3]. The incubation period is 2 to 3 weeks, and the prodromal symptoms and parotitis usually persist for 7 days. People with mumps are infectious from 5 days before to 5 days after the onset of parotitis[4]. Individuals without parotid symptoms are also infectious. Laboratory diagnosis is based on isolation of virus, detection of viral nucleic acid, or serological confirmation (generally presence of IgM mumps antibodies). Mumps is vaccine-preventable, and one dose of mumps vaccine is about 80% effective against the disease[4].

Corticosteroids may potentially modify the inflammatory response when administered early in patients with mumps[5]. Corticosteroids are very potent inhibitors of inflammation[6]. They switch off genes that encode pro-inflammatory cytokines and switch on genes that encode anti-inflammatory cytokines. Treatment with low dose corticosteroids down regulates pro-inflammatory cytokine transcription, which prevents an extended cytokine response and might accelerate the resolution of systemic and local inflammation[7]. Corticosteroids inhibit also the production of membrane-derived products such as leukotrienes and prostaglandins by inflammatory cells, with a

consequent decreases in edema and vascular permeability [8].

Aim of The Work

This study aimed at assessing the effect of intravenous dexamethasone compared with placebo on length of hospital stay in patients who were admitted to hospital with mumps complicated by orchitis.

Patients and Methods

This study was conducted between February 2010 and July 2011, at the Infectious Disease Hospital (IDH) in Kuwait, which is the only tertiary infectious hospital in Kuwait. The patients included in this study were diagnosed as mumps orchitis. Diagnosis of mumps orchitis was based clinically on the presence of parotid and scrotal swelling, and confirmed by, scrotum and parotid ultrasonography, and serologically by mumps-specific IgM.

Patients were excluded if they had a known congenital or acquired immunodeficiency or receipt of chemotherapy, any dose of corticosteroid, or immunosuppressive medications in the previous 6 weeks or malignant diseases. Eligible patients who were included in this study provided written informed consent.

This study included 30 patients their age ranged between ( ) years with mean ± years.

The patients were divided into two groups, the first one involved 15 patients who received dexamethasone and supportive treatment in the form of paracetamol, bed rest and scrotal support. Clavulanic acid/amoxicillin was administered as bacterial orchitis could not be excluded at initial presentation, and the second one involved also 15 patients but they received placebo and supportive treatment. All patients were subjected to history taking and thorough clinical examination in form of whale, liver function test (LFT), kidney profile (KP), complete blood count (CBC), blood glucose, C-reactive protein (CRP), serum amylase, serum lipase and levels of the pro-inflammatory cytokine interleukin-6 (IL-6) (Biomedix medical group, Synlab, German) on the day of presentation, after 3 days of admission and on the day

of discharge from the hospital. Patients in the dexamethasone group were given a bolus of 5 mg (1ml) of dexamethasone intravenously once on the day of admission and on next 4 days and the patients in the placebo group were given 1 ml of sterile water for injection intravenously once a day at time of admission and next 4 days.

Statistical analysis :

The data was analyzed using the statistical package for social sciences (spss) version 8.0 software. The significance of differences between mean values of the study variables was evaluated by using t-test. The significance of differences between proportions was performed using the Chi-square test. The P value less than 0.05 is considered significant.

Results

We enrolled 30 patients in this study and divided into two groups, dexamethasone group and placebo group, each of them involved 15 male patients varying in age from 14 years to 36 years (Table I). Of these, 16 patients are Indian, 6 Ethiopian, 3 Bangladesh, 2 Indonesian, 2 Nepali, and 1 Kuwaiti. Symptoms at presentation included fever, unilateral or bilateral parotid swelling, unilateral painful scrotal swelling and vomiting in some patients.

The mean length of hospital stay in the dexamethasone group was 4.5 days (±0.77) compared with 5.3 days (±0.97) in the placebo group. Length of hospital stay differed significantly between groups (p-value = 0.02, table III). All patients were treated within one hour of admission to hospital. The mean time of reliving symptoms at presentation was 2.4 days in the dexamethasone group and 3.6 days in the placebo group. The mean time of reliving symptoms at presentation was significantly lower in dexamethasone group than in placebo group (p-value = 0.001, table II).

At time of admission, there were no significant differences between the dexamethasone group and the placebo group as liver enzymes, platelets.

At time of discharge, the same

significant declines in C-reactive protein and interleukin-6 concentrations were observed between two groups (table III).

Table I: Comparison between laboratory data of both groups at time of admission.

	On admission		
	Dexamethasone group	Placebo group	P-value
ALT	94.2±48.27	84.2±47.32	0.2
AST	71.0±3.8	79.1±3.7	0.81
CRP	28.06±4.05	28.06±4.06	1.0
IL6	20.67±5.08	20.87±4.07	0.84
platelet	162.9±3.1	163.5±3.1	0.96
WBCs	5.8±1.5	6.34±1.24	0.23
s. creatinine	89.55±13.02	88.55±13.23	0.81

Table II: Comparison between laboratory data of both groups after 3 days from admission.

	After 3 days from admission		
	Dexamethasone group	Placebo group	P-value
ALT	51.1±18.2	69.1±16.2	0.06
AST	33.2±10.1	51.3±7.3	0.004
CRP	17.0±3.2	21.0±4.4'	0.002
IL6	12.3±2.8	15.2±2.5	0.002
Platelet	184.05±18	183.7±25	0.86

Table III: Comparison between laboratory data of both groups at time of discharge.

	On discharge		
	Dexamethasone group	Placebo group	P-value
ALT	50.35±10.97	51.90±8.86	0.62
AST	37.8±7.47	37.9±6.78	0.7
CRP	6.7±1.5	8.75±1.3	0.001
IL6	6.46±1.02	7.34±1.24	0.019
Platelet	191.85±17.55	188.0±20.56	0.52
WBCs	8.54±1.78	8.51±1.84	0.95
S. creatinine	84.3±11.4	85.8±11.78	0.68

Discussion

Mumps is an acute contagious RNA paramyxovirus disease and mainly affects the glands - parotid, submandibular, sublingual gland and gonads, pancreas, thyroid, and lacrimal gland, other organs such as brain, meninges, heart, liver and kidney, etc. may be involved [1].

Mumps orchitis is now rarely seen in children under 10. The incidence of mumps orchitis has declined dramatically since the introduction of the childhood vaccination programme [9]. Orchitis is the most common complication of mumps in post-pubertal males, occurring either unilaterally or bilaterally after 10 days of illness in up to 40% of this demographic with mumps [9]. When mumps complicated by orchitis, the temperature increased, chills, headache, vomiting and abdominal pain, Testicular pain and swelling occurred. Consequences of mumps orchitis include testicular atrophy (up to 50%), oligospermia or asthenospermia (up to 13%), and, rarely, sterility [3]. Less common complications of mumps include meningitis (in up to 10% of patients), encephalitis, pancreatitis, arthritis and oophoritis [3].

We postulated that adjunctive treatment of mumps-orchitis with intravenous dexamethasone might change the immune response and thereby reduce morbidity and length of stay of patient in hospital. Dexamethasone has potent anti-inflammatory effects and weak mineralocorticoid effects compared with other corticosteroid, thus avoiding interference with sodium re-absorption and water balance. Moreover, dexamethasone has a long lasting effect, allowing for a once-a-day regimen [10].

In this trial, we noted an overall reduction in mean length of hospital stay of 1 day in patients with mumps orchitis who were given intravenous dexamethasone compared with who were given intravenous placebo. These findings support our hypothesis that early administration of dexamethasone changes the immune response and thereby reduces length of hospital stay in patients with mumps orchitis. This modulation is shown in the accelerated return

to normal concentrations of C-reactive protein and interleukin-6 that we noted more in the dexamethasone group especially in the first 3 days after admission.

Steroid administration helps in diminishing pain and oedema, but it does not prevent future complications. The benefits must be weighed against the self-limiting nature of mumps orchitis and the potential side effects of steroid treatment. Bertschat et al. in his small series found that patients on corticosteroids showed better semen analysis parameters at follow up examinations, although it was not significant [11]. In this study, we noted the mean time of reliving symptoms at presentation was significantly lower in dexamethasone group than in placebo group. This finding support our hypothesis that early administration of dexamethasone reduce the time of reliving symptoms at presentation e.g pain, fever, swelling of parotid and scrotum.

Organ dysfunction can result from a systemic inflammatory response [12]. Therefore, a balanced cytokine response needs to be sufficient to control the local infection but not be excessive, to prevent systemic effect. So, adding of corticosteroids would reduce the systemic complications of the inflammatory response without affecting the resolution of local inflammation [10]. In this study, which is almost consistent with the above reports, the improvement of transaminase, and decline of CRP & IL-6 that we noted more in the dexamethasone group especially in the first 3 days after admission support our hypothesis that the use of corticosteroids as adjunctive therapy in combination with supportive measures for the treatment of mumps orchitis would reduce the systemic inflammatory response with subsequent of improvement organs dysfunction.

Conclusion

This study reveals that early administration of dexamethasone in patient with mumps orchitis reduces the time of reliving symptoms at presentation, thereby reduces length of hospital stay. The faster decline in concentrations of C-reactive protein (CRP) and interleukin-6 (IL-6) that we noted in patients given dexamethasone

compared with controls support the notion that dexamethasone reduces the systemic inflammatory response. The benefits of corticosteroids should be weight against the potential side effects of these drugs, such as super-infection, hyperglycemia and gastric disturbance.

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DEXAMETHASONE AND LENGTH OF HOSPITAL STAY IN PATIENT WITH MUMPS ORCHITIS

Mohammad Saraya MD, Mariam Al-Fadhli MD*
and Abd Elaziz Al-Fadhli MD

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ROLE OF MATERNAL PROTEIN Z IN NEONATAL RESPIRATORY DISTRESS SYNDROME

Hussein Koura MD, Ashraf Alawadi MD*, Mohamed Abdel-Aal MD, Hussein Metwali MD, Mohamed Abdel Zaher MD, Abdel-Hamead Mohammed MD*** and Mohammed El-Eraqi MD******

Departments of Pediatric (Damiatta, Cairo), Obstetrics & Gynecology ** and Clinical Pathology (Damietta****, Assuit***) Faculty of Medicine, Al- Azhar University, Egypt.*

Abstract

Background: *Respiratory distress syndrome (RDS) is one of the most common causes of respiratory distress in the newborn infant. The aim of this study was to assess the role of maternal protein Z (PTZ) and other haemostatic aspects in the pathogenesis of neonatal respiratory distress syndrome.* ***Methods:*** *This was a cross sectional study in which fifty mothers were recruited. Thirty mothers delivered newborns suffering from respiratory distress syndrome (RDS group) while the remaining 20 mothers delivered normal newborns (control group). On the day of delivery the following investigations were performed for mothers and their newborns: CBC, prothrombin time (PT), activated partial thromboplastin time (aPTT), and plasma levels of fibrinogen, plasminogen and protein Z (PTZ). Chest X-rays (postro-anterior view) were done only for the newborns.* ***Results:*** *Maternal PTZ levels were within normal range among RDS group and control group (3.4±0.2 vs 3.3±0.1µg/ml) and the difference between the two groups was statistically insignificant. Neonatal PTZ levels were comparable and within normal range among the two groups (3.3±0.4 vs 3.4±0.2µg/ml). Among RDS group, neonatal serum plasminogen level was lower than the control group (64.2±28.7 vs 82.8±31.5 mg/l) and the difference between the two groups was statistically significant (p=0.04). There was no significant correlation between neonatal serum PTZ level and neonatal coagulation parame-*

ters. ***Conclusion:*** *It is concluded that neither maternal nor neonatal PTZ has a role in the pathogenesis of neonatal respiratory distress syndrome.*

Keywords: *Respiratory distress syndrome, and protein Z.*

Introduction

Respiratory distress syndrome (RDS) or hyaline membrane disease (HMD) is one of the most common causes of respiratory distress in the newborn infant. The incidence and severity of RDS are related inversely to the gestational age of the infant [1]. The pathophysiology of RDS is complex [2].

Fibrin deposition has been demonstrated in the pulmonary micro-circulation and small airways in RDS suggesting the activation of the clotting system with a special role of protein C and protein S. Protein Z was added to these factors. Protein Z is a vitamin K-dependent plasma protein whose function remained unclear until many researchers found that its deficiency occurs in newborns affected by severe RDS due to activation of the coagulation process[3]. Hogg and Stenflo[4] were able to show that thrombin binds to phospholipid surfaces in the presence of protein Z but not in its absence.

Kemkes-Matthes et al. [5] demonstrate that protein Z was consumed during consumption coagulopathy. In humans, Protein Z is characterized by an unusual wide distribution in plasma partly explained by a genetic control. Several Protein Z gene polymorphisms influencing plasma concentration have been described. In mice, the disruption of PZ gene is asymptomatic, but in association with homozygous FV Leiden produced a severe prothrombotic phenotype[6]. Yin et al.[7] reported that the presence of protein Z dampens the coagulation response in human plasma and that concomitant protein Z deficiency dramatically increases the severity of the prothrombotic phenotype of factor V Leiden in mice.

Gris et al. reported a high frequency of protein Z deficiency in women with a first primary episode of early fetal death from the

10th to the end of the 15th week of gestation[8]. It was hypothesised that the PZ deficiency could impair the invasion of the spinal uterine arteries by the cytotrophoblast. Interestingly, PZ was detected at high expression in villous trophoblasts [9].

Data on PTZ levels in newborns could be useful, particularly in diseases such as RDS in which fibrin deposition has been demonstrated in the pulmonary microcirculation and in small airways[10]. Accordingly, the presence of fibrin deposition has been explained by the activation of the coagulation system[11].

Reduction of PTZ in RDS may contribute to this prothrombotic condition. Several studies[3,12,13,14] evaluated the role of neonatal PTZ in the pathogenesis of RDS but none of them estimate the effect of maternal PTZ level on this process. Conflicting results have been reported for maternal plasma concentrations of protein Z in normal pregnancy. Some studies found that pregnant women had a higher plasma concentration of protein Z than non-pregnant women[15,16] while others reported that normal pregnancy was associated with normal level of PTZ [17,18].

The aim of this study was to identify the role of maternal protein Z in the pathogenesis of neonatal respiratory distress syndrome.

Patients and Methods

This was a cross-sectional study which was carried out at Damietta hospital Al-Azhar University. The study population consisted of two groups. The first one included mothers who delivered newborns suffering from respiratory distress syndrome (n=30) while the second group included mothers who delivered normal newborns (n=20).

Exclusion criteria were maternal history of intake anticoagulant drugs, occurrence of hemorrhage during pregnancy and presence of diabetes mellitus or preeclampsia.

Respiratory distress syndrome was diagnosed on typical chest X-ray findings together with beginning of respiratory symptoms

before 6h of age and the need for any type of ventilatory support. On the day of delivery, maternal blood samples were collected and all women provided an informed consent prior to the collection of maternal blood. The following data were collected: mother's age, parity, gravidity, maternal diseases, history of bleeding or anticoagulant intake during pregnancy, mode of delivery, similar conditions and Apgar score at 1 and 5 minutes. Gestational age was calculated based on last menstrual period and Dubowitz assessment [19].

A thorough clinical examination was performed for all enrolled women and their newborns with special emphasis on signs of respiratory distress. Measurements of weight, length and head circumference were also recorded. At enrollment the following investigations were performed for mothers and their newborns: CBC, prothrombin time (PT), activated partial thromboplastin time (aPTT), and plasma levels of fibrinogen, plasminogen and protein Z (PTZ). Chest X-rays (postro-anterior view) were done only for the newborns. On the day of birth and after collecting blood samples from the newborns, vitamin K was given to all of them according to gestational age.

For blood samples, anticoagulated EDTA blood was used for CBC and platelets count. Citrated plasma was divided into two parts, one for the estimation of fibrinogen level, PT and aPPT. The other part was stored at -70_ until assay of PTZ and plasminogen. Platelets counting (normal range 150-400x10^3/µl) was performed using a fully automated counter (CELL DYNE 1800) and manual method. A coagulometer (SEAC S2) was used in all coagulation tests. PT was done by the Quick one-stage method[20] by using calcium thromboplastin (Diaplastin, DiaMed AG, Switzerland) (normal range 10.5-13.5 seconds). aPTT was estimated by using Diacelin, DiaMed SA, Switzerland(normal range 24.5- 40 seconds) [21].

Fibrinogen assay was performed by using kit (Diagnostica Stago, France) (normal range 200-400 mg/dl). Radial immunodiffusion (RID) with multicalibrator

concentrations is a technique that was used for measuring plasminogen level in the plasma (NL, Bindarid, Birmingham, UK) (normal range 58-140 mg/l). PTZ assay was done by using the ZYMUTEST protein Z kit (ELISA) (HYPHEN BioMed, Neuville-sur-Oise-France) (normal range 1-4 µg/ml).

Statistical analysis of data:

The collected data were organized, tabulated and statistically analyzed using Statistical Package of Social Science (SPSS), version 16. For quantitative data, mean, standard deviation (SD) were calculated and for comparison between groups, student (t) test was calculated. Differences in mean values of quantitative variables were analyzed using Mann-Whitney U test while chi-square test was used for difference in frequency between control and RDS group. Non-parametric Kendall's correlation was used to test for significance of linear relationship between different quantitative variables. A p value of <0.05 was considered statistically significant.

Results

Comparing maternal demographic and clinical characteristics, the respiratory distress group (RDS group) and control group were comparable (table 1).

Maternal coagulation parameters and PTZ were within normal ranges except for fibrinogen levels which were in both RDS group and control group higher than normal range (629.6±131.3 versus 650.5±141.1mg/dl) but the difference between the two groups was statistically insignificant (table 2).

Among RDS group maternal PTZ range varied from 2.12 to 3.89µg/ml while in control group its range varied from 2.41 to 3.89 µg/ml. Comparing clinical data of the enrolled newborns showed that there were statistically significant differences between RDS group and control group regarding all parameters (table 3).

Gestational age range in the respiratory distress syndrome group (RDS group) varied from 31 to 37 weeks while in the control group its range varied from 37 to 40 weeks. Neonatal blood cells and indices were comparable among study groups. Neonatal PT,

aPTT, fibrinogen level and platelets count were within normal ranges among study groups (table 4).

Among RDS group, neonatal serum plasminogen level was 64.2±28.7 mg/l while in control group it was 82.8±31.5 mg/l and the difference between the two groups was statistically significant (p=0.04, table 4).

Neonatal PTZ range varied from 1.71 to 3.90 (3.3±0.4) µg /ml in RDS group while its range varied from 2.51 to 3.90 (3.4±0.2) µg /ml in control group. There was no significant correlation between neonatal serum PTZ level and platelets count, PT, aPTT, serum plasminogen level or serum fibrinogen level (table 5).

Table (1): Characteristics of study population

	RDS group n=30	Control group n=20	P value
Maternal age (y)	25.32±5.33	26.52±4.93	0.41
Parity	3.15±2.31	2.96±1.82	0.71
Gravidity	4.73±2.64	4.24±2.34	0.52
Cesarean delivery (%)	17 (56.6)	7 (35)	0.11
Vaginal delivery (%)	13 (43.3)	13 (65)	0.12

Table (2): Maternal protein Z and coagulation parameters (mean ±SD)

	RDS group n=30	Control group n= 20	P value
Platelets X (10^3/µl)	220.6±85.5	208.8±78.8	0.59
PT(sec)	13.6±0.7	13.5±0.5	0.51
aPTT(sec)	31.1±0.6	32.3±0.9	0.31
Plasminogen (mg/l)	94.1±17.8	92.1±20.1	0.70
Fibrinogen (mg/dl)	629.6±131.3	650.5±141.1	0.62
PTZ (µg/ml)	3.4±0.2	3.3±0.1	0.20

Table (3): Characteristics of neonatal study cases (mean ± SD).

	RDS group n= 30	Control group n= 20	P value
Gestational age (weeks)	34.3±1.6	38.2±0.78	0.00
Post natal age (h)	3.64±0.9	2.05±0.9	0.00
Apgar score 1minute	2.54±0.7	8.13±0.6	0.00
Apgar score 5 minute	6.48±0.7	10.1±0.0	0.00
Respiratory rate/min	75.9±6.1	38.9±1.0	0.00
Heart rate /min	151.3±8.9	136.6±5.8	0.00
Weight (g)	2715.0±348.6	3570.0±283.0	0.00
Length (cm)	46.5±1.12	49.1±0.83	0.00
Head circumference (cm)	31.84±0.7	33.0±0.9	0.00

Table (4): Hematological indices, coagulation parameters and protein Z among study newborns (mean± SD).

	RDS group n= 30	Control group n=20	P value
White blood cells X (10^3/µl)	14.1±4.4	16.5±4.8	0.1
Red blood cells X (10^6/µl)	4.2±0.9	4.4±0.5	0.4
Hemoglobin (g/dl)	13.7±3.1	15.2±1.9	0.06
Hematocrit (%)	38.6±8.9	40.7±5.7	0.4
MCV (fl)	96.0±12.1	91.4±6.9	0.1
MCH (pg)	33.6±3.9	33.8±3.4	0.9
MCHC (g/dl)	36.6±0.9	36.6±1.1	1.0
Platelets X (10^3/µl)	203.7±82.3	224.1±56.9	0.3
PT(sec)	13.4±0.7	13.1±0.5	0.2
aPTT(sec)	31.27±0.7	30.95±0.8	0.1
Plasminogen (mg/l)	64.2±28.7	82.8±31.5	0.04
Fibrinogen (mg/dl)	271.2±35.3	279.4±36.3	0.4
PTZ(µg/ml)	3.3±0.4	3.4±0.2	0.3

MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin,
MCHC: Mean corpuscular hemoglobin concentration, PTZ: protein Z

Table (5): Correlation between neonatal PTZ and coagulation parameters.

	PTZ	
	r	p
Platelets X (10^3/µl)	-0.06	0.7
PT (sec)	-0.07	0.7
aPTT (sec)	-0.04	0.8
Plasminogen (mg/l)	0.06	0.6
Fibrinogen (mg/dl)	-0.10	0.3

Discussion

The results of this study show that neither maternal nor neonatal PTZ has a role in the pathogenesis of RDS. Among RDS group and control group, maternal serum PTZ levels were within normal range and the difference between the two groups was statistically insignificant. Also, this study demonstrates that maternal PTZ level did not change with advancing gestational age. This was in agreement with some studies that found the maternal plasma concentration of protein Z does not change with advancing gestational age [16,17] while other studies reported that the maternal plasma concentration of this glycoprotein increases[18] or decreases [15] with gestational age. Paidas and his colleague found that maternal PTZ levels decrease with advancing of gestational age but this change was within normal range [22].

Maternal fibrinogen levels, in both groups, were higher than normal range and this agrees with other studies[23-25]. Normal pregnancy is a hypercoagulable state[26] characterized by increased generation of thrombin, as determined by increased concentrations of fibrinopeptide A[27,28], thrombin antithrombin III (TAT) complex[29-31], and prothrombin fragments 1 and 2[30, 32, 33].

PTZ acts as an essential cofactor for PTZ-dependent protease inhibitor (ZIP), which in turn is a potent down-regulator for coagulation factor X. Lower levels of PTZ in RDS patients will result in activation of coagulation with intra-alveolar fibrin deposition which would significantly impair the surfactant's function[34]. This is correlated with the current study that showed high significant differences between neonatal RDS and control groups as regard to gestational age, post natal age, Apgar score, respiratory rate, heart rate, weight, length and head circumference (table 3). While no significant differences between them as regard to white blood cells, red blood cells, hemoglobin and other indices (table 4). These results were against Imam et al., [35] study that done to evaluate Protein Z (PTZ) and protein C (PTC) levels in Egyptian newborns

suffering from RDS. They found no effect of either gestational age, weight, APGAR score, hemoglobin %, TLCs, pH or PCO_2 on PTZ levels in all groups. Corral et al. [36], also noted no effect of either the gestational age or weight on PTZ levels.

In the present study neonatal serum PTZ level was within normal range among RDS group and control group and the difference between the two groups was statistically insignificant. These results coincide with findings of Yurdakok and his colleagues who found similar PTZ levels in newborns with RDS and in healthy preterm newborns [12,13].

In contrast Schettini and his colleagues reported that PTZ deficiency occurs early in newborns affected by severe RDS and no increase detect in its levels on the third day of disease. Schettini and his colleagues related the difference in PTZ levels between RDS patients and preterm controls to the coagulation theory, where there is activation of coagulation mechanism in RDS patients[3].

Previous studies[11,37,38] found low levels of anti-thrombin and high levels of thrombin-antithrombin complex in newborns with severe RDS, and therefore low level of PTZ could be a consequence of this prothrombotic condition. The physiological function of protein Z, which is a vitamin K-dependent protein, is still unknown.

The observation that thrombin associates with phospholipid surfaces in the presence of bovine protein Z has prompted the suggestion that this phenomenon may provide a mechanism whereby thrombin is kept from diffusing into the vascular lumen and away from the site of injury [39-41]. However, approximately tenfold higher concentrations of human protein Z are required to bind an equivalent amount of and effect of thrombin as can be bound with bovine protein Z. Therefore, considering the in vivo concentrations of protein Z, it seems unlikely that protein Z would perform this function to any significant extent in humans [4].

There was no significant corre-

lation between neonatal serum PTZ level and neonatal coagulation parameters. This was in agreement with Imam et al. [35] study who found no significant correlation between them.

Neonatal serum plasminogen level was lower among RDS group than the control group. Abnormality in the coagulation and fibrinolytic systems may play a role in the pathogenesis of RDS and contributes to the progression of the disease [42-44]. This is evidenced by fibrin deposition in pulmonary microcirculation and small airways[11,45]. Histo-pathological studies proved that fibrin constitutes a major part of the hyaline membranes, which could be regarded as a local origin for clots in RDS [46].

It is concluded that neither maternal nor neonatal PTZ has a role in the pathogenesis of neonatal respiratory distress syndrome.

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ROLE OF MATERNAL PROTEIN Z IN NEONATAL RESPIRATORY DISTRESS SYNDROME

Hussein Koura MD, Ashraf Alawadi MD, Mohamed Abdel-Aal MD, Hussein Metwali MD, Mohamed Abdel Zaher MD, Abdel-Hamead Mohammed MD and Mohammed El-Eraqi MD

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DETECTION OF SOME AUTOIMMUNE DISEASES ASSOCIATED WITH TYPE 1 DIABETES MELLITUS

Hussein Koura MD, Ashraf Alawadi MD*, Mohamed Abdel-Aal MD, Hussein Metwali MD, Abdel-Hamead Mohammed MD and Mohammed El-Eraqi MD*****

Departments of Pediatric (damiatta, cairo) and clinical pathology (Damietta***, Assuit**) Faculty of Medicine, Al- Azhar University, Egypt.*

Abstract

Background: *Type 1 diabetes often occurs in the company of other autoimmune diseases, including autoimmune thyroid disease (AITD), celiac disease (CD) and Addison's disease. The objective of this study was to estimate the relative frequency of the thyroid autoantibodies and tissue transglutaminase IgA antibodies (tTgAbs) among children suffering from T1DM.* ***Methods:*** *a cross sectional study done on 86 children with T1DM. At enrollment, the following investigations were performed: thyroid-stimulating hormone (TSH), free thyroxin (fT4), free triiodothyronine (fT3), serum total IgA, tissue transglutaminase IgA antibodies (tTgAbs), thyroperoxidase antibodies (TPOAbs), thyroglobulin antibodies (TgAbs).* ***Results:*** *Among the enrolled patients, the mean duration of diabetes was 4.1±1.2 years. Five patients (5.8%) were positive for tTgAbs and none of them was positive either for TPOAbs or TgAbs. They were diagnosed as celiac disease (CD) because of disappearance of their symptoms after feeding with gluten free diet (GFD). Seven patients (8.2%) were positive for either TgAbs and or TPOAbs and none of them was positive for tTgAbs. Six patients (7%) were positive for both TPOAbs and TgAbs and one patient (1.2%) was positive only for TPOAbs. Among those who were positive for TPOAbs and TgAbs, two patients (2.3%) were diagnosed as hypothyroidism according to the serum levels of TSH, fT4 and fT3. Mean durations of diabetes among those who were*

positive for tTgAbs and who were positive for thyroid autoantibodies were 4.2±0.3 and 4.1±0.2 years respectively. ***Conclusion:*** *It is concluded that, among type 1 diabetic patients, the relative frequency for positivity of tTgAbs and TgAbs and/or TPOAbs was 5.8% and 8.2% respectively. Also hypothyroidism was present in 2.3% of the patients. It is recommended to screen patients with T1DM for celiac disease and autoimmune thyroid disease.*

Keywords: *Type 1 diabetes mellitus; autoimmune thyroid disease; thyroid dysfunction disease; thyroid antibody.*

Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases in nearly all countries. The global prevalence of DM in the year 2010 among adults has been estimated to be 6.4%. Diabetes is the eleventh most important cause of premature mortality in Egypt, and is responsible for 2.4% of all years of life lost. Similarly, diabetes is the sixth most important cause of disability burden in Egypt[1]. It is estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes making it the country with the tenth largest population of diabetics in the world [2].

Type 1 diabetes mellitus (T1DM) is an autoimmune disease of beta cells in pancreas that result in insulin deficiency, it is often occurs in the company of other autoimmune diseases, including autoimmune thyroid disease (AITD)[3], celiac disease (CD) [4], and Addison's disease [5].

The occurrence of CD can accelerate diabetes worsening. Feature of CD specific to type 1 diabetes include poor glycaemic control, decreased bone mineral density with increased risk of developing osteoporosis and fractures in adults like in non-diabetic CD patients [6].

AIT as a more prevalent autoimmune disorder associating with T1DM is often clinically silent but it may progress to autoimmune thyroid disease (AITD), recognized as overt or subclinical hypothyroidism and hyperthyroidism. Hypothyroidism can lead to growth delay, weight gain, menstrual abnormality, hyperlipidemia, and

cardiovascular complications in diabetic patients[7]. Hyperthyroidism can worsen metabolic control of diabetes and increase its liability, often with a need for increased insulin dosage and increased chance of diabetic ketoacidosis [8].

AITD is detected most easily by measuring circulating antibodies against thyroid peroxidase (anti-TPO Ab) and thyroglobolin (anti-Tg Ab)[9]. The pathogenetic mechanism underlying the simultaneous occurrence of these autoimmune diseases has not been clearly understood yet. Evidence exists that common genetic determinants-mainly human leukocyte antigen (HLA) risk alleles or other genes outside the HLA region-could play a role Moreover, environmental factors seem to be involved in the pathogenesis of these complex diseases [10].

Presently, many would suggest that the presence of endomyseal (EMA) and tissue transglutaminase antibodies (tTgAbs) is enough to make the diagnosis of CD and that small intestinal biopsy is simply confirmatory. Nonetheless, biopsy remains the gold standard of diagnosis [11].

Studies have measured varying prevalence of associated autoimmune diseases between 3 and 50%, depending on the methodology of the study and patient's characteristics (age, sex, ethnicity, and genetic background) [12,13].

The aim of this study was to find the relative frequency of thyroid autoantibodies (TPOAbs and TgAbs) and tissue transglutaminase antibodies (tTgAbs) among infants and children with T1DM. Also the thyroid status was evaluated.

Patients and Methods

This was a cross sectional study which was carried out at Damietta university hospital. Eighty six children suffering from TIDM were recruited in the study. They are known diabetic and receiving insulin therapy. At enrollment caretakers provided informed consents and the following data were collected: age, gender, duration of T1DM, insulin treatment and hospital admission for management of diabetic ketoacidosis.

After that a thorough clinical examination was performed and nutritional status was assessed by using CDC charts[14]. Then the following investigations were performed for all enrolled patients: thyroid-stimulating hormone (TSH), free thyroxin (fT4), free triiodothyronine (fT3). Serum was divided into aliquots and they were stored at -20C till time of analysis. Determination of TSH (normal range 0.7-5.7 mIU/l), fT3 (normal range 1.5-4.1 pg/ml) and fT4 (normal range 0.8-2.3 ng/dl) were done by automated chemiluminscence immunoassay technique (Immulite autoanalyser), Thyroid antibodies were measured using ELISA technique (IMMCO diagnostics, USA).The cut point for positivity of TPOAbs was > 20 IU/ml while it was >80 IU/ml for TgAbs. Serum total IgA concentration was determined by immunoturbidmetric technique using (Integra 400 plus) analyzer with Roche Tinaquant reagents and values below 33 mg/dl were regarded as IgA deficiency. Patients with IgA deficiency were excluded from the study. ELISA (IMMCO diagnostics, USA) kit was used for estimation of tTgAbs (IgA) and the cut point for positivity was > 20 Units.

Statistical analysis :

The collected data were organized tabulate and statistically analyzed using SPSS (Statistical Package for Social Science) software computer program version 12. The results were represented in tabular forms then interpreted. Mean, standard deviation, range, frequency and percentage were used as descriptive, chi square and Fisher exact test was used for testing significance of observed differences between studied patients. The level of significance was adopted at $p < 0.05\%$.

Results

Eighty six children suffering from T1DM were recruited in the study and none of them was below 2 years old. Males were 38 (44.2%) and the mean duration of diabetes was 4.1±1.2 years (table1). Five patients (5.8%) were positive for tTgAbs and none of them was positive either for TPOAbs or TgAbs (tables 2,4). In these 5 patients, symptoms of celiac disease such as recurrent diarrhea and abdominal distension disappeared after feeding with gluten free diet

(GFD), so they were diagnosed as CD (table 4). They were advised to continue gluten free diet till intestinal biopsy was done to confirm the diagnosis. Short stature (< -2SD) was detected among all of them. Seven patients (8.2%) were positive for either TgAbs and or TPOAbs and none of them was positive for tTgAbs (tables 2, 5). Six patients (7%) were positive for both TPOAbs and TgAbs and one patient (1.2%) was positive only for TPOAbs (table 2). Among those who were positive for TPOAbs and TgAbs, two patients (2.3%) were diagnosed as hypothyroidism according to the serum levels of TSH, fT4 and fT3 (tables 6, 7). Short stature was the only manifestation of hypothyroidism (table 7). They received levothyroxin (4µg/kg/24h) and their hormonal levels returned to normal. Mean durations of diabetes among those who were positive for tTgAbs and who were positive for thyroid autoantibodies were 4.2±0.3 and 4.1±0.2 years respectively (tables 4, 5). Patients with positive levels for autoantibodies demonstrated recurrent diarrhea, abdominal distension and presence of short stature while none of those with negative autoantibodies displayed these manifestations (table 8).

Table (1): Characteristics of study cases (n=86).

	No.	**%**
Age group (y)		
< 3	2	2.3
3-	10	11.6
6-	11	12.8
9+	63	73.3
Males	38	44.2
Duration of diabetes mellitus (mean ±SD)(y)	4.1 ±1.2	
Positive family history for diabetes mellitus	4	4.6
Weight/Height < -2 SDS	0	0
Height/Age < -2 SDS	7	8.2

Table (2): Patients positive for autoantibodies (n=86).

	No.	%
tTgAbs	5	5.8
TgAbs and/or TPOAbs	7	8.2
TPOAbs+ TgAbs	6	7
TPOAbs	1	1.2

Table (3): Comorbidity with TIDM (n=86).

	No.	%
Celiac disease	5	5.8
Hypothyroidism	2	2.3

Table (4): Characteristics of patients with positive tTgAbs (n=5).

	No.	%
Age (mean ± SD) (y)	9.1 ± 1.4	
Males	1	(20)
Duration of diabetes mellitus (mean ± SD) (y)	4.2 ± 0.3	
Symptoms		
Recurrent diarrhea	3	60
Vomiting	2	40
Abdominal distension	3	60
Recurrent abdominal pain	3	60
Height/Age < -2 SDS	5	100
Disappearance of symptoms after gluten free diet	5	100
Positive TPOAbs + TgAbs	0	0
Positive TPOAbs	0	0

Table (5): Characteristics of patients with positive thyroid autoantibodies (n=7).

	No.	%
Age (mean ± SD) (y)	9.3± 1.2	
Females	5	71.4
males	2	28.58
Duration of diabetes mellitus (mean ±SD) (y)	4.1± 0.2	
Height/Age < -2 SDS	2	28.6
TSH (mean ± SD) (mIU/1)	3.9±1.2	
fT4 (mean ± SD) (ng/dl)	2.2±1.6	
fT3(mean± SD) (pg/ml)	2.7±1.3	
Positive tTgAbs	0	0

Table (6): Hypothyroidism among patients with positive thyroid autoantibodies (n=7).

	No.	Hypothyroidism	Euthyroid
TPOAbs + TgAbs (%)	6	2 (28.6)	4 (57.1)
TPOAbs (%)	1	0 (0)	1 (14.3)

Table (7): Characteristics of cases with hypothyroidism (n=2).

	Patient 1	Patient 2
Age (y)	9	10
Gender	Female	Female
Duration of diabetes mellitus (y)	4	4.5
Complaint		
Short stature	+ve	+ve
Height/Age < -2 SDS	+ve	+ve
TSH (mIU/l)	7.2	8.1
fT4 (ng/dl)	0.62	0.65
fT3 (pg/ml)	1.01	1.08
tTgAbs	- ve	-ve

Table (8): Comparison between patients with and without positive autoantibodies.

	Patients with +ve autoantibodies n=12	Patients without +ve autoantibodies n=74	T test or X^2	P
Age (mean ±SD) (y)	9.2±1.3	9.6±1.2	1.1	0.30
Males (%)	3 (25)	35 (47.3)	2.1	0.20
Duration of diabetes mellitus (mean ±SD) (y)	4.2±0.2	4.1±0.3	1.1	0.30
Positive family history for diabetes mellitus (%)	2 (28.6)	2 (2.7)	1.9	0.20
Recurrent diarrhea (%)	3 (25)	0 (0)	12	0.00
Vomiting (%)	2 (16.7)	4(5.4)	0.7	0.40
Abdominal distension (%)	3 (25)	1 (1.4)	8	0.00
Recurrent abdominal pain (%)	3 (25)	11 (14.9)	3.3	0.06
Height/Age < -2SDS (%)	7 (58.3)	0 (0)	39.5	0.00

Discussion

The results of this study demonstrate that the screening for celiac and thyroid autoantibodies appears to be essential part in the management of T1DM. It has been shown that T1DM has strong relationship with autoimmune disorders such as pernicious anemia, celiac disease, and idiopathic adrenal insufficiency. AIT is the most prevalent autoimmune disorders associated with T1DM[15]. The reason for the high prevalence of some autoimmune disorders in these patients still remains undetermined. It may be due to a generally increased tendency to react against certain antigens, or a genetically impaired ability to acquire tolerance to some autoantigens, or certain common antigens present in the tissues of individuals prone to autoimmune diseases [10].

Both T1DM and AIT are organ-specific T-cell mediated diseases, and have similar pathogenesis, which involves T-cell infiltration resulting in dysfunction of the target organ [16].

Among the study cases, the relative frequency for positivity of tTgAbs was 5.8% while it was 8.2% for either TgAbs and or TPOAbs. In the current study, five patients having tTgAbs (5.8%) were diagnosed as CD because they responded well to feeding with GFD. It has been suggested that seropositive patients at risk for CD could be started on a GFD with serial serologic testing to monitor response. Barker et al [17] proposed that, given its high specificity for CD histopathology, tTgAbs >100 units in pediatric patients at risk for CD warrant a diagnosis without biopsy. The mean duration of our diabetic patients with CD was 4.2±0.3 years. Our relative frequency for CD agreed with previous studies[18-25]. In a prospective evaluation of 273 diabetic children[23], CD was detected in 3.3% at diagnosis and in an additional 2.9% thereafter. But no patients were found to have CD >4 years after diabetes onset.

Most of the previous studies have been cross sectional surveys, with screening tests performed either at the time of diagnosis of type 1 diabetes[19,20,26-28] or at some time thereafter[21,29-31]. It

has been shown that CD can develop after the onset of type 1 diabetes in patients with no signs of CD-related autoimmunity at the time of diagnosis of type 1 diabetes[19,27,29]. In this study the use of tTgAbs as screening test is based on its highly sensitivity and specificity[32,33], close correlation between tTgAbs levels and intestinal mucosa abnormalities[34] and its efficiency among patients over 2 years of age[32-34] and majority of patients with diabetes are older than 2 years. We excluded from the study all patients with IgA deficiency because IgA antibodies may not be detected in them, resulting in a false negative test. This is particularly important because CD is more common in those with IgA deficiency than in the general population (1.7% compared with 0.25%)[35].

Presence of short stature among our patients with CD reflects the late diagnosis of CD and unawareness of its prevalence among patients suffering from T1DM. Our patients were complaining from gastrointestinal symptoms and this did not coincide with other studies where the majority of patients with CD are asymptomatic[36-41] or are not aware of symptoms. Some patients present with problems recognized only retrospectively as resulting from celiac disease; it is common for "asymptomatic" patients to report improved health or sense of well-being when following a gluten-free diet[17]. Up to one-third of patients may have unexplained failure to thrive, abdominal pain, or short stature [42].

In our study, the prevalence of AIT in female patients with T1DM was higher than that in male T1DM patients. However, it should be stated that the differences were not statistically significant. Many studies found higher prevalence of positive thyroid autoantibodies in females [13,43,44] and some studies reported similar prevalence in both genders [45,46].

The presence of hypothyroidism has been associated with thyroid autoantibodies, increasing age and diabetes duration, and female gender[3,47]. In contrast to previous studies, we found a lower relative frequency for both thyroid autoantibodies and AITD. In other

studies, the using of different tests and cut off points can explain this controversy. We screened our patients for AITD through estimating levels of TPOAbs, TgAbs, TSH, fT4 and fT3. Although there is general agreement that the high prevalence of thyroid dysfunction in type 1 diabetes subjects justifies screening in all patients [48,49], it is not clear which is the best procedure and how often to perform it.

Silverstein et al.[50] and several authors[51,52] recommend annual screening for thyroid disease in all type 1 diabetes subjects with TSH measurement; this procedure is considered the most sensitive way to identify patients with thyroid dysfunction, as autoantibodies may persist for many years without thyroid dysfunction. However, the Silverstein et al.[50] recommendations note that the presence of thyroid autoantibodies increase the risk for thyroid disease, and Hansen et al.[51] did not find any initial TPOAbs negative patients who developed thyroid disease after 3 years of follow up. Other authors[3,53] recommend screening using TSH and TPOAbs.

In a cohort of 58 type 1 diabetic patients enrolled in the Diabetes Control and Complications Trial and followed for 18 years, Umpierrez et al.[3] observed that TPOAbs positive subjects were 17.9 times more likely to develop thyroid dysfunction. These authors recommended annual screening using TSH determination, particularly in patients with positive TPOAbs.

Barker [53] screened type 1 diabetic patients with TPOAbs and thyroid function at onset and every 1-2 years thereafter and patients with positive TPOAbs every 6-12 months. Finally, a third group of authors[58,66] recommends TSH determination only in TPOAbs positive patients.

Short stature, recurrent diarrhea and abdominal distension were present only among patients with positive autoantibodies. These results reflect the importance of assessment of growth among patients suffering T1DM and paid attention for screening for CD among diabetic patients with gastrointestinal manifestations. Previous studies recommend screening for CD at any

time if a diabetic child or adolescent develops intestinal or extra-intestinal symptoms consistent with CD[23,54].

Regarding association of AITD and CD among patients suffering T1DM, the results of this study are not coinciding with previous studies[36-41]. None of our patients with positive thyroid autoantibodies was positive for tTgAbs. Jaeger and his colleagues found that a significant proportion of patients with recent-onset T1DM was positive for two or more disease specific antibodies (54/197, 27.4%). The level of coexistence for thyroid antibodies and/or celiac disease associated antibodies was 11.2 and 9.6%, respectively and 6.6% were triple positive[48]. The disagreement between our results and the others can be attributed to cross sectional design of our study in which we investigated the patients once. The appearance of autoantibodies needs years [55-57].

It is concluded that, among type 1 diabetic patients, the relative frequency for positivity of tTgAbs and TgAbs and/or TPOAbs was 5.8% and 8.2% respectively. Also hypothyroidism was present in 2.3% of the patients. It is recommended to screen patients with T1DM for celiac disease and autoimmune thyroid disease.

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REPRINT

BENHA MEDICAL JOURNAL

DETECTION OF SOME AUTOIMMUNE DISEASES ASSOCIATED WITH TYPE 1 DIABETES MELLITUS

Hussein Koura MD, Ashraf Alawadi MD, Mohamed Abdel-Aal MD, Hussein Metwali MD, Abdel-Hamead Mohammed MD and Mohammed El-Eraqi MD

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PREVALENCE OF REFLUX OESOPHAGITIS IN PATIENTS WITH CHRONIC LARYNGITIS

Lamiaa Z. Mobarak MD and Naslshah G. Kazem MD

Departments of Tropical Medicine, National Hepatology and Tropical Medicine Research Institute, Cairo. and Otorhinolaryngology, Faculty of Medicine, Banha University, Egypt

Abstract

Introduction: *Gastrooesophageal reflux disease (GERD) is a common cause of chronic laryngeal inflammation.*

Aim: *The aim of this study was to detect the prevalence of endoscopic reflux oesophagitis in patients with persistent laryngitis in the absence of typical GERD symptoms.*

Patients and Methods: *This study was conducted on 50 patients, referred from the department of otolaryngology to the gastroenterology department with persistent throat symptoms such as hoarseness, throat pain, globus sensation and excessive throat clearing, for at least 3 months, in the absence of typical GERD symptoms (heart burn, regurgitation and chest pain). After complete ENT examination, indirect and direct laryngoscopy, upper gastrointestinal tract (GIT) endoscopy was performed to all patients to detect the presence of reflux oesophagitis. The endoscopic grading of reflux oesohagitis was done according to the Los Angeles classification.*

Results: *36% of the patients had reflux oesophagitis of varying degrees.*

Conclusion: *This data suggest that in many patients suffering from chronic laryngitis, gastrooesophageal reflux disease (GERD) is associated.Patients with supra oesophageal GERD do not always have the typical symptoms of heart burn and regurgitation.*

Key words: *Reflux Oesophagitis, Chronic Laryngitis.*

Introduction

Gastrooesophageal reflux disease (GERD) is the most common oesophageal disease. It typically

presents with heartburn and regurgitation, but it may also cause atypical symptoms, either alone or in combination. About 20 to 60 percent of patients with GERD have ENT symptoms without any heartburn[1].

Gastrooesophageal reflux disease is associated with a wide spectrum of otolaryngologic disorders and extraoesophagealsyndroms[2].

It is believed that gastrooesophageal reflux disease is a common cause of chronic laryngeal inflammation, resulting in a condition known as reflux laryngitis[3].

Patients diagnosed with reflux laryngitis present with a wide variety of symptoms, including hoarseness, throat pain, globus sensation and excessive throat clearing [4].

Aim :

The aim of this study was to detect the prevalence of endoscopic reflux oesophagitis in patients with persistent laryngitis in the absence of typical GERD symptoms.

Patients and Methods

This study was conducted on 50 patients, referred from the department of otolaryngology to the gastroenterology department of the Sheikh Zayed Specialized Hospital over a total period of one year and five months with chronic laryngitis, without a definite cause. Patients presented with persistent throat symptoms such as hoarseness, throat pain, globus sensation and excessive throat clearing, for at least 3 months, in the absence of typical GERD symptoms (heart burn, regurgitation and chest pain). After complete ENT examination, indirect and direct laryngoscopy, upper gastrointestinal tract (GIT) endoscopy was performed to all patients to detect the presence of reflux oesophagitis. The endoscopic grading of reflux oesohagitis was done according to the Los Angeles classification. [5]

Results

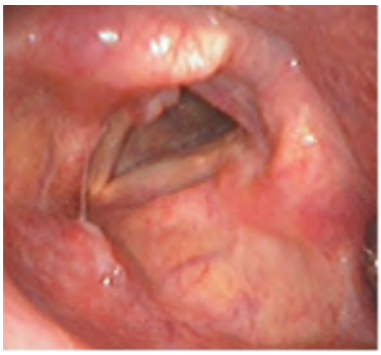
The study included 50 patients (34 (68 %) males and 16 (32 %) females).Their age ranged from (27-60). Mean age ± SD was 44.82 ± 8.6936.

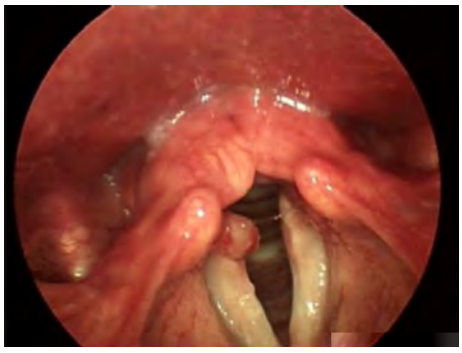
Patients presented to the otola-

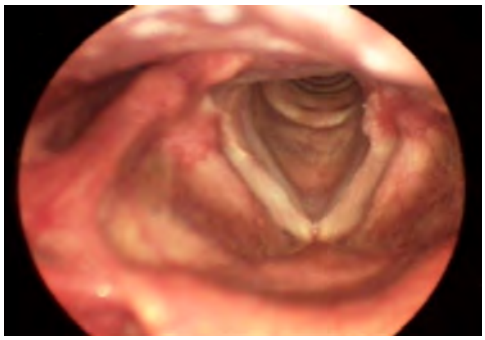
ryngology department with persistent throat symptoms for at least 3 months. Excessive throat clearing was the predominant symptom occurring in 25 patients (50%), 12 patients (24%) presented by globus sensation, 10 patients (20%) presented by hoarseness and lastly 3 patients (6%) presented by throat pain. All chronic laryngitis patients were referred for gastroenterologic evaluation after the complete ENT examination, indirect and direct laryngoscopy, where upper gastrointestinal tract (G.I.T.) endoscopy was performed to all patients to detect the presence of reflux oesophagitis, which was graded according to the Los Angeles classification. In 32 patients (64%) endoscopic reflux oesophagitis was absent, while 18 patients (36%) had reflux oesophagitis of varying degrees. 10 patients (55.55%) had reflux oesophagitis grade A, 5 patients (27.77%) had grade B, 2 patients (11.11%) had grade C, and 1 patient (5.55%) had grade D oesophagitis.

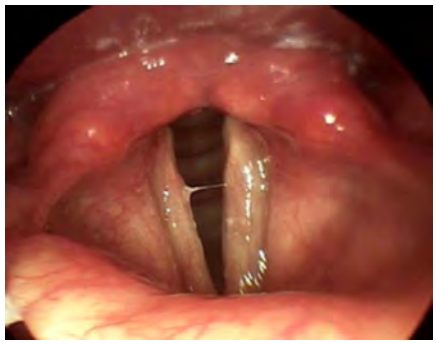
No. of patents	Range	Minimum Age	Maximum Age	Mean Age (years)		Std. Deviation
Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
50	33.00	27.00	60.00	44.8200	1.22946	8.69363

Pictures of Chronic larynitis

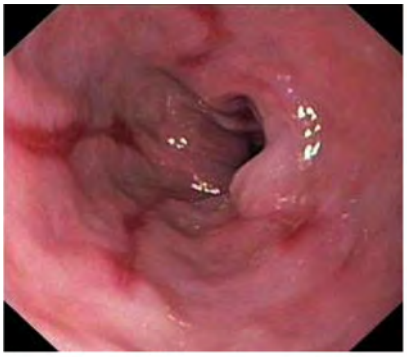


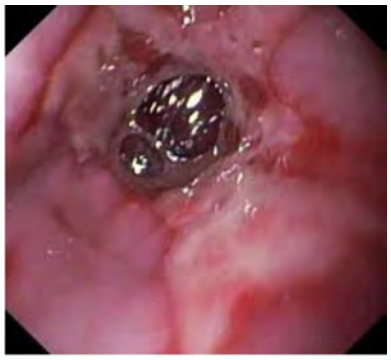


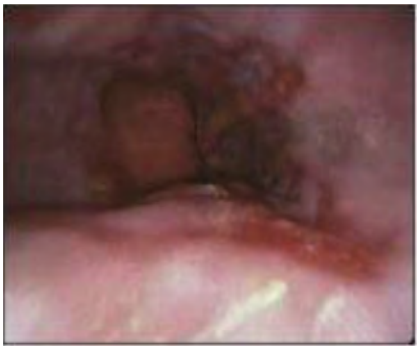


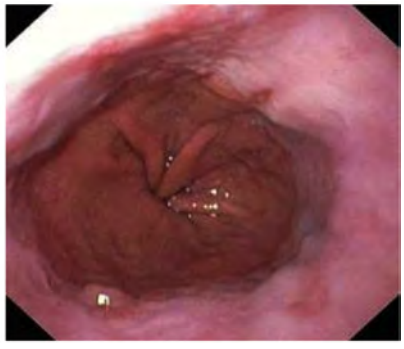


Pictures of reflux oesophagitis









Discussion

The study was conducted on 50 patients. The majority of our patients were men (38%), like other studies where most of their patients were men [6] and [7], but unlike one study, where a high proportion of their patients were women [8]. Regarding symptoms, Excessive throat clearing was the predominant symptom occurring in 50% of the patients, followed by globus sensation (24%) then hoarseness (20%). This finding is like that observed in the study conducted by [9], but unlike another study where globus sensation was the most common ENT symptom [1].

GERD can cause oesophageal and extraoesophageal syndromes [10]. Extraoesophageal manifestations are increasingly being recognized [11]. ENT become more commonly recognized or suspected by physicians, although the direct association between symptoms and acid reflux has been difficult to establish. Most patients with suspected supraoesophageal GERD do not have the typical symptoms of heartburn and acid regurgitation [12]. About 20 to 60 percent of patients with GERD have ENT symptoms without any heartburn[1]. In another study, up to 50% of patients with an endoscopically proven or negative oesophagitis suffer from symptoms other than heartburn or acid regurgitation such as laryngitis, hoarseness, chronic cough, asthma or non cardiac chest pain[11]. These findings are consistent with our results since 36% of our chronic laryngitis patients had reflux oesophagitis in absence of typical GERD symptoms. Possible mechanisms of GERD- mediated damage to extraoesophageal structures include direct-contact damage of mucosal surfaces by acid-pepsin exposure and a vagal reflex arc between the oesophagus and the upper aerodigestive tract, triggered by acid reflux [12].

Again our results agreed with Roka et al., 2008 who concluded in his study that symptom analysis showed silent GERD more frequently among GERD patients with supraoesophageal manifestations than in respiratory symptom-free GERD subjects [13].

Furthermore, in many patients

with posterior laryngitis, reflux symptoms are absent. On the other hand, the presence of symptoms of GERD in posterior laryngitis is higher than expected[14]. Based on several findings, (as in our study) chronic laryngitis has been linked to gastrooesophageal reflux disease (GERD)[8]. Epidemiological evidence indicates that laryngeal manifestations and diseases- aphonia, laryngitis and laryngeal stenosis are twice as frequent in patients with erosive oesophagitis as in controls without oesophagitis [15]. In our study, endoscopic reflux oesophagitis was graded according to the Los Angeles classification where oesophagoscopy was normal in most of our patients (64%). This agreed with Vicente et al., 2003, who found normal oesophagoscopy in 86% of his patients [8]. 36% of our patients had reflux oesophagitis of varying degrees. This was consistent with the results of one study conducted on patients complaining of persistent upper respiratory symptoms, in the absence of typical GERD symptoms that patients affected with upper airway disorders, such as posterior laryngitis, chronic sinusitis and vocal fold nodules, had a significantly higher prevalence of oesophagitis of varying degrees (31%)[15]. While in another study on typical GERD patients, Oesophagoscopy revealed oesophageal lesions in 50% of the patients[16]. Our findings were somehow different from those reported by Vicente et al., 2003 and El-Serag et al., 2001, who declared that regarding oesophageal lesions, it is widely accepted that oesophagitis is infrequent and mild in patients with laryngitis[8,7]. Regarding grading of reflux oesophagitis in our study, 55.55% of patients had reflux oesophagitis grade A, 27.77% had grade B, 11.11% had grade C and 5.55% of patients had grade D oesophagitis. Grade A and B were the most prevalent while grade C and D were the least prevalent. This finding was consistent with other studies [16] and [17] .

Conclusion

This data suggest that in many patients suffering from chronic laryngitis, gastrooesophageal reflux disease (GERD) is associated. However, laryngoscopc findings are subtle and meticulous examination is mandatory. Thus,

extraoesophageal otolaryngoscopic symptoms and laryngeal manifestations are to be considered as extragastrointestinal manifestations mainly of reflux disease. GERD has to be included into differential diagnostic approaches especially when routine treatment of laryngitis fails. Patients with supra oesophageal GERD do not always have the typical symptoms of heart burn and regurgitation.

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PREVALENCE OF REFLUX OESOPHAGITIS IN PATIENTS WITH CHRONIC LARYNGITIS

Lamiaa Z. Mobarak MD and Naslshah G. Kazem MD

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DUAL EFFECT OF SACRAL NEUROMODULATION AND POSTERIOR TIBIAL NERVE STIMULATION IN TREATMENT OF URGE INCONTINENCE DUE TO OVERACTIVE BLADDER

Basant M. Elnady MD, Shabieb A. Abdelbaky MD* and Dalia Desouky MD**

Lecturer of Rheumatology and Rehabilitation,
Faculty of Medicine Benha University,
Lecturer of Urology, Faculty of Medicine Benha University,*
*Lecturer of Public Health and Community Medicine**,*
Faculty of Medicine Menoufiya University. Egypt

Abstract

Purpose: *To investigate the effect of posterior tibial nerve electrical stimulation (PTN) combined with sacral surface therapeutic electrical stimulation (SSTES) in the treatment of overactive bladder. Patient and methods: Sixty urge incontinent patients due to over active bladder were included in this study. Their ages ranged from 14-63 years (42 ± 12.5). They were randomly divided into two equal groups. Procedures: Group (A) received a 12-week of treatment with sacral surface electrodes and posterior tibial nerve faradic electrical stimulation for 15 mins 3 times /week. Group (B) underwent pelvic floor exercises for 15 mins 3 times/week for 12 weeks. Results: This study revealed that the bladder overactivity in (A) group showed 48.69% improvement which was highly statistically significant (P<0.05), this change was non-significant (P>0.05) for group (B). Post-treatment comparisons revealed a statistical significant difference (P<0.05) between both groups with higher improvement of the bladder overactivity in group (A). Maximum flow rate significantly increased post-treatment (P<0.05) for group (A) with 25.2% improvement, while it was 12.37% more for group (B) which was also significant (P<0.05) Conclusion: Combined PTN with sacral surface therapeutic electrical stimulation (SSTES) in cases with*

overactive bladders, produced notable changes inducing urodynamic functional improvements especially in bladder overactivity, and maximum flow rate.

Introduction

Overactive bladder symptoms (urgency, frequency, nocturia and urge incontinence) are frequent complaints of patients attending the urology and gynecology clinics. In many patients, the causes are idiopathic with no obvious underlying neurological abnormality. Patients with overactive bladders also suffer from sleep disturbances, psychological distress, disruption of social, and work life. Quality of life scores (QOL) are consistently reduced in this group of patients [1].

The technology of peripheral neuromodulation is still in the relatively early stages of development. Most researches in this field have been reported in the last few years with little long-term data available. The bulk of the published studies have been uncontrolled case series. A wide variety of patient populations have been studied, and the inclusion and exclusion criteria used have been variable, as have been both the metrics used to measure responses and the parameters to establish success. Published studies have reported wide variation in degrees of success. The need exists for more randomized, controlled trials as well as data on longer-term outcomes [2].

Neuromodulation has been reported to be effective for the treatment of stress and urge urinary incontinence. The cure and improvement rates of pelvic floor neuromodulation in urinary incontinence are 30 - 50% and 60 - 90%, respectively. Pelvic floor exercises with adjunctive neuromodulation are the mainstay of conservative management for the treatment of stress incontinence. For urgency and mixed stress plus urgency incontinence, neuromodulation may therefore be the treatment of choice. As an alternative to drug therapy, it can offer improvement in patient QOL [3].

Sacral surface therapeutic electrical stimulation (SSTES) used as a therapy for urinary incontinence using the effect of neuromodulation. In this therapy, skin surface electrodes are applied on the sacral surface to provide stimulation, making the treatment very easy to perform. It has been shown that SSTES has not only an inhibitory effect on detrusor overactivity but also an efferent stimulant effect to the pudendal nerve [4].

The posterior tibial nerve contains mixed sensory and motor nerve fibers originating from L4 through S3 spinal roots, which modulate the innervation to the bladder, urinary sphincter, and pelvic floor. L4-S3 spinal roots give origin to somatic and autonomic nerves supplying also the pelvic floor. By stimulating the tibial nerve near the medial malleolus, it is believed that through this crossover tibial nerve stimulation works. Transcutaneous stimulation progressed to percutaneous stimulation and is known as posterior tibial nerve stimulation (PTNS). This was initially known as Stoller afferent nerve stimulation. PTNS look to be an easy and less expensive way to reach satisfactory results [5,6] .

PTNS is a minimally invasive neuromodulation system designed to deliver retrograde electrical stimulation to the sacral nerve plexus through percutaneous electrical stimulation of the posterior tibial nerve. The specific mechanism of action of neuromodulation is unclear, although theories include improved blood flow and changes in neurochemical balance along the neurons. Neuromodulation may have a direct effect on the detrusor or a central effect on the micturition centers of the brain [5,6,7].

Materials and Methods

This study included sixty patients who accepted to participate, and had symptoms due to overactive bladder with urge incontinence, urgency and frequency. These patients were randomly selected from the Urology department and outpatient clinic of Benha university hospitals. Inclusion criteria were: patients with overactive bladder, who have failed behavioral and/or pharmacologic

therapies and/or previous history of continence surgery. patients with any significant medical, pathological or neurological diseases which may interfere with the results of the study, e.g. diabetes mellitus, cerebro-vascular stroke, active rectal lesions or infections. Also, patients who were pregnant or planned to become pregnant during course of treatment, patients with pacemakers or implantable defibrillators, patients with uncorrectable coagulopathies, patients with nerve damage that could impact either percutaneous tibial nerve or pelvic floor function, current bladder malignancy and patients with current urinary tract infection were excluded from study.

The patients' ages ranged from 14 to 63 years old, from both sexes. A complete history had been taken. The physical examination included neurological assessment of perianal sensation, anal sphincter tone, and a brief screening for any neurological factors as, Parkinson's disease, multiples sclerosis, stroke or previous operations (mainly pelvic surgeries). Detailed analysis of the present overactive bladder symptoms had been carried out. Laboratory investigations, mainly fasting and postprandial blood glucose, complete urine analysis and urine culture had been carried out to exclude diabetes mellitus, urinary tract infection. Urodynamic studies had been carried out by the staff members of the urodynamic unit at urology department Benha university, to confirm the diagnosis of overactive bladder. All patients provided consent to participate in the study.

The patients were randomly divided into two equal groups; each group included thirty patients. Group (A) included patients suffer from overactive bladder (urge incontinence). These patients were assigned to, receive posterior tibial nerve electrical stimulation of faradic type, biphasic continuous rectangular, with a frequency of 0-10 Hz, plus superficial parasacral electrical stimulation. Surface electrodes were placed at the posterior sacral foramina of S2 and S4 with a frequency of 20 Hz. Both modalities were used for 15 minutes three days weekly, with the maximum tolerable intensity

up to 12 weeks performed by using the Sonopuls- 992 (ENRAF NONIU);. Group(B) included patients who received the routine physical therapy program of pelvic floor strengthening muscle exercise (Kegel's exercise) (8) for 15 mins three time a week for 12 weeks.

Electrical stimulation had been delivered to the posterior tibial nerve via a combination of electrodes and generator components, including a small 34-gauge needle electrode, surface electrode, lead wires and hand held electrical generator. The low-voltage stimulator (9 volts) had an adjustable pulse intensity according to patient tolerance, a fixed pulse width of 200 microseconds and a frequency of 10Hz. The device produces an adjustable electrical impulse that travels to the sacral nerve plexus by using an electrode placed near the ankle behind medial malleolus as an entry point (Figure 1), the stimulator's impulses travel along the tibial nerve to the nerves in the spine that control pelvic floor function.

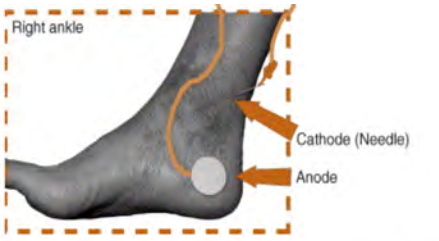

Fig. 1: PTN electrical stimulation technique (9).

Measurements were done by the Urodynamic Evaluation System. This procedure was performed by using the DANTIC UD5000/500 urodynamic investigation system which are valid and reliable, for testing the multichannel cystometry. It is comprised of a trolley-mounted unit with an integral printer and monitor, a mobile patient unit with built in H2O and CO2 pumps, a stand-mounted uroflow transducer and a stand-mounted puller mechanism. Measurements had been done by the staff of the urodynamic unit. All patients were subjected to multichannel cystometry before starting the study and at the end of the study (after 12 weeks). The variables measured were: First desire to void which reveals bladder sensation, bladder overactivity (number of uninhibited detrusor contractility), maximum flow rate ml/sec.

Data was analyzed by the SPSS version 13.0 statistical package using the appropriate statistical tests with (5%) level of significance.

Results

Sixty incontinent patients due to overactive bladder were included in this study. Their ages ranged from 14-70 years, with a mean value of 39.833±12.529 years. Female to male ratio was 78.33%, and mean disease duration was 9.5±1. 26 years.

Results of 1st desire to void through PTN and SSTES Electrical stimulation group (Group A) and (Group B):

(Group A): table (1) and figure (1), the mean value of 1st desire to void before initiation of treatment (Pre) was 147.07±26.162ml, while the mean value of 1st desire to void after application of treatment (Post) was 159.77±42.425ml. No statistical significant difference (P>0.05) between Pre and Post treatment was observed although a change or an increase of 8.64% (improvement) was observed.

Regarding (Group B): table (1) and figure (1), the mean value of first desire to void before initiation of treatment (Pre) was 132.6±27.677ml. After application of treatment (Post), the mean value of first desire to void of was 139.77±21.74ml. There was no statistical significant difference (P>0.05) before and after application of exercises with a percentage of improvement of 0.88%.

Comparative Analysis of Testing First Desire to void between both Groups of the Study:

Table (2) and figure (2), revealed no statistical significant differences (P>0.05) of mean values of first desire to void between both groups at entry and end of the study.

Results of overactivity: (Number of uninhibited detrusor contractility).

(Group A): Table (3) and figure (3), the mean value of overactivity pre-treatment was 1.33 ± 0.266, while the mean value of overactivity post-treatment was 1.943 ± 0.254.

There was a highly statistical significant difference (P<0.05) in overactivity, post-treatment when

compared with the corresponding mean value before initiation of treatment, with a percentage of change or increase (improvement) of 48.69%. (Group B):

Table (3) and figure (3), the mean value of overactivity before initiation of treatment (Pre) was 1.567±0.2, while the mean value of overactivity after application of treatment (Post) was 1.733±0.29.

There was no statistical significant difference (P>0.05) in overactivity, before and after application of treatment with a percentage of change or increase (improvement) of 4.25%.

Comparative Analysis of overactivity Testing between Groups of the Study:

Table (4) and figure (4), the overactivity at pre- treatment for electrical stimulation group (Group A) and exercises group (Group B) revealed no statistical significant differences (p>0.05) of mean value of stability among both groups at entry of the study, while at the end of the study (Post) treatment there was a statistical significant difference (P<0.05) of mean value of stability in favor of group A.

Results of Maximum flow rate; table (5) and figure (5):

In group (A): the mean value of maximum flow rate before initiation of treatment (Pre) was 12.51 ± 3.263 ml/sec, while the mean value after application of treatment (Post) was 15.663 ± 3.861 ml/sec.

There was a statistical significant difference (P<0.05) in maximum flow rate, before and after application of treatment, with a change or increase of 25.2% (improvement).

In group (B): The mean value of maximum flow rate before initiation of treatment (Pre) was 11.397±2.883 ml/sec, while the mean value after application of treatment (Post) was 12.807 ± 2.693 ml/sec.

There was a statistical significant difference (P<0.05) in maximum flow rate, before and after application of treatment (Post), with a change or increase of 12.37%. (improvement).

Comparative Analysis of Maximum flow rate Testing between Groups of the Study:

Table (6) and figure (6), the statistical analysis of maximum flow rate before treatment for electrical stimulation group (Group A) and exercise group (Group B) revealed no statistical significant difference s (P>0.05) of mean values between both groups.

After application of treatment (Post) for electrical stimulation group (Group A) and exercise group (Group B) a statistical significant difference (P<0.05) of mean values was observed between both groups.

Table (1): Statistical analysis of mean differences of first desire to void before initiation of treatment (Pre) and after application of treatment (Post) in Group (A) and Group (B).

Statistics	First desire to void(ml)			
	Group A		Group B	
	Pre	Post	Pre	Post
Mean	147.07	159.77	132.6	139.77
Standard Deviation	26.162	42.425	27.677	21.74
Mean Difference	12.7		1.167	
Paired t-value	1.397		0.61	
Probability value	0.1731		0.546	
Significance	Non Significant		Non Significant	
Percent of Change	8.64 %		0.88 %	

Pre: Before treatment.
Post: After 12 weeks of treatment.

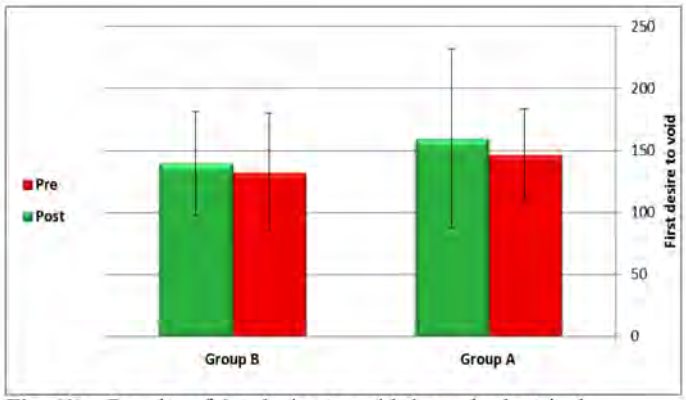

Fig. (1): Results of 1st desire to void through electrical stimulation group (Group A) and (Group B).

Table (2): Comparative analysis of the mean value of first desire to void between Group (A) and Group (B) at entry of the study and after application of treatment

Statistics	First desire to void at pre-treatment(ml)		First desire to void after application of treatment(ml)	
	Group A	Group B	Group A	Group B
Mean	147.07	132.6	159.77	139.77
Standard Deviation	42.425	27.677	26.162	21.74
Un-Paired t-value	0.91		1.93	
Probability value	0.364		0.058	
Significance	Non Significant		Non Significant	

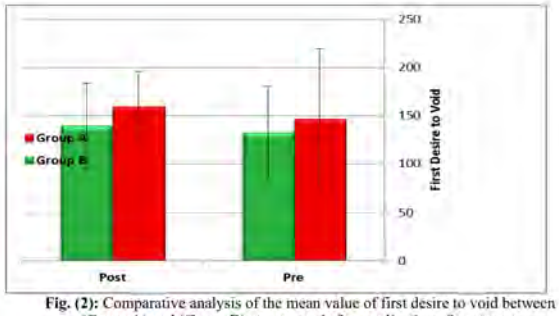

Fig. (2): Comparative analysis of the mean value of first desire to void between (Group A) and (Group B) at entry and after application of treatment.

Table (3): Statistical analysis of mean differences of bladder overactivity before initiation of treatment (Pre) and after application of treatment (Post) of Group A and Group B.

Statistics	overactivity			
	Group A		Group B	
	Pre	Post	Pre	Post
Mean	1.33	1.943	1.567	1.733
Standard Deviation	0.266	0.254	0.2	0.29
Mean Difference	0.61		0.06667	
Probability value	0.0001		0.1991	
Significance	Highly Significant		Not Significant	
Percent of Change	48.69 %		4.25 %	

Pre: Before treatment. Post: After 12 weeks of treatment.

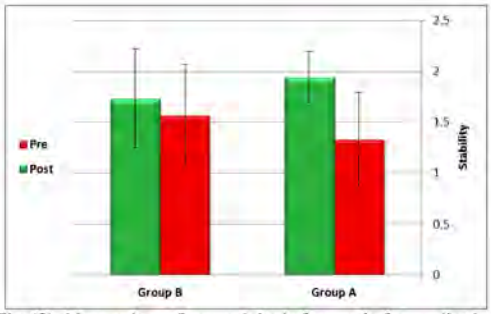

Fig. (3): Mean values of overactivity before and after application of treatment in Group (A) and Group (B).

Table (4): Comparative analysis of the mean value of bladder overactivity between Group (A) and Exercise group Group (B) before and after treatment.

Statistics	overactivity at Pre-treatment		overactivity after application of treatment	
	Group A	Group B	Group A	Group B
Mean	1.33	1.567	1.943	1.733
Standard Deviation	0.266	0.2	0.254	0.29
Student t test	1.90		2.08	
Probability value	0.0625		0.041	
Significance	Not Significant		Significant	

Post: After 12 weeks of treatment. Pre: Before treatment.

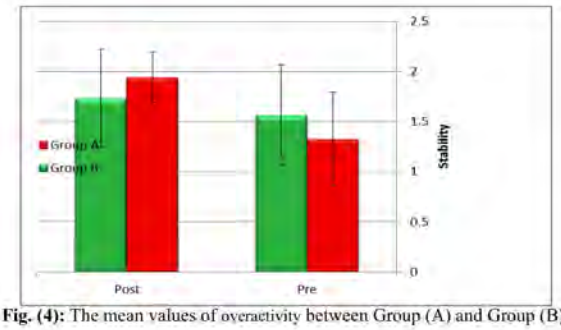

Fig. (4): The mean values of overactivity between Group (A) and Group (B)

Table (5): Statistical analysis of mean differences of maximum flow rate before initiation of treatment (Pre) and after application of treatment (Post) in both treatment groups (Group A) and (Group B).

Statistics	Maximum flow rate ml/sec			
	Group A		Group B	
	Pre	Post	Pre	Post
Mean	12.51	15.663	11.397	12.807
Standard Deviation	3.263	3.861	2.883	2.693
Mean Difference	3.153		1.41	
Paired t-value	3.277		2.871	
Probability value	0.0027		0.0076	
Significance	Significant		Significant	
Percent of Change	25.2 %		12.37 %	

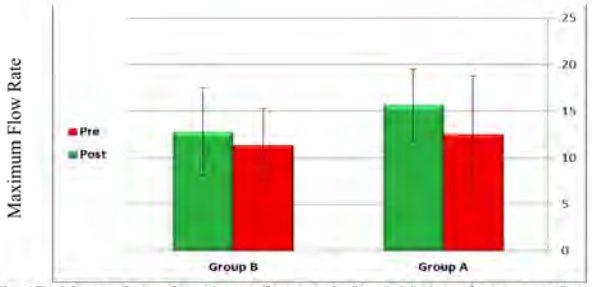

Fig. (5): Mean values of maximum flow rate before initiation of treatment (Pre) and after application of treatment (Post) between Group (A) and Group (B).

Table (6): Comparative analysis of the mean value of maximum flow rate among electrical stimulation group (Group A) and exercise group (Group B) before and after application of treatment.

Statistics	Maximum flow rate at pre-treatment ml/sec		Maximum flow rate after application of treatment ml/sec	
	Group A	Group B	Group A	Group B
Mean	12.51	11.397	15.663	12.807
Standard Deviation	3.263	2.883	3.861	2.693
Un-Paired t-value	0.7678		2.575	
Probability value	0.4457		0.0126	
Significance	Non Significant		Significant	

Pre: Before treatment. Post: After weeks of treatment.

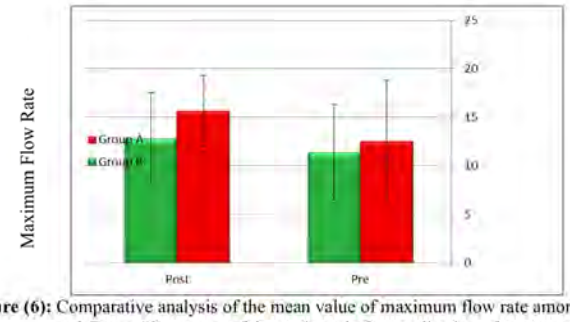

Figure (6): Comparative analysis of the mean value of maximum flow rate among Group (A) and Group (B) at entry of the study and after application of treatment.

Discussion

Overactive Bladder Syndrome (OAB) refers to individuals with the following symptoms : urinary urgency, urinary frequency, or urge incontinence. These symptoms are not life threatening, but can cause embarrassment. Incontinence, the most problematic symptom, predominantly affects women and occurs in approximately one-third of those with OAB. OAB is sometimes induced or exacerbated by drugs [10].

Despite the fact that pharmacological treatment is currently the first option for the treatment of women with clinical symptoms of overactive bladder, adherence to treatment is low, due to side effects, which lead to discontinuation in 60% of cases. Patricia et al. (2009) study noted that posterior tibial nerve electrical stimulation (PTNS) was chosen as a physiotherapeutic method because it is an interesting alternative for the treatment of overactive bladder, which is effective and without side effects. PTNS is considered to be a simpler, less invasive and easy to apply [5].

PTNS is a technique of electrical neuromodulation for the treatment of voiding dysfunction in patients who have failed behavioral and/or pharmacologic therapies. Voiding dysfunction includes urinary frequency, urgency, incontinence, and non-obstructive retention. Altering the function of the posterior tibial nerve with PTNS is believed to improve voiding function and control. While the posterior tibial nerve is located near the ankle, it is derived from the lumbar-sacral nerves (L4-S3) which control the bladder detrusor and perineal floor [11].

PTNS is a minimally invasive technique that is effective to suppress detrusor overactivity [7]. There was an objective effect of PTNS on urodynamic parameters (significant improvement in maximum cyctometric capacity and involuntary detrusor contraction [12].

In our study neuromodulation, using the dual beneficial effects of SSTES and PTN electrical stimulation offer a nondestructive alternative for patients with urge incontinence caused by over active bladder that is refractory to conservative treatment modalities.

This supported by Peters et al. (2010) who conducted a multicenter, double-blind, randomized, controlled trial comparing the efficacy of percutaneous tibial nerve stimulation to sham through 12 weeks of therapy. The improvement in global response assessment, voiding diary parameters, and overactive bladder and quality of life questionnaires was detected with significant improvement in bladder symptoms where 54.5% of patients reported moderately or markedly improved responses [13].

PTNS produces improvement in bladder overactivity, voiding frequency and bladder capacity by urodynamic evidence [14].

Capitanucci et al. (2009), evaluated the efficacy of percutaneous tibial nerve stimulation for different types of pediatric lower urinary tract dysfunction in 14 children with idiopathic overactive bladder, 14 with dysfunctional voiding, 5 with underactive bladder, 4 with underactive valve bladder and 7 with neurogenic

bladder resistant to conventional therapy. Follow up data at one and 2 years were compared with those obtained after stimulation. The investigators found that percutaneous tibial nerve stimulation is reliable and effective for non-neurogenic, refractory lower urinary tract dysfunction in children. Efficacy seemed better in dysfunctional voiding than in overactive bladder cases [15].

The application of PTNS could not abolish DO (detrusor instability). PTNS increased cystometric capacity and delayed the onset of DO. Cystometry seemed useful to select good candidate's patients without DO or with late DO onset showed to be the best candidates for PTNS [16].

In our results there was a highly statistical significant difference (P<0.05) in the mean value of overactivity in patients treated with SSTES and PTN electrical stimulation when compared with the value before initiation of treatment with an improvement of 48.69%. No significant improvement was observed for patients treated with pelvic floor exercise alone. Maximum flow rate significantly improved (P<0.05) post-treatment with neuromodulation with 25.2%, as well as for pelvic floor exercise group with 12.37%, improvement. By comparing both groups post treatment there was a statistical significant (P<0.05) improvement in neuromodulation group more than in pelvic floor exercises group.

Objective results based on frequency volume charts, voided volume, number of leakage episodes, incontinence severity, number of pads used and quality of life was reported after application of PTNS[11,14,17,18,19]. Also improvement in maximum flow rate, detrusor pressure at maximal flow, cystometric residual volume and bladder indices[20].

In a study including 10 women and five men (mean age, 60 years) with chronic pelvic pain and urinary symptoms who had failed other therapies, after 12 weekly PTNS treatments, mean visual analogue scale score for urgency changed from 4.5±1.0 at baseline to 2.7±0.7 (P<0.05). Mean visual analogue score for pain

decreased from 8.1±0.2 at baseline to 4.1±0.6 after 12 weeks of treatment (P<0.01). They found no statistically significant changes in the number of voids or bladder volume from baseline after treatment [21].

Patients with overactive bladder symptoms (urgency, frequency and urge-incontinence) after PTNS had a good results and urodynamics parameters were improved after treatment and proved statistically significant decrease in leakage episodes and frequency[16,22].

Of the 17 children treated with superficial sacral stimulation and biofeedback there was complete improvement of symptoms in 10, significant improvement in two and mild improvement in five. Six children who had no resolution of symptoms after biofeedback had salvage therapy with electrical stimulation, after which four had complete improvement of symptoms, and two a 90% and 40% improvement, respectively. Taking the two groups together, after treatment, four children developed isolated episodes of urinary tract infection. Of 21 children with nocturnal enuresis, bed-wetting continued in 13 (62%) after treatment [23].

Parasacral TENS has been shown to be more effective in randomized trials in treating LUTD not related to congenital abnormalities or neurological disease. This deserves further research to elucidate the optimal parameters and the children for whom it is most useful [23].

Peripheral nerve stimulation produce a statistical significant improvement in lower urinary tract symptoms specially day time and night time voiding frequency and volume, leakage episodes [24].

Conclusion

Combined posterior tibial nerve (PTN) with sacral surface therapeutic electrical stimulation (SSTES) is an innovative non-invasive trend in the treatment of overactive bladder and urge-incontinence). It produced objective improvements including urodynamics changes especially in bladder overactivity, bladder maximum cyctometric capacity and maximum flow rate.

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DUAL EFFECT OF SACRAL NEUROMODULATION AND POSTERIOR TIBIAL NERVE STIMULATION IN TREATMENT OF URGE INCONTINENCE DUE TO OVERACTIVE BLADDER

Basant M. Elnady MD, Shabieb A. Abdelbaky MD and Dalia Desouky MD

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IL18 GENE PROMOTER -607A/C AND -137G/C POLYMORPHISMS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Gehan G. El-Olemy MD, Eman R. Abdelgwad MD*, Yasser M. Ismail MD* and Abeer A. Aboelazm MD**

Departments of Rheumatology & Rehabilitiation,
Clinical and Chemical pathology and Microbiology and Immunology***
Benha University, Faculty of Medicine, Egypt

Abstract

Objective: *To analyze the association of IL18 promoter gene polymorphisms (-607A/C and -137C/G) with disease susceptibility and manifestations of SLE patients.*

Methods: *Forty seven SLE patients and 50 unrelated healthy subjets (a control group) were included. All SLE patients underwent thorough clinical examination and SLE disease activity assessement using SLE-DAI. The IL18 polymorphisms were genotyped by PCR amplification and RFLP analysis while, IL- 18 plasma levels were determined by ELISA for both patients and controls.*

Results: *Our data indicated that the frequency of genotype – 607/AC (P=0.04, OR: 2.58, 95% CI: 1.01-6.6) was significantly increased and the frequency of genotype -137 / CC (P=0.049, OR: 0.27, 95% CI: 0.07-1.06) was significantly decreased in SLE patients. In addition, CC (- 607) genotype frequency was significantly increased in patients with serositis (X^2=6.75, P=0.03) while genotype - 137/GG was significantly increased in patients with arthritis/arthralgia (X^2=7.06, P=0.029). Significantly elevated levels of plasma IL-18 were found in patients compared to controls (P=0.002) with significant correlation with disease activity (p <0.001). Patients with AC and CC (-607), and GC and GC (-137) genotypes have significantly higher IL-18 levels than those with AA and CC genotypes (P<0.001). Significantly higher IL-18 levels were found in control subjects with AC and CC (-607) genotypes*

(P=0.014).

Conclusion: *Our results have provided evidence that IL18 promoter gene polymorphisms at position -607 and -137contribute to genetic background of SLE susceptibility and presentation as well as enhanced production of IL-18 protein in SLE patients.*

Keywords: *Gene polymorphisms, Single nucleotide polymorphisms,*

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune connective tissue disorder characterized by loss of self - tolerance causing immune-mediated tissue destruction and various clinical presentations [1].

A large body of evidence suggests that dysregulation of the production of various inflammatory cytokines plays an important role in the downstream pathological symptoms of the disease [2].

Abnormal Th1 and Th2 cytokines profiles might be involved in the pathogenesis of the disease. Peripheral blood mononuclear cells of SLE patients show decreased production of the Th1 cytokines IL-2, IFN-γ, TNF-α and IL-12 and upregulation of the Th2 cytokines IL-4 and IL-10 [3].

Interleukin-18 (IL-18), member of the IL-1 cytokine family,was previously characterised as a Th1 cytokine because of its property in inducing IFN-γ [4]. Later, it has been shown to be a pleiotropic cytokine that can mediate both Th1 and Th2 driven immune responses [5]. In combination with IL-12, IL-18 induces IFN-γ production in Th1 cells, B cells and natural killer cells, promoting Th1 - type immune responses [6], but it can also stimulate Th2 immune responses in the absence of IL-12 [7]. Abnormalities in the production of Th1 and Th2 cytokines have been shown in SLE patients [8].

Because of its multiple functions in cytokine networks, IL-18 is likely to participate in the development of diseases. Elevated IL-18 levels have been reported in the urine of nephrotic patients, the serum of patients

with multiple sclerosis, SLE, adult - onset Still's disease, type I diabetes mellitus, viral infection, sepsis, allergic and asthma [9, 10].

IL-18 expression is regulated by the IL18 promoter gene [11], which is located within a linkage region of SLE on chromosome 11 at 11q22.2–q22 [12].

Polymorphisms in this gene could result in the imbalance of the immune response, which is a characteristic found in SLE [13].

Two single nucleotide polymorphisms (SNPs) at position -607 and -137 in the promoter gene region have been considered to be significant because the change from cytosine to adenine (C to A) at position -607 disrupts a potential cAMP-responsive element binding (CREB) protein-binding site and a change at position -137 from guanine to cytosine (G to C) changes the H4TF-1 nuclear factor binding site to a binding site for an unknown factor found in the GM-CSF promoter [14].

Therefore, we analyzed the association of IL18 promoter gene polymorphisms (-607A/C and -137C/G) with disease susceptibility and manifestations of SLE patients.

Subjects & Methods

- **Patients and Controls**

Forty seven SLE patients were recruited from outpatient clinic and inpatient of Rheumatology and Rehabilitation Department Benha university hospitals. They fulfilled the American College of Rheumatology (ACR) revised criteria for classification of SLE [15]. Fifty unrelated healthy volunteers were recruited from laboratory personnel and nursing staff, who had no history of autoimmune disease, served as controls. Informed consent was obtained from all subjects prior to the study and project approval was granted by the ethics committee of our institution.

All patients included in this study were subjected to full history taking, including ongoing medications. Clinical and serologic features of SLE, such as cutaneous manifestations, arthritis, serositis, kidney disease, neu-

rological manifestations, cardiovascular and cerebrovascular diseases, lymphadenopathy, haematological manifestations (leucopaenia, thrombocytopaenia, anaemia), erythrocyte sedimentation rate (ESR), ANA, anti-ds-DNA, complement C3/C4.

Disease activity was assessed using the SLE Disease Activity Index (SLEDAI) [16]. It is a validated disease activity measure that includes clinical and laboratory measures of SLE activity. The total SLEDAI score range from 0 to 105.

- **Genotyping**

IL18 SNPs analysis was done to all patients and control subjects. SNPs were analysed by PCR amplification and restriction fragment length polymorphism (RFLP) analysis according to [17].

One cc of venous blood was collected from each patient and control subject in EDTA tubes. All blood samples were processed on the same day of collection.

Genomic DNA was extracted from whole blood using Gene JETTM genomic DNA purification kit (Fermentas) according to the manufacturer instructions. Purified DNA samples were stored at -20°C till used in the amplification step. The reaction mixture contains the following: 1 µg of genomic DNA, 25 µL of Dream Tag PCR Mastermix(2X) (Fermentas), 0.5 mM of each primer (Fermentas), water (nuclease free) to a final volume of 50 µL. All reagents were prior vortexed and 25 µL of mineral oil was added to the reaction mixture and carried out in thermal cycler (Biometra, Germany).

IL18 -607C/A polymorphism analysis.

The polymorphism was analysed by PCR amplification and RFLP analysis. A 171 bp PCR amplification fragment was generated using the primers: 5'-GCCCTCTTACCTGAATTTTGGTAG CCCTC-3' (forward) and 5'-AGATTTACTTTTCAGTGGAACAGG AGTCC-3' (reverse). The reaction conditions were as follows: 95°C for 3 min followed by 30 cycles of (95°C for 30 sec, 56°C for 30 sec and 72°C for 1 min) and a

final elongation step carried out at 72°C for 15 min. The product was digested with Tru9I (90 min at 65°C). The -607A allele was cut into two fragments of 101 and 70 bp while the -607C allele remained uncut (171 bp).

IL18 -137G/C polymorphism analysis.

Similarly, the polymorphism at position -137 was analysed by RFLP-PCR using the forward primer : 5'-ATGCTTCTAATGGACTAAGGA-3' and the reverse primer : 5'-GTAATATCACTATTTTCATGAATT-3'. The reaction conditions were as follows : 95°C for 3 min followed by 30 cycles (95°C for 30 sec, 43°C for 30 sec and72°C for 1 min) and a final elongation step carried out at72°C for 15 min. Restriction was performed using EcoRI. The -137G allele was cut into two fragments of 107 and 24 bp while the -137C allele remained uncut (131 bp).

PCR-RFLP products were visualized using 1% agarose gel electrophoresis stained by ethidium bromide and visualized by UV light.

- **ELISA**

The plasma IL-18 levels of 42 SLE patients and 42 control subjects who were randomly selected were measured by enzyme-linked immunosorbent assay (ELISA) using the human bioactive IL-18 ELISA kit (BD Opt EIA) (USA) as described by the manufacturer instructions.

Statistical analysis

The collected data were analysed using SPSS version 16 software, categorical were presented as number and percentages using chi square (X^2) and "Z" tests for their analysis, Odds Ratio (OR) and the corresponding 95% CI were calculated when applicable. Quantitative data were expressed as mean and standard deviation using student "t" test and ANOVA (F) test for their analysis, 2 tailed P value <0.05 was considered significant.

Results

Forty seven SLE patients (age range, 9 to 52 years) and 50 healthy unrelated controls (age range, 26 to 54 years) were studied. The female-to-male ratio in SLE patients and controls were

10.75:1 and 3.2:1, respectively. The study groups' characteristics are shown in Table 1.

Association of IL18 gene polymorphism with SLE susceptibility (Table 2).

- The differences reflect statistically significant increase of genotype - 607/AC (P=0.04, OR: 2.58, 95% CI: 1.01-6.6) and decrease of genotype -137/CC (P=0.049, OR: 0.27, 95% CI: 0.07-1.06) in SLE patients. The genotypes -607/AA and -607/CC were higher in SLE patients, but were found not to be statistically significant (p=0.36 and p=0.34, respectively). For position -137, the genotypes GG and GC showed non significant increase in SLE patients (p=0.49 and p=0.5, respectively) when compared to controls.

- Non significant differences of the A and C alleles at position -607 (p=0.149 and p=0.149, respectively) as well as G and C alleles at position -137 (p=0.52 and p=0.52, respectively) were found between SLE and controls.

Association of IL18 gene polymorphism with clinical manifestations of SLE.

- The association between the clinical features profile of SLE patients with various genotypes and alleles at -607 and -137 positions was analyzed and the result was shown in Tables 3 and 4. There were significant associations between genotype - 607/AC with serositis (X^2=6.75, P=0.03) and genotype - 137/GG with arthritis/ arthralgia (X^2=7.06, P=0.029).

Plasma IL-18 levels and genotypes at SNP -607 and -137 (Table 5).

- In the patients group, the plasma level of IL-18 in patients who had AA genotype at position -607 was 124.2 ± 32.2 (mean ± SD) pg/mL, while its level for patients who had AC genotype was 295.2 ± 69.3 pg/mL. The patients with CC genotype had a level of 284.9 ± 66.8 pg/mL. Significant differences were observed among the 3 subgroups (F= 33.02, P <0.001). Also, patients with GG and GC genotypes at SNP -137 had significantly higher IL-18 plasma levels (296.8 ± 67.2 and 306.1 ± 77.5,

respectively) than those with CC genotype (79.3 ± 24.4), F=107.7 and P <0.001.

- In the control group, subjects with AA genotype at SNP -607 had significantly lower IL-18 plasma level when compared to subjects with AC and CC genotypes (F=4.66, P =0.014). The means for the 3 subgroups were 59.6 pg/mL, 115.7 pg/mL and 121.2 pg/mL respectively. Non significant differences were observed among patients with GG, GC or CC genotypes at SNP -137 (F=0.66, P=0.52).

- On the other hand , patients had significantly higher plasma IL-18 levels (283.5 ± 61.2 pg/mL) than controls (83 ± 24.3 pg/mL), p=0.002 (Fig. 1). Furthermore, a significant positive correlation between IL-18 plasma levels and SLE disease activity (SLEDAI score) were found, where r=0.64 and p <0.001.

Table 1: Characteristics of SLE patients and controls.

	SLE patients (n = 47)	**Controls (n = 50)**
Sex ,female / male	43 / 4	38 / 12
Age (years) Range mean±SD	 9 – 52 28 ± 7	 26 -54 31 ± 5
Disease duration(years) Range mean±SD	 0-16 7± 3.5	 NA
Mucocutaneous features, no(%)	34 (72.34)	NA
Serositis, no (%)	23 (48.93)	NA
Arthritis/arthralgia, no (%)	36 (76.59)	NA
Renal features, no (%)	27 (57.44)	NA
CNS features , no (%)	12 (25.53)	NA
Hematologic features, no (%)	27 (57.44)	NA
SLEDAI Range mean±SD	 5-26 15.7 ± 4.9	 NA
ANA , +ve no (%)	43 (91.48)	NA
Anti-dsDNA, +ve no (%)	42 (89.36)	NA
↓C3, no (%)	17 (36.17)	NA
↓C4, n (%)	13 (27.65)	NA

CNS; Central nervous system. ANA; Antinuclear antibodies.
Anti-dsDNA; Anti double stranded DNA. C (3 & 4); Complement (3 & 4).
N; number of patients. NA; Non applicable.

Table 2 : Distribution of *IL18* genotypes and alleles frequencies in SLE patients and controls.

Genotypes		Controls (n=50) n (%)	SLE patients (n=47) n (%)	OR	95% Confidence interval(CI) of OR	P
IL18-607A/C	AA	17 (34)	12 (25.5)	0.66	0.28-1.6	0.36
	AC	9 (18)	17 (36.17)	2.58	1.01-6.6	0.04*
	CC	24 (48)	18 (38.29)	0.67	0.3-1.5	0.34
	A allele	54 (54)	41 (43.6)	0.66	0.37-1.2	0.149
	C allele	46 (46)	53 (56.4)	1.5	0.86-2.7	0.149
IL18-137G/C	GG	21 (42)	23 (48.9)	1.3	0.59-2.9	0.49
	GC	19 (38)	21 (44.7)	1.3	0.59-2.96	0.5
	CC	10 (20)	3 (6.4)	0.27	0.07-1.06	0.049*
	G allele	66 (66)	66 (72.8)	1.2	0.66-2.2	0.52
	C allele	34 (34)	28(27.2)	0.82	0.45-1.5	0.52

OR ; odds ratio. CI; Confidence interval.
n; number of patients. *= Significant (P<0.05).

Table (3): The association of polymorphism *IL18* 607A/C with clinical manifestations in SLE patients.

Clinical features (n)	Genotype distribution			X^2	P
	AA(n=12) n (%)	AC(n=17) n (%)	CC(n=18) n (%)		
Mucocutaneous (34)	9 (75)	11 (64.7)	14 (77.8)	0.8	0.67
Serositis (23)	5 (41.7)	5 (29.4)	13 (72.2)	6.75	0.03*
Arthritis/arthralgia (36)	9 (75)	15 (88.3)	12 (66.7)	2.3	0.32
Renal(27)	7 (58.3)	10 (58.8)	10 (55.5)	0.04	0.98
CNS (12)	3 (25)	5 (29.4)	4 (22.2)	0.24	0.887
Hematologic(27)	8 (66.7)	9 (52.9)	10 (55.5)	0.58	0.75
Immunologic(43)	11 (91.7)	16 (94.1)	16 (88.9)	0.31	0.86

CNS; Central nervous system. n; number of patients.
*= Significant (P < 0.05).

Table (4): The association of polymorphism *IL18* 137G/C with clinical manifestations in patients with SLE.

Clinical features(n)	Genotype distribution			X^2	P
	GG(n=23) n (%)	GC(n=21) n (%)	CC(n=3) n (%)		
Mucocutaneous (34)	17 (73.9)	14 (66.7)	3 (100)	1.5	0.47
Serositis (23)	11 (47.8)	10 (47.6)	2 (66.7)	0.4	0.82
Arthritis/arthralgia (36)	21 (91.3)	14 (66.7)	1 (33.3)	7.06	0.029*
Renal(27)	12 (52.2)	13 (61.9)	2 (66.7)	0.53	0.76
CNS (12)	5 (21.7)	6 (28.6)	1 (33.3)	0.37	0.83
Hematologic (27)	13 (56.5)	12 (57.1)	2 (66.7)	0.11	0.94
Immunologic (43)	21 (91.3)	19 (90.5)	3 (100)	0.31	0.85

CNS; Central nervous system. n; number of patients.
*= Significant (P < 0.05).

Table (5): Comparison between plasma IL-18 levels among its promoter gene polymorphisms at position –607 and –137 in SLE patients and controls.

	Position –607			Position –137		
	Genotype(n)	Mean ± SD (pg/mL)	P	Genotype(n)	Mean ± SD (pg/mL)	P
Patients	AA(12)	124.2 ± 32.2	<0.001*	GG(23)	296.8 ± 67.2	<0.001*
	AC(17)	295.2 ± 69.3		GC(21)	306.1 ± 77.5	
	CC(18)	284.9± 66.8		CC(3)	79.3 ±24.4	
Controls	AA(17)	59.6 ± 26.8	0.014*	GG(21)	78.3 ± 27.6	0.52
	AC(9)	115.7 ± 78.4		GC(19)	83.2 ± 38.9	
	CC(24)	121.2 ± 79.3		CC(10)	92.6 ± 27.5	

n; number of patients. *= Significant (P<0.05).

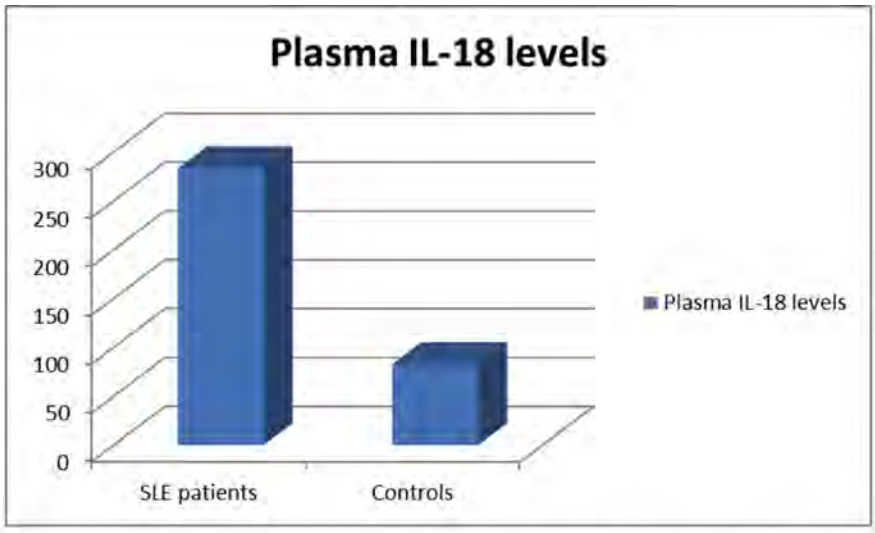

Fig. (1): Plasma IL-18 levels in SLE patients and controls.

Discussion

Many studies have examined the relationship between cytokine gene polymorphisms and the incidence of SLE, including IL-4, IL-6, IL-10, TNF-α and IFN-γ receptor [18. 19. 20. 21].

Owing to IL-18's well-established role in inflammatory and autoimmune processes [22] and that IL18 gene variation may influence IL-18 production, we conducted this study with the aim of discovering variants that may impact on SLE disease susceptibility as well as clinical presentation.

We found the genotype SNP-607/AC to be significantly higher in SLE patients when compared to control subjects. A significant decrease of genotype CC at position -137 was also observed in SLE patients compared to controls.

In the same context, [23], studied IL18 polymorphisms at position -656, -607 and -137 in a cohort of taiwan Chinese SLE patients, providing evidence for genetic association conferred by these polymorphisms with the clinical features of the disease.

Sánchez et al (2009)[24], reported an association between a putative functional genetic variant in the promoter region of IL18 (rs360719) and systemic lupus erythematosus in Spanish patients. Also, in the same study, they found an increase in the relative expression of IL18 mRNA in individuals with the rs360719 lupus-risk allele.

These results do not agree with those of [9] who reported that 607 CC and -137 CC genotypes of IL18 gene were associated with SLE susceptibility in Singapore Chinese.

Htoon et al. (2011)[24], found a genetic association between IL18 and Behçet's Disease but not lupus in turkish patients.

For position -137, a change from G to C changes the H4TF-1 nuclear factor-binding site to a binding site for an unknown factor found in the GM-CSF promoter [9. 14], postulated that because H4TF-1 nuclear factor would not bind to C allele of posi-

tion -137, the SNP-137/C allele would have lower IL-18 mRNA expression.

Our result suggests that CC genotype of IL18 (-607) is a risk for serositis and GG genotype (-137) is a risk for arthritis/ arthralgia.

However, our results are contradictory to those of [26], who did not find positive association between polymorphism in IL12 and IL18 genes and susceptibility to SLE in Thais. However, when they analyzed polymorphism of the IL12 and IL18 genes with clinical manifestations, they found significant association of the C allele of IL18 (-137) with arthritis.

Warchol et al. (2009)[27], did not observe differences in the distribution of the IL18 105 A/C polymorphism between patients with SLE and controls. Although, they found a significant association of renal symptoms with the IL18 105 AA genotype.

Takada et al. (2002)[28], reported that in patients with sarcoidosis, the C allele of IL18 at position -607 was a risk factor for sarcoidosis in the japanese population. In RA patients [29], found that the A allele at position -607, in the homozygous state, has a protective effect against the development of RA.

These large contradictions could be explained by the small samples used, the different diseases analyzed and the ethnic groups investigated.

In the same context, Love [30], postulated that the variability of the influence of the IL18 polymorphism on SLE manifestation and incidence in different populations can be due to genetic heterogeneity, which usually confounds the study of multigenic disorders. Furthermore, various environmental factors together with genetic heterogeneity may also modulate the effect of the IL-18 polymorphism on clinical manifestations.

Several functional studies have shown that the level of IL-18 production is related to the IL18 promoter gene [14]&[31].

In the present study, SLE patients had significantly higher plasma IL-18 levels that correlated well with disease activity. Our results are in line with two independent studies that have shown higher IL-18 levels in SLE patients than in control subjects, and a significant positive correlation with SLEDAI score [32, 33].

We demonstrated significantly higher IL-18 levels in patients with AC and CC (-607), and GC and GC (-137) genotypes.

Our results coincide with those of [9], who demonstrated significantly higher IL-18 levels associated with AC and CC genotypes at position -607 of IL-18 promoter gene in both SLE patients and controls.

The deficiency in enhanced gene transcription will be beneficial for the individual, as elevated levels of the proinflammatory IL-18 protein mediate many of the acute and chronic inflammatory processes. Indeed, studies in anti-cytokine therapy including TNF, IL-6, IL-18 and IFN-γ in SLE have become an important issue in preventing from tissue damage[34].

Conclusion

Our results have provided evidence that IL18 promoter gene polymorphisms at position -607 and -137 contribute to genetic background of SLE susceptibility and presentation as well as enhanced production of IL-18 protein in SLE patients.

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IL18 GENE PROMOTER -607A/C AND -137G/C POLYMORPHISMS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Gehan G. El-Olemy MD, Eman R. Abdelgwad MD, Yasser M. Ismail MD and Abeer A. Aboelazm MD

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BONE BLOCK OPERATION AFTER FAILED ARTHROSCOPIC REPAIR OF RECURRENT SHOULDER DISLOCATION

Abed A. El-Negery MD

Department of Orthopedic Surgery,
Mansoura University, Egypt

Abstract

Shoulder dislocation is common problem in orthopaedics. Management of recurrent shoulder dislocation, with different surgical procedures, having varying outcomes. Despite advances in arthroscopic techniques and implants for shoulder instability repair, the failure rate is still between 5-30%, resulting in a loss of functional performance of athletic and other shoulder activities. In general, failure of shoulder instability can be divided into failures from recurrence of instability, failure from postoperative stiffness, and failure from persistent pain. Each of these may occur individually or be part of a spectrum of issues surrounding the failed instability repair. Bone block operation was done and followed up for average of 24 months. Functional evaluation was done using Rowe score and graded as excellent, good, fair and poor. Bone block operation can be the procedure of choice for surgical treatment of recurrent traumatic anterior dislocation after arthroscopic repair and also as a salvage surgery for failed cases from other types of procedures. The only shortcoming of this procedure was some limitation in external rotation and minor loss of muscle power of that shoulder.

Key words: *Recurrent shoulder dislocation, Rowe score, Bone block operation.*

Introduction

The shoulder is the most commonly dislocated major joint, with a reported incidence of 1.7%. Symptomatic instability following dislocation is common, especially in young, active people. Recurrent instability, occurring in 50% to 96% of patients who first dislocate under the age of 20 years and in

40% to 74% of patients between the ages of 20 and 40 years, limits range of movement of the joint, requires multiple hospital and emergency department admissions for treatment, and often calls for surgical procedures to prevent further dislocation [1].

Abduction and external rotation of the shoulder can cause anterior subluxation or dislocation, resulting in anterior instability. Traumatic dislocation of the gleno-humeral joint frequently requires surgical repair [2].

Despite major advances, arthroscopic stabilization techniques are still associated with higher failure rates than traditional open procedures, especially in young adults (figure 1). Such failures are reflected in anterior shoulder pain with activities of daily living, which becomes dramatically apparent when the shoulder re-dislocates [2].

Re-dislocation after arthroscopic repair (table 1) has variously been attributed to the type and extent of the capsular lesion, chondral and bone defects, technical errors, insufficient of soft-tissue tensioning, an unhealed Bankart or anterior labro - ligamentous periosteal sleeve avulsion (ALPSA) lesion, failure of surgical devices, patient age and sex, family history, bone quality, patient maturity and self-control, participation in highly demanding sports or activities, the number of previous dislocations, the type of immobilization, the rehabilitation program, and altered scapular kinematics [2].

Persistent or recurrent glenohumeral instability after a previous arthroscopic stabilization has been reported to be caused by initial misdiagnosis of concomitant pathology in up to 84% of revision cases. This further emphasizes the importance of proper assessment of a patient with shoulder instability, especially the failure to diagnose potential underlying pathology [3].

The diagnosis of failed shoulder instability procedure begins with appropriate diagnosis and determination of mode of failure. Thus, a thorough history and complete physical examination

are essential to plan for potential future surgical intervention. Several key issues in the patient history are important, including a potential mechanism of re-injury, previous surgical treatment of the shoulder, and whether the previous shoulder stabilization ever relieved the patient's symptoms[4].

More than 150 operations have been described. Transfer of the tip of coracoid process to the anterior margin of the glenoid was first described by Laterjet for treatment of recurrent dislocation of the shoulder. Helfet used sutures to hold the coracoid tip. Mead screwed the coracoid to the anterior glenoid rim [4].

There are several reports on the Laterjet - Bristow procedure and its modification. All of them reported low incidence of re-dislocations and other complications but many reported about limitation in external rotation. A 20 years follow up study from Middlesex Hospital, London, showed that in provide good long-term shoulder stability [5].

Material and Methods

This is a prospective study carried out on patients with recurrent shoulder dislocation after arthroscopic repair. Twenty seven patients were operated between June 2007 and September 2009. They were followed up and evaluated for various periods.

Approval and consent had been taken from the patients after explaining the procedure. Deltopectoral approach was used for all cases. Cephalic vein served as a landmark for plane of dissection (figure 2) to separate and retract Deltoid and Pectoralis major on either side to expose the Coracoid process with its attached conjoint tendons of Coracobrachialis and short head of Biceps (figure 2). Coracoid process then was osteotomised just distal to the insertion of Pectoralis minor without predrilling. It was reflected down to expose the Subscapularis muscle. This muscle was then split along its fibers at junction of its mid and lower third to expose the joint capsule.

The capsule was opened along the same line to inspect the joint

cavity for any loose bodies, or any co-existing intra-articular pathology. While the joint lay open Glenoid margin was palpated to find and confirm the desired location of the screw placement. With this guide Scapular neck was drilled just below the transverse equator within one cm of the rim of Glenoid with a 3.2 mm drill bit.

The joint capsule would then be closed. Next the coracoid process was also drilled with same drill bit. A Malleolar screw was used to fix the coracoid tip to the Scapular neck through the Subscapularis split (figure 3). Closure of the wound was affected by letting separated muscles fall back and stitching the subcutaneous tissue, skin over a suction drain. Postoperatively the shoulder was then immobilized in an immobilizer for one week. Then after an arm sling was worn for another four weeks.

Then the sling was removed and pendulum exercise of the shoulder was begun. Active elbow flexion/extension was allowed only after six weeks to prevent pull out of the screw fixation. However passive flexion of the elbow was encouraged. Shoulder muscle strengthening and stretching exercises were started only after three months and continued for one-year. All activities including lifting weights were allowed after six months.

Follow up of all patients were carried out at one week for change of support, at two weeks for removal of stitches and after four weeks for removal of support and commencement of pendulum exercises. Further follow up was carried out at three months intervals for any problems (figure 4).

Antero-posterior and axillary view x-rays were taken at six weeks, three months and six months to see state of screw after the first postoperative check x-ray (Figure 5). Union of the coracoids with scapula is difficult to make out. However any pull out or loosening of the screw, if any, can be seen.

Muscle power was measured for shoulder abductors, external rotators and internal rotators according to medical research coun-

cil (MRC) grading and compared with the non-operated side. Range of motion was also noted in all directions. Function was assessed using the Rowe scoring system (table 2)[6]. It evaluated the over a success of the stabilizing procedure since half of the points were given for stability. Other points were given for range of movements and functional results.

Results

There were 21 men and 6 women. They were aged from 16 years to 35 years. 24 patients were right handed and remaining 3 left handed. All dislocations were unilateral. There were 18 right sided and 9 left sided dislocations. All patient were previously treated by arthoscopy with and without anchors.

At operation there were intra-articular pathology (bony defect) in 24 patients, and loose body (bone fragment and lose anchors) was detected in 18 of the cases. There was no radiological evidence of loosening, migration or fracture of the coracoid screw nor resorption of the coracoid tip on subsequent check x-rays. Dislocation did not occur again in any of the cases.

At twelve months, 9 patients had fair Rowe score, seven patients attained more than 75 (Good) Rowe scoring and 11 patient excellent while at two years three had fair Rowe score, 5 had good Rowe score and 19 patients had excellent Rowe score (> 90). Only three patients had poor score at one year while none had poor score at two years.

The mean loss of external rotation in operated shoulder at one year was 15 degrees which decreased to 10 degrees at the end of two years. There was no restriction of external rotation in three patients at both yearly follow-up while 18 patients had restriction in the range 10¬-20 degrees.

There were four superficial infections, no deep infection. All of them resolved with appropriate treatment with antibiotics and dressings. There was one case of operation scar hypertrophy.

TABLE 1: Common causes of failure of arthroscopic instability repair [2]
1. *Age.* 2. *Activity level* 3. *Capsular integrity* 4. *Voluntary component* 5. *Failure of recognize pathological lesion* 6. *Technical inadequacies* 7. *Bony loss (glenoid, humeral head)* 8. *Inappropriate rehabilitation*

Table 2: Rowe Scoring System

Section 1 – Stability	
1.No Recurrence, subluxation or apprehension	(50)
2.Apprehension when placing arm in certain positions	(30)
3.Subluxation (not requiring reduction)	(10)
4.Recurrent Dislocation	(0)
Section 2 – Motion	
5.100% of normal ext rotation, int rotation and elevation	(20)
6.75% of normal ext rotation, int rotation and elevation	(15)
7.50% of normal ext rotation, int rotation and elevation	(5)
8.50% of normal elevation, int rotation, No ext rotation	(0)
Section 3 – Function	
9.No limitation of work or sports, little or no discomfort (eg shoulder strong overhead, lifting, swimming, throwing) (30)	
10. Mild limitation and minimum discomfort	(25)
11. Moderate limitation and discomfort	(10)
12. Marked limitation and pain	(0)
Interpreting the Rowe Score for Instability	
13. (100 – 90) Excellent.	- (89 – 75) Good.
14. (74 – 51) Fair.	- (50 or less) Poor.

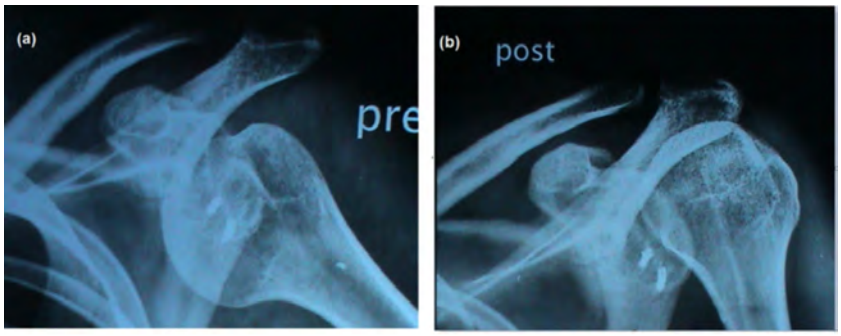

Figure (1): Failed arthroscopic manegment of instability, a. dislocated shoulder, b. relocated shoulder. Note the anchors in the Glenoid

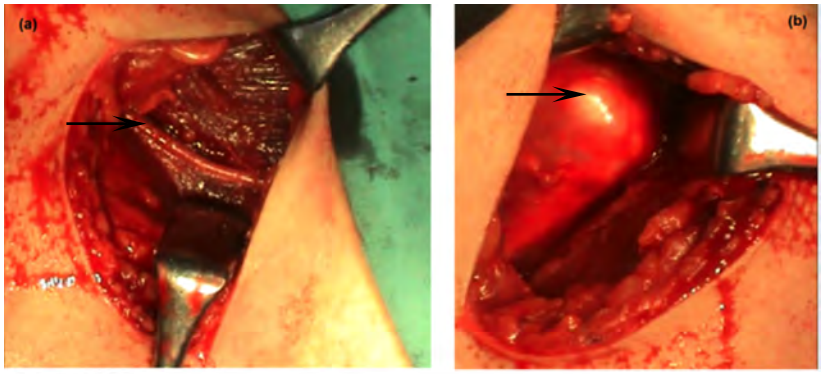

Figure (2):
a. Deltopectoral approach (arrow on cephalic vein)
b. Coracoid process (arrow) and the muscels attatched to it.

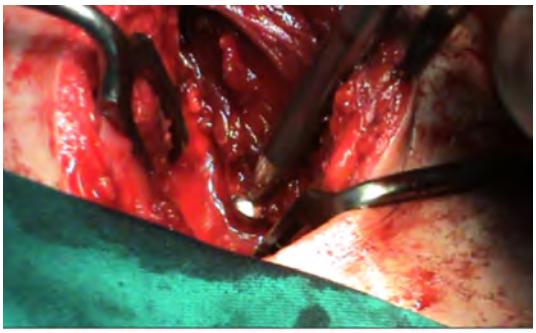

Figure (3): Malleolar screw fix the coracoid in the glenoid

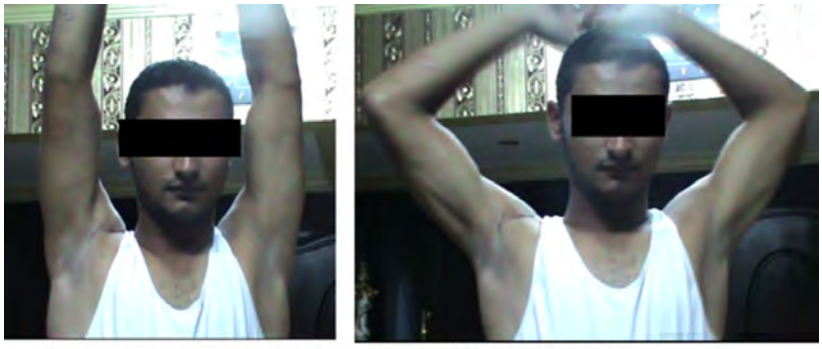

Figure (4): 3months postoperative follow up (arrow shows the operational scar)

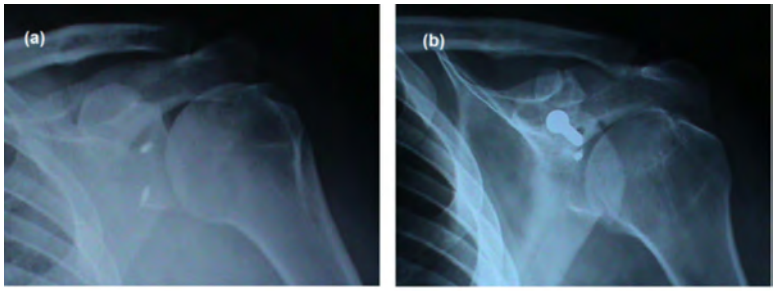

Figure (5): a. preoperative X-ray of shoulder instability previously treated by arthroscope (2 anchors)
b. postoperative X-ray (the screw in glenoid and one anchor remaining)

Discussion

Recurrence of dislocation in shoulder reaches up to 80-¬90% in young adults and are disabling. As 97% of shoulder dislocations are anterior, recurrent dislocations are also usually anterior. No single patho-anatomy is responsible for it. Surgery is the treatment of choice [1].

More than 150 operations and many modifications have been put forward for it but no single procedure is yet considered to be ideal. Although ability to detect specific pathology can lead a surgeon to correct it, choice of operation in practice is dependent upon preference of the surgeons [5].

Laterjet, Helfet and Mead were the pioneers in popularizing the coracoid transposition. The trans-

ferred conjoined tendon acts as a dynamic sling preventing forward and downward movement of the humeral head when arm is abducted [7].

During abduction of the shoulder this transfer also prevents the lower half of the subscapularis muscle to slip superiorly, and is rather slipped inferiorly to supports the humeral head inferiorly [8].

The Coracoid's piece also acts as a bone block anteriorly. Because of these effects this procedure has a good restraining to stop recurrence of the humeral head dislocation anteriorly regardless of pathology of recurrence. Thus this procedure is effective even after failure of other soft tissue procedure [9].

Much has been written in the literature about this procedure with both mid-term and long term follow¬ ups. All of them have shown satisfactory results with very few recurrences. But most of them uniformly had some degrees of limitation in external rotation of the shoulder. Few of the patients had bone and screw related complications [10].

Singer, GS et al. reported 20 year follow up of this procedure in 14 cases. One patient had recurrence; 12 had restriction of external rotation; the functional outcome by Row scoring was satisfactory but there were radiological degenerative changes in 10 shoulders. In this series, the longest follow up was two years[10,11].

No one had any re-dislocation so far. Eighteen patients had varying degrees of restriction in external rotation. Functional outcome based on Rowe scoring was excellent in 19 patients, fair in 3 patients and good in 5 patients while none patients had poor Rowe score.

Hovelius et al reported a 16% incidence of screw migration, a 14% incidence of fracture at surgery of the coracoid tip and a 28% incidence of fibrous union of the transplanted coracoid. In our series we did not encountered such complications [12].

Subsequent X-rays did not

reveal any screw pull outs. In all of the cases the coracoids process could be seen continuous with the scapular neck meaning union. Hence there were no screw related complications encountered in this series.

No radiological features of degenerative changes in the shoulder joint on subsequent X-rays.

Conclusion

Coracoid transposition following course of the conjoint tendon of short head of Biceps and Coraco-Brachialis provides mechanical block and dynamic support in positions of vulnerability thus providing good stability to the Gleno-Humeral joint. So it can be the procedure of choice for surgical treatment of recurrent post-arthroscopic anterior dislocation and also as a salvage surgery for failed cases from other types of procedures. The only shortcoming of this procedure was some limitation in external rotation.

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El-Negery A. Abed MD

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milligram (s)	mg	second (s)	S
microgram (s)	ug	centimeter (s)	cm
nanogram (s)	ng	cubic millimeter	cmm
micrometer	um	millilitre (s)	ml
millicurie(s)	mCi	milliequivalent	mEq
molar	mol/l	millimole	mmol

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