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# SELECTIVE LASER TRABECULOPLASTY AS INITIAL AND ADJUNCTIVE TREATMENT FOR OPEN ANGLE GLAUCOMA

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## Abstract

***Purpose :*** *To evaluate the efficacy of selective laser trabeculoplasty when used as an initial or adjunctive treatment in primary open angle glaucoma (POAG).*

***Methods :*** *The study included 74 eyes in 37 patients with POAG. They were grouped into 3 groups. Group I newly diagnosed eyes with POAG (Primary treatment group). Group II patients diagnosed with POAG currently on medical treatment, either intolerant of medical treatment or not well controlled to the target level (secondary treatment group). Group III control subjects: Patients with POAG with controlled IOP on prostaglandins analogues eye drops. SLT with 360$^{o}$ treatment was done in Group I and Group II.*

***Results :*** *In group I, the mean IOP was decreased from 25.5 ± 1.24 mmHg before treatment to 17.95 ± 1.23 mmHg in the last follow up visit. The average absolute reduction of IOP was 7.41 ± 0.839 mmHg. The average percent reduction of IOP was 29.8%. In group II ,the mean IOP was decreased from 21.32 ± 2.23 mmHg before treatment to 17.38 ± 1.56 mmHg in the last follow up visit.The average absolute reduction of IOP was 3.94 ± 0.7 mmHg.The average percent reduction of IOP in this group was 18.01%.*

***Conclusion :*** *SLT is nearly as effective as prostaglandins analogues, and proved a greater efficacy in IOP lowering when used as initial therapy for newly diagnosed eyes with POAG than when used as adjunctive therapy for eyes taking antiglaucoma medications with low baseline IOP.*

## Introduction

Laser trabeculoplasty (LTP) has been used as initial, adjunct, or replacement therapy to lower intraocular pressure (IOP) in patients with open angle glaucoma for 35 years ago.1 The original procedure was described using argon laser. However, argon laser trabeculoplasty (ALT) was found to produce significant tissue disruption and coagulative damage to the trabecular meshwork.2 Furthermore, a membrane formed by the migrating endothelial cells was noted on the meshwork between the laser spots was hypothesized to be responsible for the late IOP rise after ALT.3,4

In the recent years, selective laser trabeculoplasty (SLT) using Q-switched 532 nm frequency doubled Nd-YAG laser has been shown as a safe & effective therapy in reducing IOP in open angle glaucoma.5

SLT is a non thermal procedure, with short pulse and low energy. It stimulates many trabecular cells through selective light absorption and selective photothermolysis of pigmented trabecular cells, without causing any collateral structural damage to the trabecular meshwork.6,7 Ultrastructural analysis after SLT showed that it spares adjacent tissues from thermal damage, with no coaugulative damage or alteration of the collagen structure of the trabecular meshwork.8

## Patients and Methods

This prospective study was carried out on 74 eyes in 37 patients with primary open angle glaucoma attended to the outpatient clinic of Mansoura Ophthalmology center. They were grouped into 3 groups.

- **Group I:** newly diagnosed eyes with POAG (Primary treatment group).

- **Group II:** patients diagnosed with POAG currently on medical treatment, either intolerant of medical treatment or not well controlled to the target level (secondary treatment group).

- **Group III:** control subjects: Patients with POAG with controlled IOP on prostaglandins analogues eye drops.

SLT with 360º treatment was done in Group I and Group II.

**Exclusion criteria:**

1. Younger age than 18 years.
2. Any mental impairment that prevent the patient from understanding the protocol.
3. Patients with corneal disease where applanation would be inaccurate or the TM would not be adequately visible with gonioscopy.
4. Severe advanced field defect.
5. Other glaucoma than POAG.
6. Previous glaucoma operation.
7. Patients with ocular conditions which impair visualization of the trabecular meshwork.
8. Pregnant females.

All patients underwent a detailed examination before laser treatment. This include a record of the best corrected visual acuity, slit lamp biomicroscopy of the anterior segment of the eye, IOP measurement by Goldmann applanation tonometer, gonioscopy with Goldmann three mirror contact lens. The fundus was examined by direct ophthalmoscopy and with Goldman 3- mirror contact lens to assess optic disc changes in glaucoma and to exclude other retinal lesions that may produce visual field defects.

The IOP was measured by applanation tonometer 3 times in 3 different visits 1 week apart before SLT, and the average was used as baseline IOP.

**Technique of SLT:**

**Preoperative medications:**

A drop of topical anaesthetic (Benoxinate 0.2%) was given before application of the goniolens.

**Visualization of the angle:**

It was done with Latina SLT one-mirror lens, coupled with methylcellulose 1%(Thilotears gel).

**Laser parameters:**

**Spot size:** 400µm.

**Duration:** 3 nano sec.

**Power:** start with 0.5 mj.

**SLT technique:**

• The aiming beam was focused on the pigmented trabecular meshwork.

- The laser power was adjusted usually at 0.5 mj, then increased or decreased by 0.1 mj until the small champagne bubbles are just seen.
- Average of 100 shots was done over 360º of the angle (adjacent non-overlapping shots) in all cases.

**Post-SLT care:**

- Topical α-agonist (Brimonidine 0.5%) was used immediately post-SLT to avoid transient post laser IOP spikes.
- Topical steroids (dexamethazone 0.1%) was used for 3 days.
- The patients continued on antiglaucoma treatment for 10 days post SLT.

**Follow up:**

- Slit lamp examination and applanation tonometry was done at 2 days, 1week, 2 weeks, 1 month, and 6 months after SLT
- In the first follow up special attention was given for any sign of anterior chamber reaction or IOP spikes.

## Results

This study was done on 37 patients with open angle glaucoma (23 males, and 14 females) .They were classified into 3 groups:

**Group I.** Newly diagnosed eyes with POAG (Primary treatment group) included 20 eyes in 10 patients (7 males, and 3 females) aged from 42 to 55 with median age 50.40 ± 4.77 years.

**Group II.** Patients diagnosed with POAG currently on medical treatment, either intolerant of medical treatment or not well controlled to the target level (secondary treatment group) included 34 eyes in 17 patients (10 males, and 7 females) aged from 42 to 55 years with median age of 49.76 ± 4.44 years.

**Group III.** Control subjects: Patients with POAG with controlled IOP on prostaglandins analogues eye drops. They include 20 eyes in 10 patients (6 males, 4 females) aged from 42 to 58 years with median age of 49.2 ± 6.4 years.

- In group I, as shown in (table-1) the mean IOP was decreased from 25.5 ± 1.24 mmHg before treatment to

17.95 ± 1.23 mmHg in the last follow up visit. The average absolute reduction of IOP was 7.41 ± 0.839 mmHg. The average percent reduction of IOP was 29.8%.

- In group II , as shown in (table-2) the mean IOP was decreased from 21.32 ± 2.23 mmHg before treatment to 17.38 ± 1.56 mmHg in the last follow up visit. The average absolute reduction of IOP was 3.94 ± 0.7 mmHg. The average percent reduction of IOP in this group was 18.01%.

- In group III, the mean IOP was decreased from 24.85 ± 0.75mmHg before medical treatment to 17.10 ± 0.79 mmHg in the last follow up visit.The average absolute reduction of IOP was 3.94 ± 0.7 mmHg. The average percent reduction of IOP in this group was 29.31%.

6 eyes in 3 patients (30%) in the primary treatment group and 12 eyes in 6 patients (35%) in the secondary treatment group showed increase in IOP around the 3rd month, with the need of one topical glaucoma medication to control the IOP.

No specific complications occurred in the cases, and none of the patients developed any change in the visual field or gonioscopy during follow up period after SLT.

- Conjunctival redness and injection was encountered in 26 eyes and resolved in all of them within 4 days.

- Ocular pain in the first day after SLT was encountered in 10 eyes.

- Mild anterior chamber flare was encountered in 20 eyes and resolved within 2 days.

**Table 1 :** Shows comparison between IOP Pre and post-SLT in the primary treatment group.

| Time interval | IOP (Mean ± SD) | Test of significance |
|---|---|---|
| Pre-SLT | 25.50 ± 1.24 | |
| 1 Day | 9.65 ± 0.99 | t = 71.74<br>P = 0.000 |
| 1 Week | 11.20 ± 2.78 | t = 26.550<br>P = 0.000 |
| 2 Week | 13.45 ± 1.61 | t = 49.03<br>P = 0.000 |
| 1 Month | 14.45 ± 2.04 | t = 43.12<br>P = 0.000 |
| 2 Month | 15.70 ± 2.40 | t = 31.32<br>P = 0.000 |
| 3 Month | 17.15 ± 3.09 | t = 18.14<br>P = 0.000 |
| 4 Month | 16.95 ± 1.39 | t = 36.41<br>P = 0.000 |
| 5 Month | 17.75 ± 1.37 | t = 24.58<br>P = 0.000 |
| 6 Month | 17.95 ± 1.23 | t = 22.99<br>P = 0.000 |

**Table 2 :** Shows comparison between IOP pre and post SLT in the secondary treatment group.

| Time interval | IOP<br>(Mean ± SD) | Test of significance |
|---|---|---|
| Pre-SLT | 21.32 ± 2.23 | |
| 1 Day | 9.79 ± 0.98 | t = 32.58<br>P = 0.000 |
| 1 Week | 12.29 ± 1.24 | t = 31.36<br>P = 0.000 |
| 2 Week | 13.50 ± 1.69 | t = 27.48<br>P = 0.000 |
| 1 Month | 14.85 ± 2.00 | t = 21.84<br>P = 0.000 |
| 2 Month | 15.88 ± 2.37 | t = 13.96<br>P = 0.000 |
| 3 Month | 16.76 ± 2.69 | t = 10.89<br>P = 0.000 |
| 4 Month | 17.29 ± 2.57 | t = 8.73<br>P = 0.000 |
| 5 Month | 17.26 ± 1.58 | t = 12.32<br>P = 0.000 |
| 6 Month | 17.38 ± 1.55 | t = 11.86<br>P = 0.000 |

## Discussion

In the recent years, SLT has been shown as a safe & effective therapy in reducing IOP in open angle glaucoma.9 Although SLT seems to be equivalent to ALT in terms of IOP reduction, it has the advantage of less damage of the trabecular meshwork.10

In this study SLT was performed in 2 groups of patients with POAG, the first group included patients with newly diagnosed POAG (primary treatment group), and the second group included patients diagnosed with POAG currently on medical treatment, either intolerant of medical treatment or not well controlled to the target level (secondary treatment group). After SLT, patients were followed up for at least 6 months for reduction of IOP, and the need of adjuvant medical treatment.

**The first group** (The primary treatment group): The IOP was decreased from 25.5 ± 1.24 mmHg before treatment to 17.95 ± 1.23 mmHg in the last follow up visit. The reduction of IOP was greatest 24 hours after SLT. There was no significant difference compared with the control group, in which the IOP was decreased from 24.85 ± 0.75 mmHg before treatment to 17.10 ± 0.79 mmHg in the last follow up (P=0.297). The average absolute reduction of IOP was 7.41 ± 0.839 mmHg, with no significant difference compared to 7.3 ± 1.218mmHg in the control group (P=0.745). The average percent reduction of IOP was 29.8%, with no significant difference compared with 29.3% in the control group (P=0.699), showing that SLT was nearly as effective as prostaglandins analogues in reduction of IOP. This percent of IOP reduction was nearly similar to Melamed, et al. 2003 (11) who reported 30% drop of IOP (7.7mmHg) after SLT. McIlraith, et al. 2006 (12) reported that mean absolute reduction of IOP after SLT was 8.3mmHg, with average percentage of reduction 31% in the primary treatment group, compared to 7.7 mmHg mean absolute reduction of IOP in the the latanoprost group, with average percent of IOP reduction 30.6%.

In the current study, in this primary treatment group, 6 eyes in 3 patients (30%) showed increase in IOP around the 3rd month, with the need of one topical glaucoma medication (Beta-blocker) to control the IOP.

**The second group** (The secondary treatment group)The IOP was decreased from 21.32 ± 2.23 mmHg before treatment to 17.38 ± 1.56 mmHg in the last follow up

visit. The reduction of IOP was greatest 24 hours after SLT. The average absolute reduction of IOP was 3.94 ± 0.7 mmHg. This result was nearly similar to that reported by Zaninetti M, et al. (2008) (13) who reported absolute reduction of IOP of 3.3mmHg in patients with POAG uncontrolled to target IOP with medical treatment. However, McIlraith, et al. (2006) (12) reported 6.1 mmHg absolute reduction of IOP after SLT in patients intolerant of medical treatment. This difference is mostly because in their study SLT was done after 4 weeks wash-out period from glaucoma medications. The average percent reduction of IOP in this group was 18.01% compared to 29.8% in the primary treatment group and 29.3% in the control group. This significant difference (P=0.001) may be attributed to low baseline IOP before SLT in the secondary treatment group due to the effect of glaucoma medications, also this may be related to the severity of glaucoma in eyes resistant to medical treatment. Song, et al. (2006) (14) reported that prostaglandins and other glaucoma medications may blunt the response to SLT, with increasing resistance rates to SLT in eyes on prostaglandin analogues. This may also explain the decrease in percentage of IOP in the secondary treatment group.

From this secondary treatment group 12 eyes in 6 patients (35%) showed increase in IOP around the 3$^{rd}$ month, with the need of one topical glaucoma medication to control the IOP. The mean number of medications was reduced in the secondary treatment group from 1.4 ± 0.7 to 0.35 ± 0.1. This reduction in glaucoma medications is in agreement with Babighian, et al. 2010, (8) who reported reduction of the mean number of glaucoma medications from 2.2 ± 0.7 to 0.87 ± 0.8.

In both treatment groups, there was no serious immediate complications occurred after SLT, and any mild complications encountered was resolved within 4 days under cover of topical steroids.

From these results in the 2 treatment groups it was found that: SLT is nearly as effective as prostaglandins analogues. The average percent reduction of IOP

was lower in the secondary treatment group than the primary treatment group, either due to the effect of glaucoma medications in the secondary treatment group, or increased glaucoma severity in the cases resistant to medical treatment. SLT helped in either reduction of IOP to the target level, or reduction of the number of glaucoma medications, thus improving compliance.

## Conclusion

Selective laser trabeculoplasty is a safe, effective outpatient procedure for selective photothermolysis of the trabecular meshwork and lowering of IOP in open angle glaucoma. It is easy to perform with no serious side effect. SLT help in either reduction of IOP to the target level, or reduction of the number of glaucoma medications, thus improving compliance, decreasing side effects from long-term adherence to medications, and with less economic burden.

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# DIVIDE AND CONQUER VERSUS CHOP TECHNIQUES IN PHACOEMULSIFICATION : STUDY OF ENDOTHELIAL CELL LOSS

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## Abstract

***Purpose:*** *To evaluate endothelial cell loss after cataract surgery performed using the divide-and-conquer versus phaco-chop nuclear fracturing techniques.*

***Settings:*** *Mansoura Ophthalmic Center, Mansoura University, Egypt.*

***Methods:*** *This prospective study included 30 eyes of 30 patients. Eyes were randomly selected and divided into two groups: group 1 (15 eyes) undergone divide-and-conquer phacoemulsification technique and group 2 (15 eyes) undergone phaco-chop technique. During surgery, phaco time, phaco power, and grade of nuclear firmness were recorded. The endothelial cell density and central corneal thickness (CCT) was recorded preoperatively and postoperatively at one day, one week, one month and 3 months.*

***Results:*** *less phaco energy (absolute phaco time) was significantly used during phaco-chop technique than during divide-and-conquer technique. The mean absolute phaco time (APT) was 32.6 $\pm$ 13.1 S.D in divide and conquer group compared to 15.2 $\pm$ 11.8 S.D in the phaco chop group. Postoperatively, both groups had a significant but equal decrease in cell density. There were no statistically significant changes in variation in endothelial cell density, or CCT. Visual acuity increased significantly and equally in both groups. Higher endothelial cell loss had no significant correlation with age, sex, axial length (AL), APT and nuclear grade.*

***Conclusions:*** *No significant difference in endothelial cell loss was observed between the two groups in spite of lower APT (phaco energy) in the phaco-chop group.*

## Introduction

Phacoemulsification nowadays is the procedure of choice for cataract extraction for most ophthalmologists. In its beginning, phacoemulsification was performed in the anterior chamber leading to 30% endothelial cell loss during surgery. Recently, damage to corneal endothelial cells during cataract extraction has been minimized, 4% to 15%, as a result of better instrumentation and improved surgical techniques.[1]

Divide-and-conquer technique, described by Gimbel, was the first nucleofractis cracking technique developed.[2,3] It provides safer surgery with less endothelial cell loss.4 In 1993, the phaco-chop technique for cracking the nucleus was described by Nagahara. The main purpose of this technique was to mechanically break the nucleus into smaller fragments. This decreases the use of ultrasound power and limits endothelial damage.[5] Controversy of endothelial cell damage after phaco-chop and divide-and-conquer phacoemulsification have been reported.[1,6] The aim of this study is to evaluate endothelial cell loss (ECL) and postoperative results after cataract surgery performed using the divide-and-conquer versus phaco-chop nuclear fracturing techniques.

## Patients and Method

This clinical prospective study included 30 eyes of 30 patients attending Mansoura Ophthalmic center, Mansoura University-Egypt, for cataract surgery during the period from January 2009 to July 2010. Patients over 50 years having senile cataract were included in the study. Exclusion criteria were pseudoexfoliation, corneal diseases, glaucoma, uveaitis, history of ocular trauma, previous intraocular surgery, proliferative diabetic retinopathy and preoperative endothelial cell density less than 1500 cells/$mm^2$.

Patients were randomly assigned (by coin flip) to one of the 2 groups, 15 patients for each. The first group had phacoemulsifi-

cation using the divide-and-conquer technique, and the other one had phacoemulsification using the phaco-chop technique.

All patients underwent detailed examination including slit lamp and fundus examinations, best corrected visual acuity (BCVA) and intraocular pressure (IOP) measurements. Axial length (AL) measurement and IOL calculation in addition to anterior segment photography using digital camera (Sony-DFC-F85, 4.1 megapixels attached to the slit-lamp microscope). The firmness of the nucleus was graded using the Lens Opacities Classification System III (LOCS III). Endothelial cell density (cells/mm2) and central corneal thickness (CCT) were analyzed using a noncontact specular microscope (Tomy EM-3000). Patients were examined preoperatively and postoperatively at one day, one week, one month and three months.

Surgical Technique: All patients were operated on by the same surgeon using the Oertli CataRhex machine with a phaco tip 30 degree. The surgical technique was similar in all cases except for the method of nucleus fracturing. Group1 had divide-and-conquer technique while group 2 had phaco-chop technique using Nagahara chopper. Pupils were dilated with Tropicamide 1%, cyclopentolate 1% and Phenylephrine 2.5% an hour before surgery. Anesthesia was achieved locally by retrobulbar injection of 2-3ml mixture of lidocaine hydrochloride 2% and Bupivacaine 0.5% (1:1). Local anesthesia was enhanced by Facial akinesia (modified Van Lint). Self-sealing 3.25 mm superior or superior-temporal clear corneal incision and two side ports were created, followed by injection of ophthalmic viscosurgical device (OVD) (Viscoat), capsulorhexis, hydrodissection with a 27-gauge flat cannula and nuclear fracturing (divide-and-conquer or phaco-chop technique). Then cortical cleanup, implantation of a foldable hydrophilic acrylic intraocular lens (IOL), and aspiration of the OVD. The corneal wound and side ports are hydrated by balanced salt solution followed by subconjunctival injection of dexamethasone and gentamicin.

In the divide-and-conquer group two memory programs were used, a memory 1 was used for sculpting (maximum 60% ultrasound [US] power; vacuum 20 mm Hg; flow rate 20 cc/min and bottle height 75 cm). Memory 2 was used for quadrant removal (maximum 50% pulsed mode US power; maximum vacuum 300 mm Hg; flow rate 25 cc/min and bottle height 110 cm). The divide-and-conquer technique was done according to Shepherd.[3]

In the phaco-chop group, memory 1 program was bypassed and only the pulse-mode program, memory 2, was used. After the superficial cortex and epinucleus were aspirated, the phaco tip was buried in the center of the endonucleus at a high vacuum setting (100 to 120 mm Hg). Then the Nagahara phaco chopper was brought through the side-port incision and the equator of endonucleus was engaged by the chopper under the lower edge of the capsulorhexis. The chopper was moved toward the phaco probe to initiate nuclear cracking. Both instruments were moved in opposite directions, dividing the nucleus into halves. The nucleus was then rotated through 90 degrees, the phaco tip impaled in the inferior hemisection of the nucleus, and the chopper used to break this half into 2 smaller fragments, which were then emulsified. The procedure was repeated on the superior nucleus. During surgery, total phaco time (seconds) and mean phaco power (%) were measured. The total phaco energy (the absolute phaco time, APT) was calculated by multiplying the phaco time (seconds) by the mean phaco power (%). Any operative complication as posterior capsule rupture or iris trauma was reported. Postoperatively all patients received topical steroid-antibiotic (TobraDex®) drops 4 times daily for a week and then tapered over the next 3 weeks.

Statistical analysis: Results were tabulated and analyzed using the SPSS program, version 15. Differences between groups were evaluated using Mann-Whitney test for continuous variables (age, AL, cell density and CCT) and chi-square test for categorical variables (nuclear grading), Wilcoxin test for comparison within groups.

The relation between endothelial cell loss (ECL) and age, sex, nuclear grade, AL, and absolute phaco time were tested using the regression analysis test. Rates were calculated by the following equation [Rate % = (The difference between preoperative and postoperative values) X 100/ preoperative value]. Visual acuities were presented as Snellen decimal fractions. P values of 0.05 or less were considered statistically significant.

## RESULTS

Table 1 shows the baseline preoperative patient demographics and clinical data. There were no statistically significant differences between the 2 groups in age, AL, nuclear firmness, CCT and cell density. The mean total phaco energy was 32.6 ±13.1SD in the divide-and-conquer group and 15.2 ± 11.8 SD in phaco-chop group. The difference was statistically significant (P = 0.001) as shown in Table 2.

Both groups had a significant decrease in endothelial cell density at one week, one month and 3 months postoperatively (Table 3). The mean cell loss was compared postoperatively between both groups at one week, one month and 3 months, respectively. The difference between the 2 groups was non-significant at all follow-up intervals (Table 4).

Mean CCT and rate of increased CCT were compared post operatively at one day, one week, one month and 3 months. There was no significant difference in postoperative increased CCT between both groups throughout the 4 visits (Table 5).

Table 6 shows means of postoperative BCVA in both groups at the 4 postoperative visits. There was a statistically significant difference (P= 0.012) between both groups in the first postoperative day, where patient having phaco chop technique achieved better mean BCVA (0.44) compared to divide-and-conquer group (0.29).

The age, sex, nuclear grade, AL and APT had no significant association with higher endothelial cell loss as shown by regression analysis (P=.893, .590, .495, .950 and .533 respectively).

**Table (1) :** Baseline preoperative clinical data.

| Variants | Divide-and-Conquer | Phaco-chop | *P.* |
|---|---|---|---|
| | Mean ± S.D* | Mean ± S.D* | *value* |
| Age (y) | 59.6 ± 6.2 | 58.4 ± 4.4 | 0.708 |
| Axial length(mm) | 23.90 ± 6.8 | 25.21 ± 2.7 | 0.693 |
| Cell density ( cells/mm²) | 2787.5 ± 133.2 | 2627.9 ± 279.6 | 0.093 |
| CCT(µm) | 513.7± 24.8 | 511.5± 38.9 | 0.803 |

*SD = Standard Deviation

**Table 2 :** Comparison between the absolute phaco times (APT) in the 2 groups.

| | Divide-and-Conquer | Phaco-chop | P. |
|---|---|---|---|
| | Mean ± S.D | Mean ± S.D | value |
| APT | 32.6 ± 13.1 | 15.2 ± 11.8 | 0.001 |

**Table (3) :** Comparison between endothelial cell density preoperatively and postoperatively during the follow up period in both groups

| | Preoperative | One week | P value | One month | P value | 3 months | P value |
|---|---|---|---|---|---|---|---|
| Group 1 | | | | | | | |
| *Mean density* | 2787.5 ± 133.2 | 2503.7 ± 153.9 | 0.036 | 2493.3 ± 131.5 | 0.033 | 2459.0 ± 152.9 | 0.019 |
| *Mean ECL* | | 283.8 ± 115.9 | | 294.3 ± 129.1 | | 328.3 ± 150.6 | |
| Group 2 | | | | | | | |
| *Mean density* | 2627.9 ± 279.6 | 2442 ± 327.2 | 0.049 | 2387.6 ± 318.8 | 0.041 | 2374.3 ± 320.6 | 0.039 |
| *Mean ECL* | | 185.9± 127.7 | | 240.3 ± 155.8 | | 253.7 ± 159.6 | |

Group 1= Divide- and-conquer technique, Group 2= Phaco-chop technique

**Table (4) :** Comparison between postoperative rates of ECL in both groups during the follow up period .

| ECL | Divide-and-Conquer | | Phaco-chop | | P. value |
|---|---|---|---|---|---|
| | % | S.D | % | S.D | |
| One week | 10.2 | 3.9 | 7.2 | 4.9 | 0.093 |
| One month | 10.5 | 4.3 | 9.2 | 5.7 | 0.373 |
| 3 months | 11.7 | 5.2 | 9.7 | 5.9 | 0.254 |

**Table 5 :** Comparison between postoperative CCT in both groups during the follow up period.

| CCT | Divide-and-Conquer | | Phaco-chop | | P. value |
|---|---|---|---|---|---|
| | Mean ± S.D (µm) | Increased CCT (%) | Mean ± S.D (µm) | Increased CCT (%) | |
| One day | 546.7±31.4 | 6.4 | 540 ± 34.4 | 5.7 | 0.604 |
| One week | 524.1±26.6 | 2 | 520.1± 37.8 | 1.7 | 0.604 |
| One month | 517.5±25.8 | 0.7 | 516.7± 40 | 1 | 0.787 |
| 3 months | 516.7±24.5 | 0.4 | 511.9± 40 | 0.1 | 0.135 |

**Table (6) :** Comparison between means of postoperative BCVA in both groups.

| BCVA | Divide-and-Conquer | | Phaco-chop | | P . value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Day | 0.29 | 0.16 | 0.44 | 0.15 | 0.012 |
| Week | 0.54 | 0.31 | 0.68 | 0.23 | 0.098 |
| Month | 0.69 | 0.29 | 0.76 | 0.19 | 0.444 |
| 3 Months | 0.77 | 0.23 | 0.77 | 0.21 | 0.894 |

## DISCUSSION

Comparing the preoperative parameters regarding age, nuclear grade, CCT and cell density revealed that there was no biased patient selection. The present study showed shorter phaco time in phaco-chop technique

than divide-and-conquer but with equal endothelial cell loss. DeBry et al.[7] and Ram et al.[8] showed that ultrasound energy use was significantly less in phaco-chop technique than that of divide-and-conquer technique, but endothelial cell damage was not evaluated in these studies.

Pirazzoli et al.[6] and Dick et al.[9] revealed that phaco-chop technique involved shorter phaco time leading to less endothelial cell loss. They postulated that less total energy leads to less endothelial cell damage. This hypothesis was confirmed by some[10,11] while others, including the present study showed that there is no positive correlation between the total energy and endothelial cell loss.[5,12,13] Factors other than APT may play important roles in endothelial cell loss. Hayashi et al.[12] suggested that mechanical contact with nuclear fragments could be the principal cause of endothelial injury.

The lack of significant difference in cell loss between the two techniques in the current study could be explained by technical improvements in the phaco tips, machines and OVDs. Zetterstro¨m and Laurell,[1] and Hayashi et al.[12] suggested that the divide-and-conquer technique is currently as safe as the more recently developed phaco-chop technique.

In the present study, the mean endothelial cell loss after 3 months of divide-and-conquer phacoemulsification, when cell loss and reorganization have stabilized,[14] was 11.7% which is comparable to that reported by some[6,8], and much higher than that reported by others.[13] The mean endothelial cell loss after phaco-chop group in this study was 9.7%. Storr-Paulsen et al.[13] and Vajpayee et al.[15] reported higher rates, while Can et al.[16] reported much less lower rates. These differences in results may be attributed to the difference in surgeon experiences and the phaco machines used.

Some studies revealed a positive correlation between endothelial cell loss and AL or nuclear grade.[8,12,13] The present study did not find such correlation. This

may be attributed to the difference in the sample size or the patient factors.

Earlier visual recovery among the phaco-chop group was shown in the current study indicated by the significant difference in the BCVA at the first postoperative day (P=.012). The rate of increased CCT in the first postoperative day was 6.4% in the divide-and-conquer group versus 5.7% in the phaco-chop group. Although this difference did not reach statistical significance, it may explain the better BCVA among phaco-chop group.

In conclusion, no significant difference in endothelial cell loss was observed between the two groups in spite of lower APT (phaco energy) in the phaco-chop group.

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# BENHA MEDICAL JOURNAL


## DIVIDE AND CONQUER VERSUS CHOP TECHNIQUES IN PHACOEMULSIFICATION : STUDY OF ENDOTHELIAL CELL LOSS


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# EFFECT OF ACUTE NORMOVOLEMIC HEMODILUTION ON CEREBRAL HEMODYNAMICS, METABOLISM AND OXYGENATION DURING BRAIN TUMORS RESECTION

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## Abstract

***Background :*** *This prospective, randomized study was designed to evaluate the efficacy and the effect of acute normovolemic hemodilution (ANH) on cerebral hemodynamics, metabolism and oxygenation profile during brain tumours resection.*

***Methods :*** *This study was carried out on 40 patients of either sex with an age ranging from 20 to 60 years. Patients were admitted at department of neurosurgery in Mansoura University Hospital and subjected to elective resection of brain tumors. Patients were classified into 2 equal groups; hemodilution (H group)and non hemodilution or control (C group)group. Anesthesia was induced in all patients using thiopental sodium (5-7 mg/kg). Tracheal intubation with appropriate sized cuffed endotracheal tube is facilitated by the use of 0.5 mg/kg atracurium. Anesthesia was maintained using isofluorane 1%. O2: air =1 :1 and atracurium for muscle relaxation. In (H) group two units of autologous blood was withdrawn from anticubital vein into blood collection bags. Hemodynamics and O2 parameters were monitored immediately before start of hemodilution then every 10 min up to the end of hemodilution , thereafter every 30 min up to the end surgery. Cerebral metabolic rate for oxygen (CMRO2), Cerebral oxygen extraction (CEO2), Cerebral blood flow equivalent (CBFe) and Arterio-Jugular lactate difference (AJDL) were recorded at basal,40 min., end of surgery and before extubation.*

***Results :*** *This clinical trial has demonstrated that ANH resulted in systemic hemodynamic (HR & MAP) stability with comparable central blood volume to the control group. ANH was associated with comparable hematologic parameters (Hb & Hct) with no significant increase in blood loss. There was no change in seum electrolytes ($Na^+$ & $K^+$), plasma osmolarity or brain relaxation score. Furthermore, ANH resulted in significant increase in cerebral blood flow, no effect on cerebral metabolism as indicated by comparable effects on cerebral oxygen extraction, jugular venous oxygen saturation and arterio-jugular oxygen content difference*

***Conclusion :*** *ANH can be safely and effectively adopted in craniotomy for brain tumors resection, with comparable sequences to allogenic blood transfusion .*

***Key words :*** *Hemodilution, Cerebral Haemodynamics, Brain Tumor.*

## Introduction

Brain tumors constitute a major medical challenge due to the complex set of effects either on the brain itself or on different body systems. These effects are produced by alteration of cerebral blood flow either directly through compression on the adjacent blood vessels or indirectly via increased intracranial pressure and new vascularizations that lack all cerebrovascular properties as barrier function, carbon dioxide responsiveness and autoregulatory capacities [1].

Allogeneic blood transfusion is crucial in major surgery with considerable blood loss, however the potential for adverse effects, high costs and intermittent shortage mandates a wise use of allogenic blood transfusion especially in less extreme situations[2]. Increased awareness of risks related to homologous blood transfusion such as immunological reactions[3], Transmission of infections[4] and immunosuppresion[5] has necessitated search for alternative techniques to deal with the need of intraoperative blood transfusion. Acute normovolemic hemodilution (ANH) is one of these techniques. Others include, preoperative autologous blood donation, preoperative use of erythropoietin, intraoperative cell salvage and retransfusion and the prophylactic

use of aprotinin, ?-amino caproic acid or tranexamic acid.

ANH is a blood conservation technique which involves intraoperative removal and storage (at room temperature) of whole blood prior to surgical blood loss with simultaneous replacement with an appropriate volume of crystalloid and/or colloid solution[6]. Augmented ANH is a new concept in which we use preoperatively erythropoietin or artificial hemoglobin in addition to ANH. Though ANH is commonly employed in many surgical procedures, there is a hesitation to apply this blood saving technique in patients undergoing intracranial surgery, two main concerns discourage its use in neurosurgical patient: (i) the possibility of enhancing brain edema resulting from hemodilution and (ii) increased risk of bleeding due to dilution of clotting factors [7].

Serum osmolality and plasma oncotic pressure changes are major determinants of brain edema formation in patients with intracranial lesions[8]. The risk of exaggerating brain edema will be eliminated durig ANH, if osmolar and oncotic changes due to the procedure are minimized. Hydroxy-Ethyl Starch (HES) is a plasma volume substitute with a wide range of macromolecules with molecular weights ranging from 30.000-2.400.000 Daltons. HES 6% in balanced electrolyte solution has an osmolality of 308 mosm/kg, Kiran and his colleagues have been concluded that ANH using HES 6% does not cause hemodynamic or osmolar changes which are likely to enhance the risk of brain edema[7].

ANH reduces the red cell mass lost during surgery by reducing the hematocrit (Hct) level in the lost blood[9]. Benefits of ANH are avoidance of potential hazards of homologous blood transfusion, reduce the total transfusion cost and overcome blood donation shortage in some situations. ANH provides the only chance to transfuse patients with fresh whole blood containing viable platelets, and high level of clotting factors. Also ANH improves tissue perfusion through reduction of blood viscosity which is specially required in microvascular and

plastic surgeries [10].

Acute normovolemic hemodilution (ANH) proved to improve tissue perfusion through reduction of blood viscosity and this beneficial rheological property may enhance the microcirculation including cerebral vasculature affecting the cerebral metabolism and oxygenation. So, the aim of this study is to evaluate the efficacy and the effect of ANH on cerebral hemodynamics, metabolism and oxygenation profile. All possible and available safety guards against any trivial hazards related to ANH will be taken into consideration.

## Patients and Methods

This prospective, randomized study was carried out after approval by the local ethical and scientific committee of Department of Anesthesia and Surgical Intensive Care, Faculty of Medicine, Mansoura University. This study was carried out on 40 patients of either sex with an age ranging from 20 to 60 years. These patients were admitted to the department of Neurosurgery for elective resection of brain tumors. According to closed envelope randomisation they were classified into 2 equal groups; hemodilution (H group) and non hemodilution or control (C group) group.

Exclusion criteria includes age under 20 years old or above 60 years, ASA physical status class IV, haemoglobin (Hb) level less than 12 gm/dl and presence of contraindication to hemodilution such as patients with uncontrolled systemic hypertension, ischaemic heart disease, heart failure, valvular heart disease, ECG rhythm other than regular sinus rhythm, uncontrolled diabetes mellitus, significant respiratory disease ($PaO_2$ <8KPa on room air), acute infection, coagulopathies: platelets count <$100x10^3$/cc, INR >1.2, chronic renal impairment; serum creatinine >1.5 mg/dl, and hepatic dysfunction (serum bilirubin >1.5 mg/dl and aspartate aminotransferases level more than 2 fold upper normal level).

Also we excluded patients with known allergy to HES, obese patient (BMI >30), pregnant or post partum woman, surgery in sitting or prone position and patients

with altered level of consciousness.

**Patient preparation:**

**a- The day before surgery:**

After having informed and written consent, all patients were evaluated for, full medical history, clinical examination, ECG and investigated for complete blood picture, complete liver functions, serum creatinine, random blood glucose, prothrombin time, activity and INR.

**b- The day of surgery:**

On arrival to the recovery room, a wide bore i.v. cannula (18 G) was secured into a suitable peripheral veins (usually an anticubital vein). Patients were premedicated with intravenous midazolam 0.05 mg/kg plus 1.5 μg/kg fentanyl 10 min before induction.

Preanesthetic monitoring for basal reading included, ECG and HR, pulse oximetry and non invasive blood pressure.

**Induction of anesthesia:**

Preoxygenation with 100% $O_2$ for 3-5 min, sleep inducing dose of thiopental sodium (5-7 mg/kg) then tracheal intubation with appropriate sized cuffed endotracheal tube is facilitated by the use of 0.5 mg/kg atracurium.

Maintenance was done by isoflurane at a concentration of 1%, $O_2$ : Air (1:1) and Fentanyl in repeated doses (20 μg) when needed.

Lungs were mechanically ventilated to maintain $PETCO_2$ around 30 mmHg and $SpO_2$ more than 95%. Mannitol (20%) 1 gm/kg just before opening the dura via central line. Atracurium in incremental doses 0.1 mg/kg every 20 minutes. Crystalloids (normal saline solution) in addition to the blood to keep CVP ≈ 0-5 mmHg.

**Cannulation:** Immediately after induction of anesthesia using seldinger technique the following catheters were secured.

- **Arterial cannula :** Under complete aseptic conditions 20 G cannula was inserted into the radial artery of non-dominant hand after performing Modified Allen's test and local infiltration of 0.5ml xylocaine 2%.
- **Central venous catheter:** A

suitable intravenous catheter was secured into Rt. subclavian vein under complete aseptic technique,its correct position was confirmed by chest X-Ray.

- **Jugular bulb catheterization :** Under strict sterile technique the right internal jugular vein was cannulated in a retrograde technique with confirmation of the catheter tip position using X-Ray (C-arm).

**Jagular Bulb Catheter Placement:**

The technique for retrograde cannulation of the internal jugular vein is relatively simple. The patient is positioned supine with the head in a neutral position. The right internal jugular vein was cannulated in the cephalad direction, at the level of the cricoid ring. The Seldinger technique was used. The catheter is then advanced through the introducer to the jugular bulb, approximately at the level of the mastoid process.

Once the catheter was inserted, it is critical that the correct position be attained in order to limit the contamination from extra cerebral blood. The catheter should sit as close to the roof of the jugular bulb as possible. Even a 2-cm difference can lead to as much as 10% contamination. This rises exponentially as the tip is withdrawn further. The position of the catheter tip can be confirmed by either a lateral or an antero-posterior (AP) neck radiograph [11]. On the lateral film, the catheter tip must be above the disc of C1/C2 and as close to the skull base as possible. On AP view, a correctly placed tip should lie cranial to a line extending from the atlanto-occipital joint space and caudal to the lower margin of the orbit. The catheter tip should also lie cranial to a line connecting the tips of the mastoid processes.

**Estimated allowable blood loss:**

The maximum volume of blood that could be collected was derived from the following formula:

$$V = EBV \times \frac{H_o - H_f}{H_{av}} \quad (12)$$

Where V is the maximum volume of blood to be removed.

EBV is the estimated blood vol-

ume of the patient (B.W x 75 ml for male or B.W x 70ml for female).

$H_o$ is the initial hemoglobin .

$H_f$ is the desired hemoglobin .

$H_{av}$ is the average hemoglobin (average of $H_o$ and $H_f$).

**Hemodilution technique:**

In hemodilution group,after application of all monitoring devices and zeroing of transducers, hemodilution was started after induction of anesthesia with stable hemodynamic parameters and before the start of surgery. ANH was carried out in accordance with the published guidelines for ANH[6]. Two units of autologous blood was withdrawn from anticubital vein(veins) into blood collection bags containing citrate-phosphate-glucose with adenine. At the same time HES 6% (Voluven-130/0.4) was infused into a central line in a rate equal to the rate of blood collection (1:1 volume). The harvested blood was continously shaked to ensure proper mixing of blood with the anticoagulant then was labelled in a serial manner and stored in sterile condition at operating room temperature. The reinfusion was in the reverse order of collection; the second collected unit was reinfused first. Irrespective of Hct and Hb levels all the harvested autologous blood was reinfused and no unit was wasted.

**Time of blood transfusion:**

**Hemodilution group:**

Autologous blood was reinfused on reaching maximum allowable blood loss (the calculated volume) or with starting surgical haemostasis. Allogenic blood was infused if negative balance between harvested blood and blood loss exceeding 500 ml.

**Control group:**

In the control group allogenic blood was transfused when blood loss exceeded more than 500 ml.

**Monitoring and sampling:**

**1- Hemodynamic and $O_2$ parameters:**

HR, NIBP, $SpO_2$ (pulse oximetry), $P_{ET}CO_2$ and CVP.

Immediately before start of hemodilution then every 10 min up to the end of hemodilution (about 45 min), thereafter every 30 min up to the end surgery.

**2- Brain edema score (subjective surgeon score):**

Brain relaxation was noticed by the surgeon and recorded at the time of dural opening on a grade of 1-to-3 (grade 1 denoting brain bulging above the level of craniotomy, grade 2 at the level of craniotomy and grade 3 means brain surface well below craniotomy level) .

**3- Blood sampling for laboratory assessment:**

- **Site:** Simultaneous radial artery and jugular bulb blood samples.
- **For:** Arterial and jugular blood gases (AVL, Compact3, Rouch), Hb level and Hct (Sysmex KX-21, Rouch), S. lactate (Integra 800, Rouch), S. osmolality (Azmomat 030, Rouch)and $Na^+$ & $K^+$ (jugular).

**- Time:**

1. After induction of anesthesia with stable hemodynamic parameters and immediately before hemodilution (sample 1).
2. After completion of ANH technique or 45 min from start of surgery in the control group (sample 2).
3. After finishing surgical manipulation of the brain and just before closure of the dura (sample 3).
4. Before reversal of residual neuromuscular blocking (sample 4) .

**4- Calculated parameters:**

**a- Estimated cerebral metabolic rate for O2: (eCMRO2) as follow:**

$CaO2 = (SaO_2 \times Hb \times 1.39) + 0.003 \times PaO_2$.

$CJVO_2 = (SJVo_2 \times Hb \times 1.39) + 0.00 \times PJVO_2$.

$Ca\text{-}jO_2 = CaO_2 - CJVO_2$.

$$eCMRO2 = Ca\text{-}jO_2 \times \frac{PaCO_2}{100} \quad (13)$$

Where $CajO_2$ is arterio-jugular $O_2$ content difference.

$PaCO_2$ is arterial $CO_2$ tension.

**b- Cerebral Extraction Rate of $O_2$ ($CRO_2$):**

Calculated as the difference between arterial and jugular bulb $O_2$ saturations, $CEO_2 = SaO_2 - SjO_2$ [14].

**c- Cerebral Blood Flow equivalent (CBFeq.):**

Which is an index of flow metabolism relationship, was calculated as a reciprocal of arterio-jugular O2 content difference (15)

$$\text{CBF eq.} = \frac{1}{CaO_2 - C_{jv}O_2}$$

**5- Other parameters to be recorded:**

Body weight, maximum allowable blood loss, withdrawn blood volume, blood loss volume, duration of surgery and total blood transfusion.

**Postoperative period:**

All patients were transferred to surgical intensive care unit for at least 24 hs for full monitoring and follow up and any intraoperative or postoperative complications were reported.

**Data Analysis :**

Data entry and analyses were performed using SPSS statistical package version 10 (SPSS, Inc., Chicago, IL, USA). Qualitative data were presented as number and ratio while, quantitative data were presented as mean and standard deviation. The chi-square test ($x^2$) was used to test the association between raw and column variables of qualitative data. Student t-test (unpaired) was conducted to compare the mean of continuous variable for the hemodilution and control groups at each point of time while, paired t-test was conducted to evaluate the impact of time on the mean of continuous variable in each group. The t-test was used in data proved to be normally distributed (Kolmogrove - Smirnov test), while Man-Whitney-U test was used for data away from normal distribution. The P value of $< 0.05$ and $<0.001$ indicate significant and highly significant results respectively.

## Results

This study was conducted on 40 patients of either sex, with an age ranging from 20 to 60 years. Patients were randomly allocated into 2 equal groups; control (C group, n=20) and hemodilution (H group, n=20). Patients of either group showed no significant changes concerning age, sex and duration of surgery as well (table 1).

Intraoperative HR did not differ significantly during the whole study period when comparing the two studied groups. While, the HR showed no intragroup significance in the hemodilution group, in the control group HR was significantly lower at 60 min from start of surgery when compared to control value (immediately after induction of anesthesia) as p.value was 0.02 (table 2).

Mean arterial blood pressure (MAP) showed no significant difference between the two studied groups throughout the whole study period.In the control group MAP was significantly lower at 20 min, 30 min, 40 min after anesthesia, start of surgery and up to 120 min after start of surgery compared to that immediately after induction of anesthesia (p.value, 0.013-0.049) (table 2). In the hemodilution group, there was significant decrease in MAP at 20 min, 30 min and 40 min after induction of anesthesia (p.value, 0.002, 0.018 and 0.009 respectively) as well as MAP at start of surgery, 30 min and 120 min after start of surgery (p.value, 0.002, 0.006 and 0.049 respectively) compared to MAP immediately after induction of anesthesia (table 2).

Although, central venous pressure (CVP) did not show any significant difference on comparing the two studied groups, there was significant changes within each group at different study intervals. In the control group CVP was significantly lower at 20min after induction of anesthesia and throughout the study period up to 120 min after start of surgery compared to the control value (immediately after induction of anesthesia) (p.value, <0.001-0.004). In the hemodilution group CVP was significantly lower 10 min after start of ANH and the subsequent readings up to 120 min after start of surgery compared to CVP at start of ANH (p.value, <0.001-0.008) (table 3).

Both Hb and Hct were significantly lower in the hemodilution group at the end of hemodilution compared to 40 min after induction of anesthesia in the control group (p.value, 0.014 for Hb and 0.032 for Hct). In the control group Hb and Hct were

significantly lower at 40 min after induction of anesthesia,end of surgery and before endotracheal extubation compared to the control value (p.value, <0.001-0.002). Also, in the hemodilution group Hb and Hct were significantly lower at the end of hemodilution, the end of surgery and before endotracheal extubation compared to the start of hemodilution (p.value, <0.001-0.009) (table 4).

**Hemodilution parameters, Surgical blood loss and brain relaxation score:**

Preoperative Hb and Hct showed no significant difference between the two studied groups. Estimated blood volume (EBV), surgical blood loss and maximal allowable blood loss (MABL) showed no significant difference between the two groups of the study. Regarding the subjective surgeon score (SSS) of brain relaxation, there was no significant difference between both studied groups (table 5).

Serum $Na^+$ and $K^+$ showed no significant difference between both studied groups. While, the control group showed no intragroup significant change in serum $K^+$, in the hemodilution group serum $K^+$ was significantly higher at the end of surgery and just before endotracheal extubation compared to start of hemodilution (p.value, 0.024 and 0.004 respectively) (table 6).

Redarding plasma osmolarity, there was no significant difference between both studied groups. While, the control group showed no significant change in plasma osmolarity throughout the study period, the hemodilution group showed significant difference at some study intervals. In the hemodilution group, plasma osmolarity was significantly higher at the end of hemodilution, end of surgery and before endotracheal extubation compared to the start of hemodilution (p.value, 0.018, 0.015 and 0.004 respectively) (table 7).

Arterial oxygen saturation ($SaO_2$) showed no significant change between both studied groups. In the control group, $SaO_2$ was significantly lower 40min after induction and at the end of surgery compared to that

after induction of anesthesia (p.value, 0.005 and 0.041 respectively) (table 8). There was no significant change in jugular venous oxygen saturation on comparing both studied groups (table 8).

Arterial carbon dioxide tension ($PaCO_2$) showed no significant difference on comparing both studied groups. While, the hemodilution group showed no intragroup significant difference at all study intervals,In the control group $PaCO_2$ was significantly lower at the end of surgery and before endotracheal extubation compared to that immediately after induction of anesthesia (p.value, 0.002 and 0.009) (table 8).

**Calculated cerebral parameters and Jugulo-arterial lactate difference:**

Jugulo-arterial oxygen content difference ($Cj\text{-}aO_2$) did not change significantly between both studied groups. In the control group $Cj\text{-}aO_2$ was significantly lower at the end of surgery and before endotracheal extubation compared to that immediately after induction of anesthesia (p.value, 0.003 and 0.002 respectively). In hemodilution group, $Cj\text{-}aO_2$ was significantly lower after hemodilution than at the start of hemodilution (p.value, 0.032) (table 9).

Cerebral extraction of oxygen ($CEO_2$) did not change significantly on comparing both studied groups at all study intervals. while, there was no significant difference in the control group throughout the whole study period, In the hemodilution group, there was lower $CEO_2$ after hemodilution compared to the start of hemodilution (p.value, 0.023) (table 9).

Regarding the estimated cerebral metabolic rate for oxygen ($eCMRO_2$) there was no significant difference between the two studied groups throughout the whole study period. In the control group, $eCMRO_2$ was lower 40 min after induction of anesthesia, the end of surgery and before endotracheal extubation compared to the basal value (p.value, < 0.001-0.021). In the hemodilution group, $eCMRO_2$ was significantly lower at the end of hemodilution, and the end of surgery compared to that at the start of hemodilution (p.value,

0.005 nd 0.034 respectively) (table 10).

Cerebral blood flow equivalent (CBFe) showed no significant difference between both studied groups throughout the whole study period. In the control group, CBFe was significantly higher at the end of surgery and before endotracheal extubation compared to that immediately after induction of anesthesia (p.value,0.005 and 0.022 respectively).In the hemodilution group, CBFe was significantly higher after hemodilution than at start of hemodilution (p.value,0.042) (table 10).

Jugulo-Arterial lactate concentration difference (J-A Lactate) did not show any significant change either, on comparing both studied groups or at different sampling times within each group (table 7).

**Table (1):** Patients Characteristics and Surgical Duration Of Control **(C)** and Hemodilution **(H)** Groups. Values are in mean ± SD and ratio (M/F).

| | C | H |
|---|---|---|
| **Age(Y)** | 44.20 ± 8.7 | 42.75 ± 12.5 |
| **Sex (M/F) ratio** | 9 / 11 | 11 / 9 |
| **B.Wt(Kg)** | 80.00 ± 5.5 | 80.95 ± 7.1 |
| **Duration Of Surgery (min)** | 186.00 ± 54.7 | 177.00 ± 53.2 |

B. Wt (kg) = body weight in kilograms.

**Table (2):** Intraoperative Heart rate "HR" (bpm) and Mean arterial blood pressure "MAP" (mmHg) for the studied groups. Values are in mean ± SD And (p.value).

| Time(min) | HR (bpm) | | MAP (mmHg) | |
|---|---|---|---|---|
| | C | H | C | H |
| Basal | 83.00 ± 11.1 | 82.85 ±10.5 | 88.70 ± 6.3 | 89.15 ± 6.7 |
| 10 min after | 82.95±10.7 | 81.90±11.1 | 87.95 ± 6.2 | 87.80 ± 5.0 |
| 20 min after | 81.55±11.4 | 81.75±10.1 | 86.20 ± 6.1* (0.049) | 85.95 ± 5.6* (0.002) |
| 30 min after | 81.00 ± 12.6 | 82.75±9.9 | 85.80 ± 6.2* (0.013) | 86.25 ± 4.2* (0.018) |
| 40 min after | 81.50 ± 10.9 | 82.10 ± 11.2 | 84.60 ± 7.1* (0.024) | 85.55 ± 5.3* (0.009) |
| Start of surgery | 81.40 ± 10.7 | 83.80 ± 9.9 | 85.25 ± 5.8* (0.014) | 84.90 ± 5.5* (0.002) |
| 30 min after | 81.15 ± 11.1 | 82.55±8.7 | 84.85 ± 7.5* (0.047) | 84.35 ± 8.0* (0.006) |
| 60 min after | 79.10 ± 11.3* (0.02) | 83.05 ± 8.5 | 84.05 ± 6.3* (0.013) | 87.00 ± 6.2 |
| 120 min after | 80.35 ± 11.1 | 83.60 ± 10.9 | 85.25 ± 5.8* (0.040) | 85.85 ± 6.6* (0.049) |

Basal : immediately after induction of anesthesia.
* : significant when compared to basal value of the same group.
Significance : P. value < 0.05.

**Table (3):** Central venous pressure "CVP" (mmHg). Values are in mean ± SD And (P.value).

| Time (min) | CVP(mmHg) | |
|---|---|---|
| | C | H |
| Basal | 3.30 ± 1.38 | 3.50±1.36 |
| 10 min after | 3.50±1.92 | 3.15±1.31* (0.005) |
| 20 min after | 2.70±1.13* (0.004) | 3.00±1.21 * (0.004) |
| 30 min after | 2.80±1.00* (0.004) | 2.95±1.36 * (0.008) |
| 40 min after | 2.50±0.89* (0.002) | 2.55±1.54 * (<0.001) |
| Start of surgery | 2.20±0.89* (<0.001) | 2.45±1.57 * (<0.001) |
| 30 min after | 2.05±0.89* (<0.001) | 2.25±1.25* (<0.001) |
| 60 min after | 2.00±1.08* (<0.001) | 2.00±1.17 * (<0.001) |
| 120 min after | 2.15±0.88* (<0.001) | 2.30±1.08* (<0.001) |

Basal : immediately after induction of anesthesia.

* : significant when compared to basal value of the same group.

Significance : P. value < 0.05.

**Table (4):** Intraoperative Hemoglobin "Hb" (gm/dl) and hematocirt "Hct" (%). Values are in mean ± SD And (P.value).

| Time | Hb(gm/dl) | | Hct (%) | |
|---|---|---|---|---|
| | C | H | C | H |
| Basal | 13.03 ± 0.9 | 13.47±1.2 | 38.86±2.5 | 39.86±3.8 |
| 40 min. After | 12.48±1.3* (0.002) | 11.36±1.5*† (<0.001) (0.014) | 36.55±3.4* ( 0.001) | 33.66±4.7*† ( <0.001) (0.032 ) |
| End of surgery | 12.04±1.4* (<0.001) | 11.85±1.8* (<0.001) | 35.71±3.4* (<0.001) | 35.19±5.5* (<0.001) |
| Before extubation | 12.20±1.1* (<0.001) | 12.15±1.6* (<0.001) | 36.32±2.8* (<0.001) | 36.31±5.3* (<0.001) |

*Basal : immediately after induction of anesthesia.*
*40 min. after induction of anesthesia (C group) or end of ANH (H group).*
** : significant when compared to the basal value of the same group.*
*† : significant when compared to the control group at the same time .*
*Significance: P. value < 0.05.*

**Table (5):** Estimated Blood Volume "EBV" (ml), Maximal Allowable Blood Loss "MABL" (ml), Surgical Blood Loss and Subjective Surgeon Score for Brain Relaxation(SSS). Values are in mean ± SD.

| | C | H |
|---|---|---|
| EBV (ml) | 5776.00 ± 467.6 | 5886.75 ± 570 |
| MABL (ml) | 1505.55 ± 412.6 | 1733.15 ± 600.4 |
| Blood loss (ml) | 1100 ± 500 | 1210 ± 307 |
| SSS | 2.3 ± 0.47 | 2.55 ± 0.51 |

**SSS** = subjective surgeon score for brain relaxation.

**Table (6):** Serum sodium "$Na^+$" , Serum potassium "$K^+$" (meq/L) and Plasma Osmolarity "Osmo"(ml Osmo/L). Values are in mean ± SD and (P.value).

| Time | $Na^+$(meq/L) | | $K^+$(meq/L) | | Osmo.(m Osmo/L) | |
|---|---|---|---|---|---|---|
| | C | H | C | H | C | H |
| Basal | 140.40±6.7 | 141.00±7.3 | 3.73±0.45 | 3.75±0.45 | 297.8±8.5 | 295.6±9.6 |
| 40 min. After◊ | 138.85±6.1 | 142.20±6.6 | 3.73±0.36 | 3.66±0.48 | 299.4±9.9 | 299.9±9.0* (0.018) |
| End of surgery | 139.25±6.9 | 140.20±7.2 | 3.85±0.41 | 3.99±0.46* (0.024) | 300.5±8.9 | 300.2±8.6* (0.015) |
| Before extubation | 140.95±6.1 | 139.45±7.5 | 4.03±0.53 | 4.01±0.42* (0.004) | 300.8±9.6 | 302.6±9.4* (0.004) |

Basal : immediately after induction of anesthesia.
◊ : 40 min. after induction of anesthesia (C group) or end of ANH (H group).
* : significant when compared to the basal value of the same group.
Significance: P value < 0.05.

**Table (7):** Arterial carbon dioxide tension "$PaCo_2$" (mmHg),Arterial oxygen saturation (SaO2) and Jugular venous oxygen aturation(SjvO2).Values are in mean ± SD and (P.value).

| Time (min) | PaCo2 (mmHg) | | $SaO_2$ (%) | | $SjvO_2$ (%) | |
|---|---|---|---|---|---|---|
| | C | H | C | H | C | H |
| Basal | 39.1±2.4 | 39.2±2.5 | 99.6 ± 0. 5 | 99.6 ±0.4 | 71.9 ± 3.7 | 72.4 ± 3.8 |
| 40 min. after◊ | 38.4±1.9 | 38.4±2.6 | 99.2 ± 0.9* (0.005) | 99.5 ± 0.4 | 72.2 ± 4.6 | 72.5 ± 3.8 |
| End of surgery | 37.3±1.9* (0.002) | 38.6±2.7 | 99.3 ± 0.7* (0.041) | 99.6 ± 0.4 | 71.7 ± 3.9 | 72.8 ± 4.1 |
| Before extubation | 37.5±2.3* (0.009) | 37.9±2.6 | 99.4 ± 0.7 | 99.6 ± 0.3 | 72.5 ± 4.1 | 72.4 ± 4.3 |

Basal : immediately after induction of anesthesia or start of ANH.
◊ : 40 min. after induction of anesthesia (C group) or end of ANH (H group).
* : significant when compared to basal value of the same group.
significance : P. value < 0.05.

**Table (8):** Arterio- jugular oxygen content difference "Ca-j$O_2$"(ml O2/100ml blood) and Cerebral extraction ratio of oxygen "$CEO_2$" (%).Values are in mean ± SD And (P.value).

| Time | Ca-j$O_2$(ml O2/100ml blood) | | $CEO_2$ (%) | |
|---|---|---|---|---|
| | C | H | C | H |
| Basal | 5.27±0.68 | 4.97±0.54 | 27.62±3.49 | 26.83±3.75 |
| 40 min. After$^{\Diamond}$ | 4.93±0.69 | 4.60±0.52$^{*}$ (0.032) | 26.90±4.43 | 27.20±3.91 |
| End of surgery | 4.89±0.74$^{*}$ (0.003) | 4.65±0.52 | 27.44±4.03 | 26.67±4.06 |
| Before extubation | 4.88±0.62$^{*}$ (0.002) | 4.85±0.55 | 26.94±3.88 | 26.79±4.20 |

Basal : immediately after induction of anesthesia.
$^{\Diamond}$ : 40 min. after induction of anesthesia (C group) or end of ANH (H group).
* : significant when compared to the basal value of the same group.
Significance: P value < 0.05.

**Table (9):** Estimated cerebral metabolic rate for oxygen "e$CMRO_2$" (ml/100gm/min), Cerebral blood flow equivalent "CBFe"(ml blood/ml O2) and Jugulo-Arterial Lactate difference" J-AL" (mg/dl). Values are in mean ± SD and (p.value).

| Time | eCMRO2(ml/100gm/min) | | CBFe(ml blood/ml O2) | | J-A Lactate (mg/dl) | |
|---|---|---|---|---|---|---|
| | C | H | C | H | C | H |
| Basal | 2.06±0.24 | 1.96±0.29 | 0.192±2.94 | 0.203±2.32 | 3.90±1.48 | 3.70±2.11 |
| 40 min. After$^{\Diamond}$ | 1.89±0.27$^{*}$ (0.021) | 1.76±0.20$^{*}$ (0.005) | 0.207±3.28 | 0.220±2.49$^{*}$ (0.042) | 3.20±2.07 | 3.75±2.07 |
| End of surgery | 1.82±0.29$^{*}$ (<0.001) | 1.80±0.23$^{*}$ (0.034) | 0.209±3.17$^{*}$ (0.005) | 0.217±2.43 | 3.75±2.43 | 3.30±1.63 |

Basal : immediately after induction of anesthesia.
$^{\Diamond}$ : 40 min. after induction of anesthesia (C group) or end of ANH (H group).
$^{*}$ : significant when compared to the basal value of the same group.
Significance: P value < 0.05.

## Discussion

The major goals in neuroanesthesia are; smooth and hemodynamically stable induction of anesthesia and operative course, acceptable operative field and rapid emergence that allow early neurologic assessment[16]. One of the most important goals is intraoperative fluid and volume management. Acute normovolemic hemodilution (ANH) is widely practiced in different surgical procedure[17], however, it is underutilized in craniotomy for brain tumors resection.

Because of the adverse effects of allogenic blood transfusion, there is a need for safe and effective alternative. ANH is simple, inexpensive and potentially effective alternative to allogenic blood transfusion[18]. It represents an effective blood conservation technique together with optimization of oxygen delivery to the vital organs espcially in major surgeries[19].

This prospective, controlled, randomised and opened study was designed to evaluate different effects (systemic and cerebral) of ANH technique, when used in brain tumors resection, in comparison with the routinely used allogenic blood transfusion for blood loss compensation (transfusion or conservation). ANH was investigated regarding intraoperative systemic and cerebral hemodynamic profile, cerebral metabolism and cerebral oxygenation. This clinical trial has demonstrated that ANH resulted in, stable systemic hemodynamics (HR & MAP) with comparable central blood volume to the control group. ANH was associated with comparable hematologic parameters (Hb & Hct) with no significant increase in blood loss. There were no significant changes in seum electrolytes ($Na^+$ & $K^+$), plasma osmolarity or brain relaxation score. Furthermore, ANH resulted in significant increase in cerebral blood flow, no effect on cerebral metabolism and comparable effects on cerebral oxygen extraction, jugular venous oxygen saturation and arterio-jugular oxygen content difference. Changes in jugulo-arterial lactate concentration difference were not significantly different from the control group.

**Systemic Hemodynamic parameters (HR, MAP and CVP):-**

During anesthesia, the effects of drugs, surgical stimulation, changes in position, temperature and blood volume, all could influence the autonomic functions with subsequent effects on systemic hemodynamics[20]. In this study, heart rate (HR) did not differ significantly in the hemodilution group when compared to the control group at all study intervals. In the hemodilution group HR did not significantly change throughout the whole study period compared to the basal value . This stability in HR reflects adequate analgesia and proper intraoperative fluid management in either studied group with minimal effect of ANH on autonomic nervous system in this study. This finding is in agreement with the previous results that ANH up to 15% of estimated blood volume had no effect on HR in urologic surgeries [21,22].

Mean arterial blood pressure (MAP) did not differ significantly in the hemodilution group when compared to the control group throughout the whole study period. Both groups showed significant decrease in MAP starting 20 min after induction of anesthesia or starting hemodilution. This decrease in MAP continued allthrough the study period. This could be explained by the effect of isoflurane anesthesia. This result was supported by the previous reports that isoflurane administered to human volunteers produced dose-dependent decrease in MAP (approximately 25% at 1 MAC isoflurane)[23,24] due to decreased systemic vascular resistance. Also this may be due to minimal myocardial-depressant effect of isoflurane[25]. In previous studies, ANH was accompanied with an increase in stroke volume and cardiac index[26,27]. This increase in cardiac performance may be expected to increase MAP, in contrast to the results of the current study. This increase in cardiac performance may be overcome by direct vasodilating effect of isoflurane as well as its minimal negative inotropic effect, explaining our results. The finding of the current study is confirmed by the results of other two studies, where anesthesia significantly reduced the cardiac output response associat-

ed with ANH[28,29]. Despite this decrease in MAP, all the decreased reading values remained within the physiologic range.

Central venous pressure (CVP) did not differ significantly in the hemodilution group when compared to the control group throughout the whole study period. This might be due to proper (volume and rate) voluven infusion while blood was collected during hemodilution technique and proper fluid volume management after that. In either group, CVP showed significant reduction in different intervals when compared to the basal value in the same group. This reduction could be explained by induced and ongoing diuresis in addition to fluid restriction policy aiming at having clear surgical field and relaxed brain. All the changes recorded in CVP are still within the predetermined protocol of the study. Another study supporting our results, had been demonstrated no hemodynamic compromise with ANH in healthy adult volunteers,[30].

All the hemodynamic parameters, measured in this study showed good stability and the hemodilution technique was accompanied with hemodynamic changes comparable with those recorded in the routinely used allogenic blood transfusion technique.

**Hematologic parameters (Hb & Hct):**

Both hemoglobin (Hb) and Hematocrit (Hct) were significantly lower in the hemodilution group after completion of hemodilution compared to 40 min after induction of anesthesia in the control group. This finding is self explained as hemodilution reduces Hb and Hct. Blood withdrawal reduces Hb content and colloid infusion reduces the Hct.

In the control group, Hb and Hct were significantly lower at 40 min after induction of anesthesia, end of surgery and before endotracheal extubation compared to the basal value. This could be explained by the surgical blood loss and crystalloid infusion, diluting the Hb content. In the hemodilution group, both Hb and Hct were significantly lower after ANH, end of surgery and before endotracheal extubation compared to that

at the start of ANH (basal value). This reduction in Hb and Hct is in accordance with the previous findings that, in surgical patients low Hb & Hct are usually encountered after advanced surgical blood loss and asanguinous fluid infusion therapy to maintain normovolemia as a result of dilution effects of crystalloids or colloids [31,32].

It is worth noting that the mean value of Hb and Hct in the hemodilution group was more than 11.3 and 33.6 respectively. These values are accepted for adequate oxygen delivery for different organ tissues including the brain [33].

**Hemodilution parameters, surgical blood loss and brain relaxation score:**

Preoperative (Basal) Hb and Hct were comparable in the two studied groups (13.03±0.9 Vs 13.47±1.2 for Hb & 38.86±2.5 Vs 39.86±3.8 for Hct). This reflect proper randomization of the study. Both estimated blood volume (EBV) and maximal allowable blood loss (MABL) showed no significant difference between the two groups of this study. Surgical blood loss was comparable in both studied groups with no significant difference but with more amount in the hemodilution group (1210±307mL in H group Vs 1100±500mL in C group). This insignificant increase in blood loss in the hemodilution group could be explained by the dilution of platelets and clotting factors or the effect of the diluent (Voluven) itself on platelets functions. With retransfusion of collected blood this potential side effect may be partially corrected as retransfused blood contains fresh viable platelets and clotting factors.

Insignificant coagulopathy and blood loss related to ANH is supported by the previous reports that coagulation remained unchanged despite significant plasma dilution during ANH[34,35]. Also, a number of studies of ANH failed to demonstrate association between moderate reduction of hematocrit (Hct ± 30%) and surgical blood loss[36,37]. While, no patients in the hemodilution group received allogenic packed RBCs, 40% of patients (8 from total 20 patients) in the control group

received allogenic blood. This result is supported by the previous studies which concluded that ANH reduced the likehold of allogenic blood exposure and the total units of allogenic blood tansfused in major surgical procedure[38,39]. A meta-analysis studied twenty-four randomised prospective studies confirmed the last finding [40].

Subjective surgeon score for brain relaxation was comparable in both studied groups. Brain oedema results mainly from increased brain water due to decrease in either plasma oncotic pressure or plasma osmolarity. In this study, hemodilution with voluven did not result in any significant difference in plasma osmolarity compared to the control group (299.9 ± 9.01 In H group Vs 299.4 ± 9.9 in C group). Furthermore, ANH resulted in statistically significant increase in plasma osmolarity compared to that before ANH (299.9 ± 9.01 Vs 295.6 ± 9.6). This finding is supported by the previous report of Kiran and his colleagues (2000) where they demonstrated no significant difference in brain relaxation as a result of hemodilution.

**Serum electrolytes (Na+ and K+) and plasma osmolarity:**

Changes in serum electrolytes ($Na^+$ & $K^+$) in neuroanesthesia for brain tumors resection are complex due to the preoperative and intraperative administration of mannitol, corticosteroids and furosemide in addition to fluid restriction protocol for brain dehydration. Transfusion of stored blood may increase serum $K^+$. The increase in serum $K^+$ is supported by some studies reported that hyperkalemia is well-recognized complication of blood transfusion and can lead to cardiac arrest[41,42]. This is because the $K^+$ concentration in stored blood steadily increases with time and serum $K^+$ in stored blood may be as high as 19-30 meq/L for 21 days storage. Also, the transfusion rate exceeding 100ml/min of banked blood (regardless of its age) can cause significant hyperkalemia to occur clinically [43].

In this study, serum $Na^+$ and $K^+$ showed no significant difference in the hemodilution group compared to the control group at all study intervals. While, there was no significant change either

in serum $Na^+$ or $K^+$ in the control group throughout the whole study period, in hemodilution group, Serum $K^+$ showed significant increase at the end of surgery and before endotracheal extubation. This significant increase was only of statistical value, but not of clinical one; since all the recorded $K^+$ levels were within the physiological range. Keeping serum $K^+$ & $Na^+$ within the physiological range could be attributed to transfusing fresh blood in the hemodilution group, and the use of limited volume of allogenic blood in the control group. Good fluid replacement therapy (volume and type) shared in stabilizing the patient electrolytes level.

Plasma osmolarity did not differ significantly in the hemodilution group compared to the control group throughout the whole study period. This finding is in accordance with the previous reports that ANH did not significantly affect plasma osmolality during craniotomy for brain tumors resection[7]. While, in the control group there was no significant change in plasma osmolarity at all study intervals, in the hemodilution group, plasma osmolarity was significantly higher at the end of hemodilution, end of surgery and before endotracheal extubation compared to the basal value. This may be explained by the use of voluven rather than crystalloids as a replacement fluid for hemodilution. Voluven has an osmolality of 308 mosm/kg and remains in the circulation for longer times than crystalloids. This finding is supported by the previous results, where they expermintally demonstrated that ANH using normal saline (twice the volume of shed blood) was associated with higher brain water content than replacement with 6% hexaethyl starch, both in the normal and injured brain[8]. Decrease in plasma osmolarity will lead to increased brain water.

The increase of osmolarity in the hemodilution group, was of statistical and not clinical significance, since it was of very small magnitude (295-302 m.osmo/L). This minimal increase can explain good operative field, no brain oedema and slightly higher SSS, but not significant in this group.

**Blood Gases ($SaO_2$, $SjvO_2$ and $PaCo_2$):**

In this study, arterial oxygen saturation ($SaO_2$) showed no significant difference between both studied groups and was within the physiological ranges throughout the whole study period. This indicates good ventilation and oxygenation of all patients enrolled in the study. While, there was no significant change in arterial oxygen saturation in the hemodilution group, in the control group, $SaO_2$ was significantly lower at 40min after induction of anesthesia and at the end of surgery compared to the basal value (99.2 ± 0.9 and 99.3 ± 0.7 Vs 99.6 ± 0.5). Despite this statistically significant decrease the arterial oxygen saturation still within the accepted physiologic range (statistical bias).

Jugular venous oxygen saturation $SjvO_2$ has been proposed as a simple, inexpensive and reliable method of assessing the adequacy of cerebral blood flow (CBF) for cerebal metabolism. $SjvO_2$ mirrors global cerebral perfusion and cerebral oxygen supply-demand balance. Jugular desaturation ($SjvO_2$ <50%) suggest that CBF is insufficient for the metabolic requirements of the brain (44). $SjvO_2$ did not show any significant difference on comparing both studied groups at all study intervals, this means that ANH did not affect cerebral blood flow in way that cerebral extraction of oxygen did not change resulting in no significant change in $SjvO_2$. So, cerebral oxygen balance was preserved with this technique

Arterial carbon dioxide tension ($PaCo_2$) did not show significant change in hemodilution group compared to the control group at all study intervals. This reflects proper controlled mechanical ventilation. While, the hemodilution group showed no intragroup significant change in $PaCo_2$ at different study intervals, in the control group, $PaCo_2$ was lower at the end of surgery and before endotracheal extubation compared to the basal value. These changes deserve no explanation since it is still within the predetermined range achieved by mechanical ventilation.

**Calculated cerebral parameters and jugulo-arterial lactate difference:**

Arterio- Jugular oxygen content difference ($Ca\text{-}jo_2$) has an inverse relationship with cerebral blood flow and direct relationship with CMR in absence of ischemia, but in presence of ischemia this relation is unpredictable. When cerebral blood flow decrease, the brain extract more oxygen and $Ca\text{-}jo_2$ increase. However, when oxygen extraction is maximal, more decrease in CBF result in cerebral oxygen uptake becomes supply dependent and the relation between $Ca\text{-}jo_2$ and CBF is unpredictable[45]. $Ca\text{-}jo_2$ did not differ significantly in the hemodilution group compared to the control group throughout the whole study period. This finding means that ANH did not interfere with the oxygen delivery-demand balance, at least through CBF variation. This finding is in accordance with the previous reports that ANH, despite decrease in red blood cell mass, maintain cerebral oxygen delivery-demand balance[46,47].

In both groups, control and hemodilution, $Ca\text{-}jo_2$ showed a decrease, significant in some events and insignificant in others, when compared to the basal value in the same group. As mentioned before, this decrease might be attributed to increased CBF or increased metabolism. In this study, it is shown that cerebral metabolism ($CMRO_2$) was not increased, it was even decreased in most of the events. Meanwhile, CBF (CBFe) showed significant increase on the same events, of $Ca\text{-}jo_2$ decreases. So, the decrease of $Ca\text{-}jo_2$ could be attributed to the increase in CBF. This CBF increase, in the two groups could be caused by the cerebral vasodilating effect of isoflurane anesthesia [23,24]. Another explaination is the decrease in blood viscosity caused by blood withdrawal, in hemodilution group, and blood loss in control group[31,32] and asanguinous fluid replacement.

Cerebral oxygen extraction ($CEO_2$) together with jugular venous oxygen saturation ($SjO_2$) reflect the adequacy of cerebral blood flow to meet the metabolic requirements of the brain. $CEO_2$ did not show any significant difference either on comparing both

studied groups or within each group throughout the whole study period. This finding means that ANH maintain the cerebral oxygen supply-demand balance. This finding was supported experimintally, where they reported that ANH from Hb of 11 to 8 gm/dL increase CBF to maintain sufficient $CMRO_2$ in anesthetised hypocapnic dogs[48]. In the present study, patients had higher hemoglobin levels than that in the previous experiment as a safeguard against any possibility for the occurence of cerebral ischemia.

Changes in cerebral metabolism are associated with parallel changes in CBF (Flow-Metabolism coupling). This coupling is the metabolic explanation of cerebral autoregulation, which maintain a constant CBF over a wide range of cerebral perfusion pressure[49]. This coupling exists between the CBF and $CMRO_2$ under normal circumstances or even under some altered physiological conditions like fever and seizures while, it is disturbed under other pharmacological and pathological influences like brain tumors[50].

In the current study, there was no significant difference in $CMRO_2$ in the hemodilution group compared to the control group at all study intervals. In the control group, $CMRO_2$ was lower at 40 min after induction of anesthesia, at the end of surgery and before endotracheal extubation compared to the basal value (1.89 ± 0.27, 1.82 ± 0.29, 1.84 ± 0.26 Vs 2.06 ± 0.24 respectively). Also, In the hemodilution group, CMRO2 was lower after hemodilution and at the end of surgery compared to the basal value (1.76 ± 0.20 and 1.80 ± 0.23 Vs 1.96 ± 0.29). This decrease in $CMRO_2$ in both groups could be explained by the effect of general anesthesia (especially isoflurane) and good analgesic level. This finding is in accordance with the previous finding that general anesthesia decreases $CMRO_2$ to 1.5-2 ml / 100gm / min in healthy anesthetised adults compared to 3.4 - 3.5 ml/ 100gm/min in young healthy awake adults [51,52].

Cerebral blood flow equivalent (CBFe) did not show any significant difference in the hemodilu-

tion group compared to the control group throughout the whole study period. An increase in CBFe was recorded in the two groups when compared with the basal value in the same group, in most of the events. This increase was earlier in hemodilution group. This increase might be explained by cerebral vasodilating effect of isoflurane[23,24], decreased blood viscosity by blood loss or withdrawal and asanguinous fluid replacement[31,32] or it may be due to cranial decompresion. These results are supported by the previous findings that hemodilution resulted in maintenance of adequate tissue oxygenation due to the underlying improvement of blood fluidity[53,54,55]. Another clinical study reported that ANH using HES 6% increased middle cerebral artery blood flow velocity without any significant change in cerebrospinal fluid pressure[56]. Earlier increase in hemodilution group, support the effect of lowered viscosity more.

High level of lactate generally indicates anerobic metabolism. Lactic acid is formed as a result of anerobic glycolysis that takes place secondary to tissue ischemia either absolute or relative. Adding lactate determination to oxygen transport or utilization parameters monitoring provide a more complete and reliable assessment of tissue oxygen balance. Because lactate level in whole blood and plasma are equivalent, both measurements are called blood lactate. The normal blood lactate concentration is less than 2 mmol/L (0.5 - 2 mmol /L or 4.5 - 20 mg/dl). But the threshold for significant elevated blood lactate is 4 mmol/L[57,58]. The use of lactate as an indicator of cerebral oxygenation imbalance is in agreement with a previous study, stated that moderate cerebral ischemia associated with lactate production may be used as a reliable indicator of cerebral ischemia in acute brain injury[59,60].

Brain has the enzymes required for both the anerobic production of lactate and aerobic utilization of lactate[61]. Hyperlactemia has proven to be protective in states of hypoglycemia[62]. Lactate croses blood brain barrier (BBB) by both simple diffusion and a carrier mechanism making

simple blood lactate interpretation difficult[63].

In the beginning of this work and through a pilot study, we found a very high jugular venous lactate concentration, that might be due to a lag time between blood sampling and analysis so, it is considered that Jugulo -Arterial lactate difference (J-ALD) may overcome time-induced changes in lactate level. Hemodilution did not show any significant difference in J-ALD compared to the control group at all study intervals. This finding is supported by the previous reports that, at the micro circulatory level ANH increase red blood cell velocity in capillaries and enhance flow motion, this allow for better spatial and temporal distribution of red blood cell within the capillary network leading to more homogenous delivey of oxygen to the tissues[64,65]. Also, ANH is reported to maintain cerebral oxygen supply-demand balance[46,47]. The last finding could be confirmed, in the current study, by the stable or even slightly lower lactate level in the hemodilution group.

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# BENHA MEDICAL JOURNAL


## EFFECT OF ACUTE NORMOVOLEMIC HEMODILUTION ON CEREBRAL HEMODYNAMICS, METABOLISM AND OXYGENATION DURING BRAIN TUMORS RESECTION


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# INTRATHECAL FENTANYL VERSUS INTRATHECAL FENTANYL MAGNESIUM IN PEDIATRIC CARDIAC SURGERY

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## Abstract

***Introduction:*** *Fast track cardiac anesthesia improves patient outcome. Magnesium is (NMDA) receptor antagonist used as an adjuvant for postoperative analgesia. There are several studies comparing the efficacy of the different routes of administration of magnesium, such as I.V., Intrathecal (IT), or epidural, in improvement of anesthetic and analgesic quality.*

***Aim of the work (Objective):*** *We aimed to study the effects of both IT fentanyl and IT fentanyl -magnesium on peri-operative analgesic requirements, stress response and the extubation time after elective pediatric cardiac surgery.*

***Patients & Methods:*** *Eighty pediatric patients undergoing elective open cardiac surgery were randomly allocated into four groups (20 patients each) : Intrathecal placebo (control) group (A), intrathecal fentanyl group (B) (received IT 1 μg/kg of fentanyl citrate), intrathecal fentanyl and magnesium (0.5mg) group (C) (received IT 1 μg/kg of fentanyl citrate and 0.5mg/kg magnesium sulphate) and intrathecal fentanyl magnesium (1mg) group (D) (received IT 1 μg/kg of fentanyl citrate + 1 mg/kg magnesium sulphate). The perioperative anesthetic management was standardized. Peri-operative analgesic requirements, weaning from CPB, extubation time and stress response [represented by serum cortisol, and blood glucose levels] were recorded.*

***Results:*** *The results of this study demonstrated that the analgesic profile tended to be better with ITF, ITF-Mg 0.05mg/kg and ITF-Mg*

*1mg/kg groups than the I.V. (control) group. As these groups were associated with less total intraoperative I.V. fentanyl used, less postoperative analgesic requirements (Postoperative fentanyl consumption µg / 24 h) and prolonged time to 1st analgesic requirement when compared with control group. HR and MAP were maintained in group D intraoperatively and early postoperatively ,and time to 1st analgesic requirement is prolonged and time to extubation is shorter in ITF-Mg 1mg/kg group (D) as compared with ITF and control groups.*

***Conclusion:*** *In conclusion, the use of intrathecal fentanyl-magnesium (1mg/kg) in pediatric patients undergoing open cardiac surgery provided superior hemodynamic stability, reduce intra and postoperative analgesic consumption, prolong the time to first analgesic requirement and allows early tracheal extubation when compared with intravenous fentanyl, intrathecal fentanyl or intrathecal fentanyl-magnesium (0.5mg/kg).*

***Keywords:*** *Intrathecal, fentanyl, magnesium, pediatric.*

## Introduction

The trend of early extubation is often used in the perioperative management of adult patients undergoing cardiac surgery. Nowadays, several retrospective studies concerning the feasibility of an early extubation approach in pediatric cardiac patients were published, including patients at high risk for a complicated perioperative course[1,2]. The advantages of this approach may be primarily related to the avoidance of adverse effects of prolonged mechanical ventilation and lower hospitalization costs related to lower ICU and hospital stay[2].

Intrathecal (IT) morphine allows intense postoperative analgesia but has delayed onset of action and may cause delayed extubation after cardiac surgery because of prolonged ventilatory depression[3]. On the contrary, lipid-soluble opioids such as fentanyl and sufentanil have a rapid onset but shorter duration of action [4].

Magnesium is the fourth most plentiful cation in the body. It has antinociceptive effects in animal and human models[5]. These effects are primarily based on the regulation of calcium influx into

the cell, which is natural physiological calcium antagonism, and antagonism of N-methyl-D-aspartate (NMDA) receptor[6]. These effects have prompted the investigation of magnesium as an adjuvant for postoperative analgesia. There are several studies comparing the efficacy of the different routes of administration of magnesium, such as I.V., IT, or epidural and its reflection on anesthetic and analgesic quality[7]. I.V. Magnesium, even high doses, is associated with limited passage across the blood-brain barrier[8].

Studies in humans in which intrathecal magnesium was given to various different groups of patients found that none had symptoms suggestive of neurotoxicity,[9-13] nor did they exhibit signs of systemic toxicity such as hypotension, arrhythmias, somnolence or weakness, and even on long term follow up.

Buvanendran et al in 2002. found that intrathecal 50 mg magnesium prolongs spinal fentanyl analgesia during labour and suggested that the availability of an intrathecal NMDA antagonist could be of clinical importance for pain management[9]. Also, Bilir et al, in 2007 found that co-administration of epidural magnesium for postoperative epidural analgesia provided a pronounced reduction in patient-controlled epidural fentanyl consumption without recorded side-effects[7].

This study hypothesized that preoperative administration of IT fentanyl, and magnesium in different doses in pediatrics undergoing open heart surgery for congenital heart diseases may facilitate the weaning from CPB, improve the quality of postoperative analgesia, decrease stress response and shorten the extubation time. The aim of this study is to compare the effects of both IT fentanyl and IT fentanyl -magnesium in two different concentration on perioperative analgesic requirements, weaning from cardiopulmonary bypass, stress response and the extubation time in elective pediatric cardiac surgery.

## Materials & Methods

After approval of the local ethical committee of Anesthesia And Surgical Intensive Care Depart-

ment, Mansoura University, this prospective double blinded controlled randomized study(using closed envelop method) was conducted on 80 patients of either sex, aged 3 - 14 years were submitted for elective cardiac surgery (VSD, ASD, Subaortic membrane) using CPB at Cardiac Surgery Unit in Children Hospital, Mansoura University. All parents of the patients were given written informed consent prior to enrollment after explanation.

**Exclusion criteria include:** coagulopathy, platelet count <100,000 $mm^3$ and the recent use of anticoagulant drugs. Also the administration of preoperative vasoactive medications as inotropic drugs or preoperative intra-aortic balloon pump, preoperative mechanical ventilation, re-do, heart block, preoperative impaired left ventricular ejection fraction <40%, current clinical diagnosis of either systolic or diastolic congestive heart failure were excluded. Signs of interstitial or alveolar edema on chest x-ray, body mass index > 35 $kg \cdot m^{-2}$, preoperative serum creatinine > 1.5 mg/dl, were excluded from the study. Known or anticipated difficult airway, current use of clonidine or steroids (for their possible confounding effects on postoperative analgesia), and any known contraindication to spinal anesthetic administration were not included.

The intraoperative exclusion criteria were: need of application of deep hypothermic circulatory arrest, CPB times exceeding 90 min. The postoperative exclusion conditions were prolonged (>24 h) mechanical ventilation, hemodynamic instability, and need for large-dose opioids or sedatives, and none happened.

All patients were subjected to preoperative clinical examination for assessment of cardiovascular function. Laboratory investigations includes CBC, electrolytes analysis, ABG, urine analysis, coagulation survey including prothrombin time, partial thromboplastin time, and bleeding time, clotting time, activated clotting time (ACT), blood glucose level, liver and renal function tests. Radiological work, ECG and echocardiographic investigations were fulfilled. Patients were randomly

classified into 4 groups: [A-Control group]: (n=20) Anesthesia was maintained with isoflurane with intrathecal 0.15 ml/Kg preservative free sterile water as a whole volume. [B-ITF (fentanyl) group]: (n=20) Anesthesia was maintained with isoflurane with intrathecal fentanyl 1 µg/Kg. [C-IT fentanyl-0.5 mg/kg magnesium group]: (n=20) Anesthesia was maintained with isoflurane with intrathecal fentanyl 1µg/Kg with magnesium sulfate 0.5 mg/Kg. [D-IT fentanyl-1.0mg/kg magnesium group]: (n=20). Anesthesia was maintained with isoflurane with intrathecal fentanyl 1µg/Kg with magnesium sulfate 1 mg/Kg. All injectate were given 0.15 ml/Kg as whole volume in preservative free sterile water with maximum volume 4 ml.

All children were fasted according to fasting protocol with age for at least 4 hours before the surgery. Intake clear fluids were allowed up to 3 hours before the induction of anesthesia.

All patients were pre-medicated in the pre-operative area with I.M. 0.05 mg/Kg midazolam and 0.015 mg/Kg atropine sulfates 15 minutes before induction of general anesthesia. five leads ECG, peripheral oxygen saturation [$SpO_2$] and non - invasive blood pressure [NIBP] were monitored. Supplemental oxygen were provided via a face mask. Peripheral intravenous indwelling cannula was inserted one hour after application of EMLA cream.

In all groups Anesthesia was induced by slow I.V. administration of fentanyl 10 µg/Kg, propofol 1-2 mg/Kg and 0.1 mg/Kg vecuronium to provide neuromuscular blockade and facilitate tracheal intubation. With loss of consciousness, positive pressure ventilation was started via face mask at a rate of 20-28 breathes per minute. Patients were intubated orally with an appropriately sized endotracheal tube such that no audible air leak was present below 35 $cmH_2O$ peak inspiratory pressures.

Patients were mechanically ventilated with 100% $O_2$ and the end-tidal $CO_2$ was monitored by main-stream capnograph and maintained between 30-35 mmHg.

Anesthesia was maintained with isoflurane, incremental 0.02 mg/Kg doses of vecuronium to maintain muscle relaxation and additional I.V. boluses of fentanyl (1 μg/kg) were given if blood pressure and heart rate increased (>20% above baseline) and it was assumed that the increases were due to insufficient depth of anesthesia.

A radial artery catheter (22 - 24 G) was inserted, after performance of a modified Allen's test in the non dominant hand, to monitor the arterial blood pressure and blood gases sampling during the entire procedure. A urinary catheter was placed to monitor urine output and rectal and nasopharyngeal temperatures were continuously monitored, an appropriate size central venous catheter was inserted under complete aseptic condition for central venous pressure monitoring [insertion done after intrathecal injection to allow maximum time (more than 45 to 60min) between intrathecal and heparin injection].

The patient was placed in the right lateral decubitus position. The skin over the injection area was cleaned with a chlorohexidine 0.5% containing solution, which was allowed to dry. Then, using sterile technique a 2-inch, 25-gauge Quincke spinal needle was inserted at L3-4 or L4-5. Successful dural puncture was confirmed by observation of a free flow of cerebrospinal fluid, and the injection was performed with the bevel of the needle oriented in the cephalic direction and the table turned with head up position for 5 mins [hypobaric solution(specific gravity measured for different injectant solution using combitest strips and was about 1000 for placebo (water),about 1000-1005 for fentanyl in water and about 1005 for both fentanyl-0.5mg magnesium and fentanyl- 1mg magnesium)] to get high level.

The heart was approached through a standard median sternotomy in all patients. The ascending aorta, SVC and IVC were cannulated for cardiopulmonary bypass (CPB) using a membrane oxygenator and a roller pump with an arterial line filter after elevation of the ACT above 480 second using heparin (300-400

i.u./kg). Perfusion was a non-pulsatile pump flow with average rate around 2-2.4 L/min/$m^2$. An α-stat carbon dioxide management strategy (blood gas measurements corrected to body temperature) were employed. Moderate hemodilution and moderate systemic hypothermia (32$^oC$) were used. Once the target core temperature was achieved, 10 mg/kg of sodium thiopental was added to the CPB circuit because of its cerebroprotective benefits. After aortic cross clamping, all patients were received cold crystalloid cardioplegia, that was delivered (20 ml/kg for the first dose and 10 ml/kg for subsequent doses every 25-30 min) in anterograde manner in the aortic root. Surface cooling was used by packed iced saline. Cardioplegia was repeated every 25 minutes. Hematocrit was maintained between 20-25% during CPB.

H.R, MAP, CVP, $EtCO_2$, $SpO_2$, nasopharyngeal and rectal temperatures, arterial blood gases and electrolytes (Na and K), were recorded at the following time intervals: Before induction of general anesthesia [baseline], after induction, after skin incision , after sternotomy, each 15 minutes until CPB, and then each 15 minutes after discontinuation of cardiopulmonary bypass. Hypertension and hypotension were considered clinically significant when systolic arterial pressure differed by more than 30% compared with baseline values for more than 1 minute. Bradycardia was considered clinically significant when heart rate decreased by more than 30% compared with baseline values for more than 1 minute. Requirement for additional bolus doses of fentanyl (1μg/kg) was determined by the increases in blood pressure and heart rate by more than 20% of baseline values where other factors were excluded, and it was believed that the increases were caused by insufficient depth of anesthesia. ACC (minutes), CPB (minutes), spontaneous recovery of the heart, need for DC shocks and the inotropic and / or vasopressor support to wean the heart from CPB were recorded in all patients.

After surgery, patients were transferred to the ICU, where they were monitored by other team who

were blinded to the anesthetic protocol that had been used. In the ICU. H.R, MAP, CVP were recorded at one hour intervals after admission to ICU, for 12 hours.

Post - extubation hourly pain score was assessed using objective pain discomfort score (10 points scale 0= no pain &10 = maximum pain) in children for 12 hours (14).

**Blood pressure:**

2 → > 20 % pre - operative.
1 → (11 - 20 %) above pre - operative.
0 → ≤10 % pre - operative.

**Crying:**

2 → crying not responding to TLC [Tender, Loving, Care].

1 → crying but responds to TLC.
0 → not crying.

**Moving:**

2 → Thrashing.
1 → Restless.
0 → None.

**Agitation:**

2 → Hysterical.
1 → Mild.
0 → patients asleep or calm.

**Verbal:**

2 → Moderate pain [localized verbally or by pointing].
1 → mild pain [can't localize].
0 → Asleep or no pain.

Bromage score, was assessed after extubation and graded as: I - Free movement of legs and feet [Nil (0%)], II - Just able to flex knees with free movement of feet [Partial (33%)], III - Unable to flex knees, but with free movement of feet, [Almost complete (66%)], IV - Unable to move legs or feet [Complete (100%)].

Extubation time, the need for re - intubation (Tracheal extubation was accomplished when standard extubation criteria were fulfilled. Criteria for extubation included a responsive and cooperative patient, negative inspiratory force of > 20 cm $H_2O$, vital capacity > 10 mL/kg, $PaO_2$ > 80 mmHg with fraction of inspired oxygen < 0.5, pH > 7.3, core temperature > 36.5 Co, hemodynamic stability, limited chest tube drainage and no uncontrolled dysrrhythmia,). First request for analgesic requirement and the total dose of analgesic consumption

[i.v. fentanyl μg/Kg], the length of ICU and hospital stays were recorded. Neurological evaluation were done on daily basis to exclude any signs of neurological abnormality.

In this study the Stress response to surgery and CPB was determined by changes in serum cortisol, and blood glucose concentrations. Venous blood samples were collected at four times: preoperatively, 5 min after sternotomy, 1 and 24 hours after CPB. These times were based on likely physiological responses over time and local economic limitations. The blood samples were centrifuged and serum were drawn off and were stored at 4 $C^o$ until assayed within 2 weeks of collection. Serum cortisol levels were determined using a radioimmunoassay technique (Streptavidin Coated microparticles). The sensitivity (Lower detection limit) is 0.018 μg/dL (0.5 nmol/dL) and the coefficient of variation 9%.

Serum magnesium level was measured preoperative and postoperative to exclude any systemic effect of intrathecally given magnesium.

**Statistical Analysis:**

The statistical analysis of data done by using excels program and SPSS (SPSS, Inc, Chicago, IL). program statistical package for social science version 10. To test the normality of data distribution K-S (Kolmogorov-Smirnov) test was done only significant data revealed to be nonparametric. The analysis of the data was done to test statistical significant difference between groups. ONE WAY ANOVA test to compare more than two groups, followed by Post Hoc test LSD (least significant difference) for inter groups comparisons. For quantitative data 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. P value was considered significant if <0.05 at confidence interval 95%.

## Results

Table 1 shows the patients demographics of the four studied groups, control group (I.V.), ITF group, ITFMg (0.5mg) group, and

ITFMg (1mg) group, there were no significant differences between the four groups as regards age,body mass index (BMI), male to female ratio and the type of surgical procedure.

In this study intraoperative parameters, (ACC (min), CPB (min), lowest temperature on CPB ($C^o$), and operative time (min)] showed no significant differences between the four groups but the total dose of intraoperative I.V. fentanyl used was significantly lower in ITF group, ITFMg (0.5mg) group, and ITFMg (1mg) group when compared with the control group. (Table 2).

In this study, no significant differences between the four groups as regard to criteria of weaning from CPB (Table 3).

In the present study, most of the patients in the four groups were extubated early (within 6 hours). However, the number of patients who were extubated in the operating room was significantly higher in the ITFMg (1mg) group (6 patients) when compared with the other groups (Table 4). The time to extubation was significantly lower in the ITFMg (1mg) group when compared with the control and ITF groups (Table 4). Postoperative fentanyl consumption was significantly lower in ITF group, ITFMg (0.5mg) group, and ITFMg (1mg) group when compared with the control group (Table 4). The time to 1st analgesic request was significantly longer in ITF group, ITFMg (0.5mg) group, and ITFMg (1mg) group when compared with the control group and also significantly longer in ITFMg (1mg) group when compared with the ITF group and ITFMg (0.5mg) group (Table 4). There were no significant differences between the four groups as regard ICU and hospital length of stay. There were no significant differences between the four groups as regard postoperative Bromage score and adverse effects as nausea and vomiting, pruritus, and neurologic deficit (Table 4).

Table 5 showed laboratory evaluation of the stress response (serum cortisol and blood glucose) and serum magnesium. There were no significant differences

between the four groups in serum cortisol and blood glucose at different stages of evaluation, but there were significantly high serum cortisol and blood glucose at 1 hour after termination of CPB when compared with their basal values in the four groups (Table 5). There were no significant differences between preoperative and postoperative serum magnesium (Table 5).

In this study, postoperative modified objective pain score was significantly lower in the ITFMg (1mg) group in the early postoperative period when compared with the other groups (Table 6).

In the current study, the mean arterial blood pressure (Table 7) and heart rate (Table 8) was significantly lower in the ITFMg (1mg) group introperatively and early postoperatively when compared with the other groups. Also, there were no significant differences between the four groups as regard the perioperative central venous pressure, $SaO_2$, $PaO_2$ and $PaCO_2$.

**Table 1 :** Patients characteristics in the studied groups. Data are expressed as Mean ± SD and number.

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group(*n = 20*)** | **ITFMg(1mg) group(*n = 20*)** |
|---|---|---|---|---|
| Age (year) | 5.28 ± 2.94 | 4.04 ±1.51 | 4.86 ± 2.34 | 4.91 ± 2.62 |
| Body mass index ($kg/m^2$) | 18.33 ± 5.18 | 22.13 ± 6.04 | 17.40 ± 4.09 | 19.53 ± 6.89 |
| Gender (M/F) | 12/8 | 13/7 | 11/9 | 13/7 |
| Surgical procedure | | | | |
| ASD | 10 | 11 | 9 | 10 |
| VSD | 8 | 8 | 9 | 8 |
| Subaortic membrane | 2 | 1 | 2 | 2 |

**Table 2 :** Intraoperative variables in the studied groups. Data are expressed in Mean ± SD.

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group (*n = 20*)** |
|---|---|---|---|---|
| ACC (min) | 29.00±6.81 | 27.00±5.41 | 29.26±12.61 | 29.53±7.18 |
| CPB (min) | 43.00±6.72 | 44.06±7.77 | 44.40±16.07 | 45.26±9.39 |
| Minimum temperature on CPB( C°) | 32.06±1.75 | 32.26±1.79 | 31.66±1.79 | 32.13±1.50 |
| Surgical time (min) | 146.13±11.32 | 147.33±9.50 | 146.07±11.51 | 149.93±10.50 |
| Total Intraoperative IV fentanyl used (µg/kg) | 18.06 ± 2.43 | 15.60±2.84* | 14.40±2.02* | 12.26±1.38* |

*P < 0.05 Significant when compared with control group
ACC : Aortic cross clamp time
CPB : Cardiopulmonary bypass time

**Table 3 :** Weaning off CPB parameters in the studied groups. Data are expressed in Mean ± SD, number, and percentage.

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group(*n = 20*)** |
|---|---|---|---|---|
| Spontaneous recovery of heart | 19(95%) | 18 (90%) | 18(90%) | 19(95%) |
| DC shock | 1(5%) | 2(10%) | 2 (10%) | 1 (5%) |
| Dopamine use | 7 (35%) | 6 (30%) | 6 (30%) | 8 (40%) |
| Dopamine dose (µg/kg/min) | 5 ± 1.9 | 6 ± 1.2 | 5 ± 1.6 | 5 ± 2 |
| Nitroglycerine use | 20 (100.0%) | 20(100.0%) | 20 (100.0%) | 20 (100.0%) |
| Nitroglycerine dose (µg/kg/min) | 2.76±1.29 | 2.83±1.33 | 2.66±1.34 | 2.80±1.26 |

**Table 4 :** Postoperative variables in the studied groups. Mean ± SD, number, percentage.

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group (*n = 20*)** |
|---|---|---|---|---|
| OR extubation | 0 (.0%) | 0 (.0%) | 3 (15%) | 6 (30%) # |
| Extubation time (hr) | 4.80 ± 1.43 | 4.10 ± .84 | 3.69 ±1.18 | 1.84 ± .57# |
| Time to 1st analgesic requirement(hr) | 2.86 ±1.64 | 7.30 ± 2.02* | 8.42 ± 2.04* | 10.33± 5.36* † |
| Postoperative fentanyl consumption µg /kg/ 24hr | 7.06 ± 2.31 | 4.93 ± 2.37* | 4.66 ± 2.09* | 3.76 ± .97* |
| ICU length of stay(hr) | 32.60 ±3.26 | 31.80 ± 3.50 | 30.60 ± 3.54 | 30.33 ± 3.99 |
| hospital length of stay (days) | 6.13 ± .63 | 5.80 ± .67 | 5.80 ± .67 | 5.80 ± .67 |
| Nausea and Vomiting | 2 (10%) | 2 (10%) | 1 (5%) | 1(5%) |
| Pruritus | 0 (.0%) | 0 (.0%) | 0 (.0%) | 0 (.0%) |
| Neurologic deficit | 0 (.0%) | 0 (.0%) | 0 (.0%) | 0 (.0%) |
| Bromage score | I | I | I | I |

* P < 0.05 Significant when compared with control.
† P < 0.05 Significant when compared with ITF and ITFMg (0.5mg) groups.
# P < 0.05 Significant when compared with control and ITF groups.

**Table 5 :** Laboratory evaluation [[serum Cortisol (µg/dl), Blood glucose (mg/dl), Serum $Mg^{+2}$(mg/dl)]. Data are expressed in Mean ± SD

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group (*n = 20*)** |
|---|---|---|---|---|
| **SerumCortisol** (µg/dl) | | | | |
| Basal | 14.82 ± 3.18 | 15.14 ± 5.33 | 15.20 ± 4.07 | 15.52 ± 4.18 |
| Poststernotomy | 15.22 ± 4.52 | 17.76 ± 4.56 | 15.35 ± 4.13 | 14.45 ± 4.78 |
| 1hr post CPB | 59.28±16.60* | 57.08±13.78* | 60.92± 9.74* | 61.78 ± 6.41* |
| 24hr postoperative | 16.41 ± 8.11 | 17.27 ± 7.00 | 18.12 ± 6.42 | 17.82 ± 3.75 |
| **Blood glucose** (mg/dl) | | | | |
| Basal | 79.26 ± 5.03 | 79.40 ± 5.74 | 79.60 ± 5.48 | 78.26 ± 5.92 |
| Poststernotomy | 117.33 ± 6.94 | 115.13 ± 6.34 | 111.47 ± 5.76 | 110.80 ± 6.48 |
| 1hr post CPB | 206.13 ±11.89 * | 213.27± 12.63 * | 207.40±14.48 * | 202.13± 27.86 * |
| 24hr postoperative | 81.86± 5.40 | 82.33± 5.82 | 80.40± 5.35 | 79.86± 5.33 |
| **Serum $Mg^{+2}$** (mg/dl) | | | | |
| preoperative | 1.50 ± .58 | 1.34 ± .42 | 1.36 ± .48 | 1.47 ± .48 |
| postoperative | 1.43 ± .53 | 1.31 ± .36 | 1.27 ± .46 | 1.40 ± .37 |

* $P < 0.05$ Significant when compared with their basal value

**Table 6 :** Postoperative Pain score in the studied groups, Data are expressed in median ( range)

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group (*n = 20*)** |
|---|---|---|---|---|
| After1 hr | 5(3-7) | 5(3-8) | 5(3-7) | 2(0-5)* |
| After 2 hr | 4(3-6) | 4(3-6) | 4(3-5) | 2(0-4)* |
| After 3 hr | 4(3-6) | 4(1-5) | 4(1-5) | 2(0-4)* |
| After 4 hr | 4(2-5) | 4(1-5) | 3(1-5) | 1(1-4)* |
| After 5 hr | 4(2-5) | 4(1-5) | 3(1-4) | 2(1-3)* |
| After 6 hr | 4(2-5) | 3(0-5) | 3(0-5) | 2(1-3)* |
| After 7 hr | 4(2-5) | 3(1-4) | 3(1-4) | 1(0-3)* |
| After 8 hr | 3(2-5) | 3(1-5) | 3(2-4) | 2(0-3)* |
| After 9 hr | 3(2-4) | 3(0-5) | 3(1-5) | 1(0-3)* |
| After10hr | 3(2-5) | 3(0-5) | 2(0-4) | 1(0-2)* |
| After11 hr | 3(2-4) | 3(0-4) | 2(0-4) | 1(0-2)* |
| After12 hr | 3(2-4) | 3(0-4) | 2(0-4) | 1(0-2)* |

* $P < 0.05$ Significant when compared with control, ITF and ITFMg (0.5mg) groups.

**Table 7 :** Perioperative MAP (mmHg) in the studied groups, Data are expressed in Mean ± SD

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group (*n = 20*)** |
|---|---|---|---|---|
| **Basal** | 70.80 ± 5.15 | 71.46 ± 4.54 | 70.66 ± 4.83 | 70.33 ± 3.26 |
| **Intraoperative** | | | | |
| Post induction | 81.86 ± 5.01 | 80.73 ± 6.95 | 79.73 ± 5.04 | 82.00 ± 5.00 |
| After skin incision | 80.46 ± 5.39 | 78.00 ± 5.95 | 79.13 ± 5.16 | 75.20 ± 4.27* |
| Post sternotomy | 82.46 ± 4.38 | 77.66 ± 5.36 | 78.66 ± 4.13 | 72.93 ± 3.76* |
| 30 min post CPB | 81.33 ± 4.32 | 77.73 ± 5.89 | 76.20 ± 5.89 | 70.80 ± 4.76* |
| 60 min post CPB | 83.00 ± 5.05 | 76.86 ± 6.41 | 77.13 ± 5.15 | 72.00 ± 5.87* |
| **Postoperative** | | | | |
| After 1 hr | 82.86 ± 5.11 | 78.86 ± 6.77 | 77.73 ± 4.81 | 71.80 ± 5.47* |
| After 6 hr | 80.66 ± 6.60 | 79.00 ± 6.67 | 78.40 ± 5.09 | 74.20 ± 5.93* |
| After 12hr | 79.33 ± 6.44 | 79.86 ± 5.97 | 79.86 ± 5.48 | 75.06 ± 5.61 |

* Significant when compared with control group

**Table 8 :** Perioperative HR (beats/min) in the studied groups, Data are expressed in Mean ± SD

| | **Control group (*n = 20*)** | **ITF group (*n = 20*)** | **ITFMg(0.5mg) group (*n = 20*)** | **ITFMg(1mg) group (*n = 20*)** |
|---|---|---|---|---|
| Basal | 106 ± 5 | 105 ± 8 | 106 ± 7 | 106 ± 6 |
| **Intraoperative** | | | | |
| Post induction | 115 ± 4 | 113 ± 6 | 115 ± 4 | 109 ± 22 |
| After skin incision | 119 ± 26 | 117 ± 8 | 119 ± 7 | 115 ± 5 |
| Post sternotomy | 122 ± 4 | 118 ± 7 | 117 ± 6 | 111 ±4* |
| 30 min postCPB | 126 ± 7 | 120 ± 7 | 116 ± 8 | 113 ± 6* |
| 60 min postCPB | 129 ± 5 | 121 ± 6 | 120 ± 9 | 115 ± 8* |
| **Postoperative** | | | | |
| After 1 hr | 128 ± 10 | 120 ± 7 | 121 ± 9 | 114 ± 7* |
| After 6 hr | 125 ± 8 | 121 ± 6 | 119 ± 11 | 114 ±7* |
| After 12hr | 125 ± 7 | 121 ± 6 | 121 ± 6 | 119 ± 8 |

* Significant when compared with control

## Discussion

The results of this study demonstrated that the use of intrathecal fentanyl/magnesium (1mg/kg) in pediatric patients undergoing open cardiac surgery provided superior hemodynamic stability, reduce intra and postoperative analgesic consumption, prolong the time to first analgesic request and allows early tracheal extubation when compared with intravenous fentanyl, intrathecal fentanyl or intrathecal fentanyl/magnesium (0.5mg/kg) but did not affect the stress response to CPB.

In this study, the analgesic profile tended to be better with ITF, ITF-Mg 0.05mg/kg and ITF-Mg 1mg/kg groups than the control group in pediatric patients undergoing cardiac surgery. As this groups were associated with less total intraoperative i.v. fentanyl used, less postoperative analgesic requirements (Postoperative fentanyl consumption µg / 24hr) and prolonged time to 1st analgesic requirement when compared with the control group. Also time to 1st analgesic requirement is prolonged in ITF-Mg 1mg/kg group compared with ITF and ITF-Mg 0.05mg/kg groups. This is in accordance with earlier clinical human investigations reported an increase in duration of analgesia with intrathecal magnesium in various obstetric and non-obstetric populations[9-13]. O¨ Zalevi et al.[13] concluded that ; in patients undergoing lower extremity surgery, IT $MgSO_4$ (50 mg), when added to spinal anaesthesia induced by bupivacaine and fentanyl, delayed the onset of both sensory and motor blockade and prolonged the duration of anaesthesia, without increasing the incidence of side-effects. However other study found that the addition of $MgSO_4$ to spinal anaesthesia prolonged the time to first analgesic requirement, but did not reduce total analgesic consumption in the first 24 h[10].

In another study, Pirat et al.[15] compared cardiovascular and neurohumoral responses associated with I.T. fentanyl (2 µg/kg), or I.V. (10 µg/kg followed by an i.v. infusion of 10 µg / kg/ hr) fentanyl in pediatric patients undergoing cardiac surgery. They found that patients receiving combination of both I.V. and IT fentanyl had less

hemodynamic response to sternotomy than those in either of the other groups, in his study the three groups required similar amounts of additional fentanyl during the operation (Group I.V., 2.5 ±4.0 μg/kg; Group IT, 2.2 ±3.6 μg/kg; and Group IT + I.V. 2.4 ± 3.5 μg/kg), also there were no significant differences among the groups with respect to postoperative analgesic requirement.

In the current study, all patients in the four groups extubated early (less than 6 hours), however, the number of patients who were extubated in the operating room was significantly higher in ITF-Mg 1mg/kg group than other groups, and the time to extubation was shorter in ITF, ITF-Mg 0.05mg/kg and ITF-Mg 1mg/kg groups than the I.V. control group and this may be explained may by the decrease in the total intraoperative intravenous fentanyl used in these groups (18.06 ± 2.43 μg/kg in the control group, 15.60±2.84 μg/kg in ITF group, 14.40±2.02 μg/kg in ITF-Mg 0.05mg/kg group and 12.26±1.38 μg/kg in ITF-Mg 1 mg/kg group) as explained by other studies[16].

In contrast, Pirat et al.[15] cannot explain the significantly shorter extubation times in pediatric cardiac surgery that were recorded for Group IT + I.V., although using high dose of intravenous fentanyl (49.7 ± 6.1μg/kg). Davis et al.[17] defined intraoperative variables predictive of early extubation, CPB time, aortic cross clamp time and circulatory arrest time.

The present study showed that no significant differences as regard ICU and hospital length of stay. We found no significant hemodynamic effect (hypotension) following the addition of magnesium to our spinal solution and addition of intrathecal magnesium to fentanyl provides hemodynamic stability. This may be attributed to the absence of systemic vasodilator effects of spinal magnesium in this dose and this was shown in normal $Mg^{+2}$ serum level in the postoperative values.

As regard the stress response to cardiac surgery in this study, we used serum cortisol and whole-blood glucose as hormonal and metabolic indicators of the stress

response. The four groups showed significantly higher serum cortisol and blood glucose levels at one hour after CPB when compared with basal values. These findings concur with the results of other study[15] and are believed to be related to several factors as, systemic inflammatory response, hypotension, and hypothermia associated with CPB, independent of the opioid dose used. The results indicate that the above 4 mentioned anesthetic regimen were not enough to blunt stress response.

Considering respiratory depression, pruritus, nausea and vomiting all of which are well recognized adverse effects of spinal opioid usage. These side effects were nearly absent and there were no significant differences between studied groups. The decreased incidence of respiratory depression (as the postoperative arterial blood gases indicated no significant difference between the 4 groups), nausea and vomiting may be explained by the pharmacokinetics of fentanyl, which is lipid-soluble opioid, binds fairly rapidly with the opioid receptors in the dorsal horn of the spinal cord leaving only small amounts of substance for cephalad migration to the fourth ventricle, and chemoreceptor trigger zone, in contrast with the less lipid-soluble morphine, being hydrophilic drug, which spread rostrally in the CSF more rapidly and to a greater extent than do hydrophobic opioid[18].

This in accordance with the result of Pirate et al.[15] who found no problems with this adverse effects on similar group of patients although using larger doses of fentanyl both intrathecal and intravenous.

Varassi et al.[19] reported that the subarachnoid administration of 25 μg of fentanyl during spinal anaesthesia in non-pre-medicated men did not cause early respiratory depression in elderly patients. An increased risk of respiratory depression in labouring parturient has been reported with systemic $MgSO_4$ therapy[20], and an increased incidence of respiratory depression may be expected when these two drugs are combined; however, we did not observe this in this study, and this in accor-

dance with other studies that investigate neuroaxial magnesium[9,10,13], all of this studies proved that absence of respiratory depressant effect of neuroaxial magnesium.

Urinary retention is another side effects of intrathecal opioids, we recorded no cases of urinary retention because all patients had urinary catheter in place inserted before surgery an remained in place in the ICU.

In this study, there were no neurological deficit, or signs suggestive epidural haematoma, nerve compression or neurotoxicity even on long term follow up (up to 2 months), the absence of epidural haematoma may be due to use of a 25-gauge spinal needle inserted at L3-4 or L4-5 approximately one hour before administration of heparin and this coincides with other studies[15,17,21].

The current study showed no significant differences between the studied groups in the criteria of weaning off CPB as regard the spontaneous recovery of the heart, the need for DC shocks for defibrillation and the need for inotropes or vasodilators. This is explained by other study that reported that myocardial injury in pediatric cardiac surgery in infants and children was dependent on age and ischemic time and type of surgical procedure[22]. Also previous ventricular function determine the need for inotropic support during weaning off CPB (23). Since that the age, type of surgical procedure and aortic cross clamp time were nearly the same in the four studied groups, there were no significant differences in the weaning parameters between the studied groups. Thus, this study indicates that weaning off CPB was not affected by intrathecal fentanyl or intrathecal fentanyl-magnesium.

The present study showed that, heart rate and mean arterial blood pressure both intra and post operatively were significantly lower in intrathecal fentanyl-magnesium 1mg/kg group than the control group. The explanation for this is that spinal opioids may attenuate increased sympathetic nerve activity elicited by myocardial ischemia

and spinal opioids may only partially attenuate the stress response in patients undergoing cardiac surgery[24] and addition of magnesium had synergistic effects to spinal opioid in this dosage.

The current study indicates that preoperative administration of intrathecal fentanyl-magnesium 1mg/kg in pediatrics submitted to open heart surgery for congenital heart repair provided lower pain scores in the early postoperative period with subsequent lower dosages of fentanyl consumed postoperatively when compared with the control group. Because no published study has assessed the effectiveness of intrathecally injected $MgSO_4$ in preventing post-operative pain in pediatric cardiac surgery, no reported data are available for comparison.

In conclusion, the use of intrathecal fentanyl-magnesium (1mg/kg) in paediatric patients undergoing open cardiac surgery provided superior hemodynamic stability, reduce intra and postoperative analgesic consumption, prolong the time to first analgesic request and allows early tracheal extubation when compared with intravenous fentanyl, intrathecal fentanyl or intrathecal fentanyl-magnesium (0.5mg/kg).

**Recommendation :-** Further studies are recommended to confiram this results and also using different doses of magnesium, large number of patients, and long term follow up for subsequent years.

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# BENHA MEDICAL JOURNAL


## INTRATHECAL FENTANYL VERSUS INTRATHECAL FENTANYL MAGNESIUM IN PEDIATRIC CARDIAC SURGERY


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# VALUE OF CD56 IMMUNOHISTOCHEMISTRY IN DISTINGUISHING PAPILLARY THYROID CARCINOMA FROM OTHER LESIONS OF THE THYROID GLAND

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## Abstract

***Background and aim of the work:*** *Pathological diagnosis is essential for optimal treatment of thyroid tumors. Diagnostic difficulties are caused by morphological similarities of biologically diverse lesions, such as papillary hyperplasia versus encapsulated papillary carcinoma. Also there may be difficulties in differentiation between follicular adenoma and minimally invasive follicular carcinoma or differentiating follicular variant of papillary thyroid carcinoma (FVPTC) from follicular adenoma. The purpose of this study was to evaluate the diagnostic value of CD56 immunohistochemical staining in distinguishing papillary thyroid carcinoma (PTC) from other thyroid malignancies and benign lesions.*

***Materials and Methods:*** *Immunohistochemistry using the chain polymer conjugated method was performed for antibody against CD56 in 129 cases of both benign and malignant thyroid lesions. Membranous staining only was considered as positive. The data were submitted for statistical analysis.*

***Results:*** *Statistical analysis showed significantly different expressions of CD56 in PTC versus other lesions of the thyroid gland except anaplastic carcinoma (AC). The sensitivity and specificity of CD56 for diagnosing PTC vs other thyroid lesions were 100% and 92% respectively. It also showed a specificity of 100% and a sensitivity of 22% for follicular adenoma (FA) vs follicular carcinoma (FC).*

***Conclusion:*** *CD56 is a negative diagnostic marker for PTC including*

*its follicular variant and can significantly differentiate PTC from other malignant and benign lesions of the thyroid gland. It is also a highly specific marker for benign lesions and is able to differentiate them from malignant lesions. On the other hand, it is of low sensitivity, but with higher specificity for follicular adenoma vs follicular carcinoma. So, we recommend the use of CD56 immunohistochemistry with other marker such as galectin-3, which is of value in differentiating follicular adenoma from follicular carcinoma.*

***Key Words:*** *CD56, papillary thyroid carcinoma, immunohistochem-*

## Introduction

Thyroid cancer represents the most common malignancy of the endocrine system and the seventh most frequent human malignancy; with Papillary carcinoma is the commonest thyroid cancer[1]. It represents 1.5% of all cancers in Egypt[2]. In the recent decades, an obvious increase in the incidence of papillary thyroid carcinoma has occurred, which is due to advances in medical surveillance of impalpable nodules. The pathological diagnosis of this cancer is usually easy in the majority of cases. However, there may be misinterpretation of the follicular variant of papillary carcinoma as follicular adenoma[3,4]. Also there may be difficulties in differentiation between follicular adenoma and minimally invasive follicular carcinoma5. Distinction of hyperplastic epithelial pseudopapillae from true papillary neoplasia can represent a significant diagnostic problem[6].

Recently, immunohistochemistry has been investigated for its ability to differentiate benign from malignant thyroid lesions such as antibodies against HBME-1, galectin-1, galectin- 3, Rb-1, S100 protein, CD56, ceruloplasmin, lactoferrin and secretory components[3,6,7.8.9].

D56 is a homophilic binding glycoprotein of the Ig-super family in which its antibody targets an isoform of the neural cell adhesion molecule (NCAM) and is expressed normally in NK cells, activated T cells, large granular lymphocytes, specific endocrine, and brain tissue[10,11]. It has been reported that reduced expression of NCAM affects the formation of metastasis

with poor prognostic outcome in some malignancies by stimulating lymphangiogenesis through the increased production of VEGF-C and VEGF-D by tumor cells[12,13].

## MATERIALS AND METHODS

A total of 129 cases that underwent total or subtotal thyroidectomy between January 2004 and July 2010 (both retrospective and prospective) were retrieved from the archives of the pathology department, and oncology center, faculty of medicine, Mansoura University. All the clinical charts and histopathology reports were reviewed for data regarding the patient age & sex, tumor size, the presence or absence of extrathyroid extension, distant metastasis and the presence or absence of lymph node involvement.

The study included 36 cases of papillary thyroid carcinoma, 14 cases of follicular carcinoma,7 cases of medullary carcinoma, 7 cases of anaplastic carcinoma, one mixed medullary papillary carcinoma, one insular carcinoma, 18 cases of follicular adenoma, 7 cases of Hurthlre adenoma, 7 cases of hashimoto thyroiditis, 19 cases of colloid nodular goiter, 5 cases of Grave's disease, one dyshormonogenetic goiter and 2 cases of lymphocytic thyroiditis. Cases of papillary carcinoma were divided into 2 groups: (i) overt papillary carcinoma more than 1cm in size, and (ii) papillary micro carcinoma less than or equal 1cm in size. All neoplastic and non neoplastic cases and carcinomas metastasized to lymph nodes were immunostained for CD56.

### Immunohistochemical stains and interpretation

The selected paraffin blocks (included both the representative sections of the analysed tumor and the rims of morphologically normal thyroid tissue around them, which served as an internal control) were sectioned at 4-5μm and stained by haematoxyline and eosin (H&E) to revaluate and subclassify tumors according to the World Health Organization (WHO) classification, (2004)[14]. Immunohistochemistry using the chain polymer conjugated method for CD56 antibody was used.

Sections were deparaffinized, rehydrated in graded alcohols,

and subjected to an antigen retrieval procedure. Antigen retrieval was performed by placing the slides in boiling EDTA solution at PH 9.0 for 15 minutes using a microwave. The antibody used was CD56 IgG1/k (clone 123C3.D5) mouse monoclonal antibody; Cell Marque corporation product Cat. #156M-88 (7.0ml) ready-to-use. Positive external control used was neuroblastoma and positive internal control was normal thyroid follicular cells.

Evaluation of immunohistochemical staining was performed in a semi-quantitative fashion according to the estimated percentage of positive tumor cells: 0, stained in less than 10% of the tumor cells; 1, stained in 10-25% of the tumor cells; 2, stained in 26-50% of the tumor cells; 3 stained in more than 50% of the tumor cells. A score of 0 was considered as negative, and scores of 1 to 3 were considered as positive. Membranous expression only was considered positive.

**Statistical analysis :**

Data entry and analyses were performed using SPSS statistical package version 10 (SPSS, Inc., Chicago, IL, USA). Quantitative data were presented as mean and standard deviation and the Student t-test was used to compare means of two groups. Qualitative data were presented as number and percentage and chi-square ($\chi^2$) was used to test the association between raw and column variables. The sensitivity, specificity and accuracy of CD56 in differentiation of the different thyroid lesions were performed. P value of < 0.05 and of < 0.001 indicate a significant result and a high significant result respectively while P value of >0.05 indicates non significant result.

## Results

Most of the patients were women (67%), with a mean age of 42.5 years. The peak incidence of benign cases was less than 40 years of life, while in the malignant cases it was between 40 and 60 years. It was found that there is a significant association between malignancy and the age category more than 60 years (P= 0.032). When the neoplastic lesions were divided into tumors less than and those more than 4 cm 51.4% of

malignant lesions were more than 4 cm, while 80% of follicular adenomas were less than 4 cm. It was found that, there is a statistical significant association between the size group more than 4 cm and malignancy (p= 0.029).

From table (1), CD56 expression was higher in benign than malignant lesions with 96.61% and 31.4% respectively. The metastatic deposits of PTC within lymph nodes showed also absent CD56 expression, as seen in their thyroid primaries. In the mixed medullary papillary carcinoma, the medullary component was positive, while the papillary component was negative for CD56.

Table (2) showed that, CD56 was able to differentiate PTC including the follicular variant from FC, MC, FA and benign non neoplastic lesions to a statistically significant degree. It was also able to differentiate benign lesions from malignant lesions. Regarding follicular adenoma, CD56 showed no statistical significance in differentiating this lesion from follicular carcinoma, and Hurthle carcinoma. There was also no statistically significant difference between overt papillary carcinoma and papillary micro carcinoma.

As seen in table (3), CD 56 was 100% sensitive and 85% specific in differentiating PTC from other thyroid lesions. The specificity, positive predictive values and accuracy increased to 92%, 84% and 94% respectively when AC cases were excluded, which showed similar expression patterns. It was also noticed that, it had 100% sensitivity for FVPTC when it was compared for both follicular adenoma and carcinomas. Regarding its specificity for FVPTC vs follicular adenoma and carcinomas, it was 100% and 78% respectively. Table (3) also showed that, this marker is of limited value in differentiating follicular adenoma from follicular carcinoma, with 22% sensitivity, but of 100% specificity. It was found that, CD56 had a specificity of 97% and a sensitivity of 69% for benign vs malignant lesions.

**Table (1):** Immunohistochemical staining for CD56 in a variety of thyroid diseases.

| Diagnosis | | Immunohistochemical expression, n (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | | 3 | | Total positive | |
| PTC | | 36 (100) | | 0 (100%) | | 0 (100%) | | 0 (100%) | | 0 (100%) | |
| FC | CFC | 4(22.2%) | 3(21.4%) | 5 (27.7%) | 4 (28.6%) | 3(16.6%) | 2 (14.3%) | 6(33.3%) | 5 (35.7%) | 14(77.7%) | 11(78.6%) |
| | HC | | 1(25%) | | 1 (25%) | | 1 (25%) | | 1(25%) | | 3 (75%) |
| AC | | 7 (100%) | | 0 (100%) | | 0 (100%) | | 0 (100%) | | 0 (100%) | |
| MC | | 0 (100%) | | 0 (100%) | | 2 (28.6%) | | 5 (71.4%) | | 7 (100%) | |
| MMPC | | 0 (100%) | | 1 in medullary component only | | 0 (100%) | | 0 (100%) | | 1 in medullary component only | |
| IC | | 1 (100%) | | 0 (100%) | | 0 (100%) | | 0 (100%) | | 0 (100%) | |
| T M L | | 48 (68.57%) | | 6 (8.5%) | | 5 (7.1%) | | 11 (15.7%) | | 22 (31.4%) | |
| CNG | | 2 (10.5%) | | 2 (10.5%) | | 7 (36.8%) | | 8 (42.1%) | | 17 (89.5%) | |
| FA | CFA | 0 | 0 (0%) | 8(32%) | 7(38.9%) | 11(44%) | 6(%33.3%) | 6(24%) | 5(27.8%) | 25(100%) | 18 (100%) |
| | HA | | 0 (0%) | | 1(14.3%) | | 5(71.4%) | | 1(14.3%) | | 7(100%) |
| HT | | 0 (100%) | | 1 (14.3%) | | 1 | | 5 (71.4%) | | 7 (100%) | |
| TG | | 0 (100%) | | 0 (100%) | | 1(20%) | | 4 (80%) | | 5 (100%) | |
| LT | | 0 (100%) | | 0 (100%) | | 0 (100%) | | 2 (100%) | | 2(100%) | |
| DG | | 0 (100%) | | 1 | | 0 (100%) | | 0 (100%) | | 1 (100%) | |
| T B L | | 2 (3.3%) | | 12 (20.3%) | | 20 (33.89) | | 25 (42.37%) | | 57 (96.61%) | |

PTC papillary thyroid carcinoma. **FC** follicular carcinoma. **CFC** classic follicular carcinoma. **HC** Hurthle carcinoma. **AC** anaplastic carcinoma. **MC** medullary carcinoma. **MMPC** mixed medullary papillary carcinoma. IC insular carcinoma. **T M L** total malignant lesions. **CNG** colloid nodular goiter. **CFA** classic follicular adenoma. **HA** Hurthle adenoma. **HT** Hashimoto's thyroiditis. **TG** toxic goiter. **LT** lymphocytic throiditis (focal). **DG** dyshormonogenetic goiter. **T B L** total benign lesions.

**Table (2):** Statistical analysis regarding CD56 immunohistochemical expression.

| | P -value |
|---|---|
| PTC vs FA | .000 |
| PTC vs FC | .000 |
| PTC vs hashimoto | .000 |
| PTC vs Hurthle carcinoma | .000 |
| PTC vs Hurthle adenoma | .000 |
| PTC vs medullary carcinoma | .000 |
| PTC vs anaplastic carcinoma | * |
| PTC vs toxic goiter | .000 |
| PTC vs CNG | .000 |
| PTC vs Other lesions | .000 |
| PTC vs Other lesions excluding AC | .000001 |
| FA vs FC | .073 |
| FA vs Hurthle adenoma | * |
| FA vs Hurthle carcinoma | .182 |
| Bg vs mg lesions | .000001 |
| Bg neoplastic vs mg lesions | 0.000001 |
| Bg non neoplastic vs mg lesions | 0.000001 |
| FVPTC vs FC | .000 |
| FVPTC vs FA | 0.000 |
| OPC vs PMC | * |

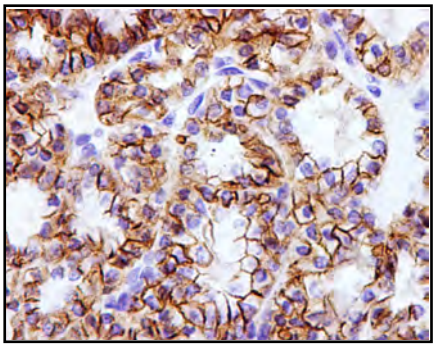
* No statistics are computed because CD56 is a constant

PTC papillary thyroid carcinoma. FA follicular adenoma. FC follicular carcinoma. CNG colloid nodular goiter. AC anaplastic carcinoma. Bg benign. mg malignant. FVPTC follicular variant papillary thyroid carcinoma. OPC overt papillary carcinoma. PMC papillary microcarcinoma.

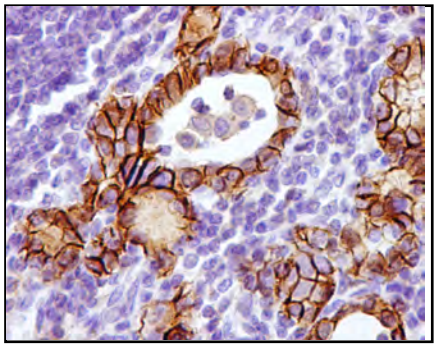
**Table (3):** Sensitivity, specificity, positive and negative predictive values, and accuracy of CD56 in differentiating different thyroid lesions from each others

| Lesions | Sensitivity | Specificity | PPV* | NPV* * | Accuracy |
|---|---|---|---|---|---|
| PTC vs Other lesions | 100% (100%) | 85% (92%) | 72% (84%) | 100% (100%) | 89% (94%) |
| Bg vs. mg lesions | 69% | 97% | 96% | 72% | 81% |
| FVPTC vs FC | 100% | 78% | 86% | 100% | 90% |
| FVPTC vs FA | 100% | 100% | 100% | 100% | 100% |
| FA vs mg | 69% | 100% | 100% | 53% | 77% |
| Bg non neo vs mg | 69% | 94% | 96% | 59% | 77% |
| FA vs FC | 22% | 100% | 100% | 64% | 67% |
| PTC vs FC | 100% | 78% | 90% | 100% | 93% |

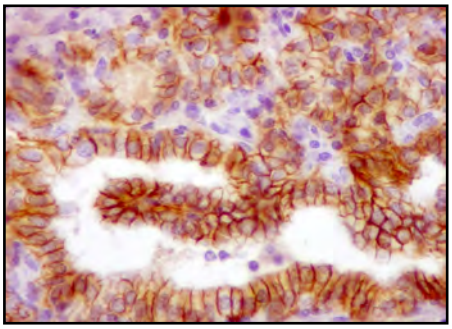
( ); calculated values when AC cases were excluded from the study.
*positive predictive value;** negative predictive value



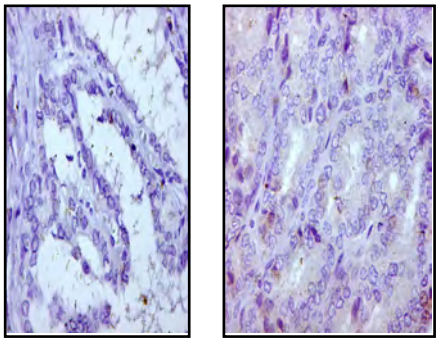
**Photomicrograph (1) :** Hyperplastic nodule in colloid nodular goiter showing strong membranous CD56 (immune stain x 400).



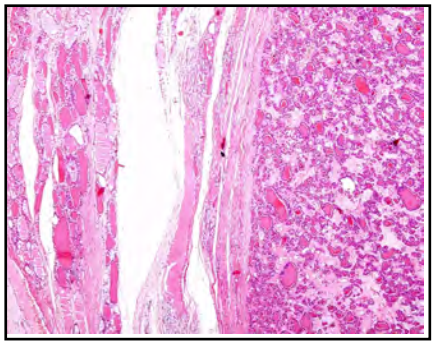
**Photomicrograph (2) :** Hashimoto thyroiditis showing strong membranous CD56 staining: (Immune staining x400).



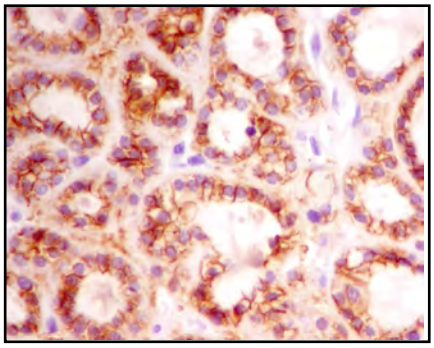
**Photomicrograph (3) :** Toxic goiter with papilla formation showing strong membranous CD56 staining (immunostain x 400) .



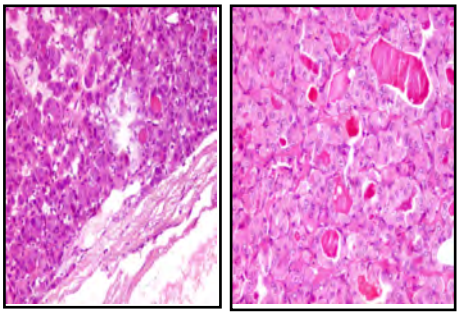
**Photomicrograph (4) :** Papillary thyroid carcinoma showing papillae on the left and follicular pattern on the right both negative for CD56 immunostaining (x400).



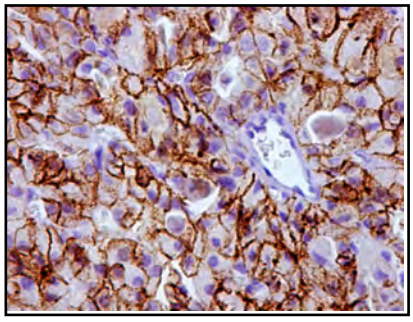
**Photomicrograph (5) :** Follicular adenoma showing a capsule and compressed thyroid tissue (H&E x40).



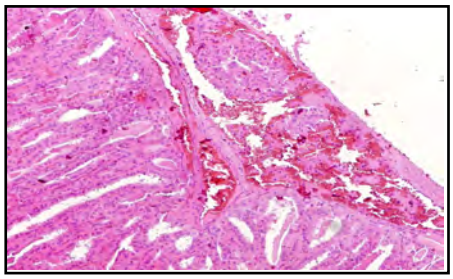
**Photomicrograph (6) :** Follicular adenoma showing strong membranous CD56 staining (immunostaining x 400).



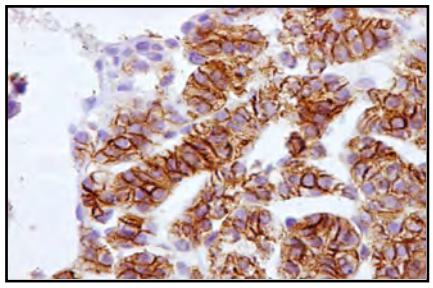
**Photomicrograph (7) :** Hurthle cell adenoma (H&E) showing a capsule (left x 200) and cells with abundant esinophilic granular cytoplasm (right x 400).



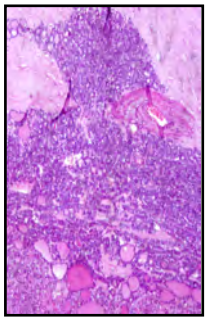
**Photomicrograph (8) :** Hurthle cell adenoma showing strong membranous CD56 staining (immunostaining x 400).



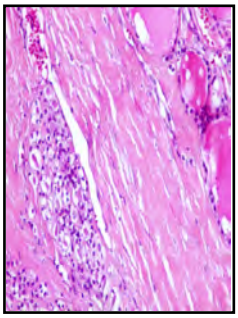
**Photomicrograph (9) :** Hurhle cell carcinoma showing vascular emboli outside the capsule (H&E x100) .



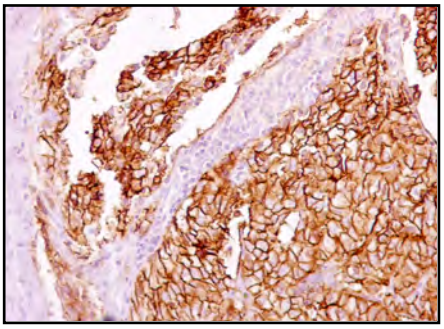
**Photomicrograph (10) :** Hurhle cell carcinoma showing strong membranous CD56 staining (immunostaining x 400),



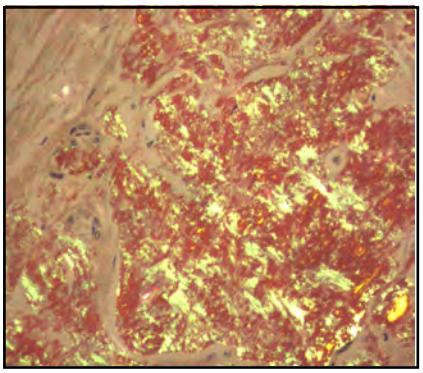
**Photomicrograph (11) :** Follicular carcinoma with muschroom pattern of capsular invasion (H & E x 40).



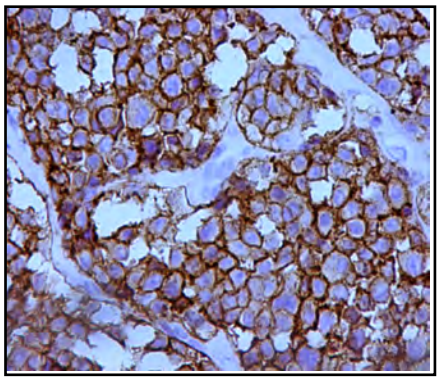
**Photomicrograph (12) :** Follicular carcinoma with thick capsule and vascular emboli outside the capsule (H & E x 200)



**Photomicrograph (13) :** Follicular carcinoma showing strong membranous CD56 staining (immunostaining x 200).



**Photomicrograph (14) :** Medullary carcinoma showing apple green birefringens underpolarized light after Congo red staining (x 400) .



**Photomicrograph (15) :** Medullary carcinoma showing strong CD 56 membranous immunostaining staining (x400).

## Discussion

The current diagnostic method of surgically resected thyroid nodules is pathologic evaluation of routine hematoxylin and eosin stained slides[15]. Diagnostic difficulties are caused by morphological similarities of biologically diverse lesions, such as papillary hyperplasia versus encapsulated papillary carcinoma, hyperplastic versus neoplastic nodule or follicular variant of PTC versus follicular adenoma. Also there may be difficulties in differentiation between follicular adenoma and minimally invasive follicular carcinoma[3, 5.16].

For the previous reasons, investigators have focused during the last several years on finding molecular or immunohistochemical markers that can help in differentiation between benign and malignant lesions of the thyroid gland[3, 17]. As a result, many surgeries for benign lesions can be avoided and patients can be managed medically as needed[18].

In the present study, CD56 expression was higher in the benign than the malignant lesions with 96.61% and 31.4% of cases respectively. This slightly differs from Park et al,[19] who found that, CD 56 was present in 91.6% of the benign lesions and 37.5% of the malignant lesions. In the latter study, the lower expression in the benign cases can be attributed to the lower expression of CD56 in follicular adenoma than our study. On the other hand, the higher expression in the malignant lesions can be explained by the higher expression of CD56 in PTC and follicular carcinoma cases.

The expression of CD56 protein in our study was diffuse and strong in the normal follicular cells and negative in all variants of PTC, insular carcinoma and all anaplastic carcinoma cases with no difference in different tumor stage. The metastatic deposits of PTC within lymph nodes also showed negative CD56 expression, as seen in their thyroid primaries, which is consistent with El Demellawy et al[3]. Others reported the expression of CD56 in 5 cases (7.5%) of PTC and most cases were grade 1[19]. This slight difference from our results could be at-

tributed to the higher number of PTC cases involved in their work. Migita et al[20], reported CD56 expression in 2/6 (33%) cases of papillary thyroid carcinoma. The higher percentage in the latter study could be explained by the fewer number of the papillary carcinoma than the recent study and that performed by El Demellawy et al and Park et al[3,19].

Within the PTC group, occasional CD56 positive cells were identified. In all cases, these cells were located at the tumor non-tumor interface i.e. at the periphery of the PTC <5%. These results agree with El Demellawy et al,[3] who interpreted that, these positive cells at the periphery may represent the infiltrative tumor non tumor interface and may rarely be residual non-tumor cells.

Regarding the follicular carcinoma, CD56 expression was present in 77.7% of cases. This is slightly lower than that reported by Park et al,[19] where follicular carcinoma positive cases were 82.5%. The results of our study and that of Park et al[19] are slightly lower than that of El Demellawy et al,[3] where CD56 expression was present in 100% of all follicular carcinoma cases. This difference could be attributed to the fewer number of follicular carcinoma in the study performed by El Demellawy et al [3] or due to difference in the techniques of the immunohistochemistry among the different literatures.

As regard medullary carcinoma, CD56 expression was present in 100% of medullary carcinoma cases, which is consistent with Park et al[19]. In the only one case of mixed medullary papillary carcinoma, the medullary component was positive, while the papillary component was negative for CD56, but there were no previous studies concerning this type to compare these results with them.

Regarding anaplastic carcinoma the result of our study was consistent with other literature, where CD56 was absent in this type of carcinoma[19]. Min et al,[21] reported CD56 expression in 1/9 cases (11%) of insular carcinoma, which was absent in the insular carcinoma case in our study and this can be attributed to the few

number of this type of carcinoma in our study.

It was found that, CD56 expression was present in 96.61% of all benign lesions, with all subtypes of benign cases showed 100% expression of CD56 except colloid nodular goiter, where it was present in 89.5% of cases. This is near to that found by Park et al,[19] where this marker was present in 91.6 % of all benign lesions, and in 90.5% of nodular hyperplasia, but they found that, 93.3% of follicular adenomas were positive in contrast to 100% of our follicular adenoma cases. On the other hand, other investigators reported that CD56 expression was present in 100% of all subtypes of the benign lesions[3,20].

In the present study, grade 3 of CD56 expression accounted for 27.9% of cases and it was the most common in both benign and malignant lesions accounting for (42.37%) and (15.7%) respectively. However, in follicular adenomas grade 2 was the most common accounting for 44% and the only case of dyshormonogenetic goiter which was grade 1. Other study found also that grade 3 represented 43.2% of the studied cases and like the present study this grade was the most common in both benign and malignant lesions, accounting for 86.1% and 29.4% respectively[19]. The difference in the grade between the present study and the latter study could be attributed to difference in the biological behavior of the thyroid lesions in the different races.

Our study showed that, CD56 is a highly significant marker in differentiating all subtypes of PTC from other lesions whether benign or malignant including medullary carcinoma ($P<0.001^{***}$), except anaplastic carcinoma where CD56 was absent in all cases of the both lesions. This agrees with Park et al,[19] who reported p values $<0.001^{***}$ and 0.571 for other lesions and anaplastic carcinoma respectively. The same results were also reported by El Demellawy et al,[3] except that they did not involve anaplastic or medullary carcinomas in their study.

The present study also showed that, this marker had a high statistical significance in differentiat-

ing FVPTC from follicular carcinoma including hurthle carcinoma and from follicular adenoma including Hurthle adenoma (P=.000), which agrees with El Demellawy et a[13]. Regarding follicular adenoma, CD56 showed no statistical significance in differentiating this lesion from follicular carcinoma, and Hurthle carcinoma (P=.073 and .182 respectively). This finding is consistent with the finding noticed by Park et al,[19] where they found p value of 0.339. It was also found in our study that, this marker is highly significant in differentiating benign whether neoplastic or non-neoplastic from malignant lesions (p< 0.001***).

Regarding the validity of CD56 antibody in the present study, it was found that, CD56 had a sensitivity (100%), specificity (85%), positive (72%) &negative (100%) predictive values, and accuracy (89%) in differentiating papillary carcinoma from other thyroid lesions, This agrees with El Demellawy et al,[3], who concluded that, lack of CD56 expression in PTC was constant specific, and extremely sensitive.

In the study performed by Park et al,[19] the sensitivity of CD56 was 92.5%, which is lower than our study. This is because they found CD56 positivity in 7.5% of PTC, while in ours all cases of papillary carcinoma were negative. On the other hand, they reported a specificity of 86.4%, which is higher than that of our study. This may be explained by the higher number of papillary carcinomas in their study. They also reported a PPV higher than the recent study accounting for 84.9%, and lower NPV (93.3%) and similar accuracy 89.2%. When anaplastic carcinomas were excluded, the specificity, positive predictive values, and accuracy of CD56 for differentiating PTC from other lesions were increased to 92%, 84% and 94% respectively. This agrees with Park et al, 19 where they noticed an increase in these values (90%, 89% and 91.7%) respectively.

In an effort to utilize CD 56 in differentiating benign from malignant thyroid lesions, it had a sensitivity of 69% and specificity of 97%. On the other hand, others[19] noticed that, CD56 has a higher

sensitivity (95.9%) and much lower specificity (less than 50%) than our results. This difference could be attributed to the large number of PTC included in their study (62 cases of PTC versus 36 cases in the recent study).

As regard the differentiation of FVPTC from follicular carcinoma, CD56 had a sensitivity of 100% and a specificity of 78%. The lower specificity can be attributed to that there were negative cases of follicular carcinoma. It was also found that CD56 is a highly sensitive and specific marker of 100% each in the differentiation of FVPTC from follicular adenoma.

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# BENHA MEDICAL JOURNAL


## VALUE OF CD56 IMMUNOHISTOCHEMISTRY IN DISTINGUISHING PAPILLARY THYROID CARCINOMA FROM OTHER LESIONS OF THE THYROID GLAND


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# RAPID VERSUS CONVENTIONAL PERIOPERATIVE MANAGEMENT OF HIGH BLOOD PRESSURE IN HYPERTENSIVE PATIENTS FOR ORTHOPEDIC OPERATIONS

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## Abstract

***Introduction:*** *There is general agreement that stages 1and 2 hypertension are not an independent risk factor for perioperative cardiovascular complications. On the contrary guidelines for stage 3 preoperative hypertension are still vague and not clear enough.*

***Aim of the work (Objective):*** *This study aimed to test the hypothesis that rapid control of preoperative high blood pressure (stage 3) is safe as regard perioperative haemodynamic responses, morbidity and mortality and that the conventional control over several days is not feasible.*

***Patients & Methods:*** *This study was carried out on 120 patients of either sex with stage 3 hypertension who were admitted at the Emergency Hospital in Mansoura University and subjected to orthopaedic operations. Patients had no previous myocardial infarction, angina, previous coronary revascularization, renal failure, left ventricular hypertrophy, preoperative dysrhythmias, conduction defects, or stroke. Patients were randomly divided into 2 major groups via sealed envelope assignment; Group A (study group) It included patients whose blood pressure was going to be managed acutely within 6 hours. These patients were further subdivided into 2 subgroups, group A1 and group A2, Group B (control group) it included patients whose surgery was postponed until their blood pressure was controlled.*

***Measurements and Main Results:*** *Rapid control was shown to be*

*as safe as its comparator conventional slow control for the management of stage 3 preoperative hypertension based on absence of significant difference in the primary outcome of the hemodynamic changes, dysrhythmia, myocardial ischemia, MI, renal dysfunction, neurologic dysfunction and perioperative blood losses. However we did observe a significant increase in the period of hospitalization in the control group compared to the two study groups. Also there was a significant decrease in both total (TND) and mean (MND) nitroglycerin dose in the control group compared to the two study groups.*

***Conclusion:*** *Rapid control of preoperative high blood pressure (stage 3) is safe as regard perioperative haemodynamic responses, morbidity and mortality and conventional control over several days is not feasible.*

***Key words:*** *arterial pressure; complications, hypertension; risk, preoperative.*

## Introduction

Worldwide hypertension affects as many as 1 billion people and is responsible for 7.1 million deaths per year[1]. The prevalence of hypertension in Egypt is unusually high (30.4%) for a developing country[2]. In addition, improved surgical and anesthetic techniques result in the ability of older patients to have surgery. Anesthesiologists, therefore, now face a larger quantity of hypertensive patients[3].

Adequate preoperative blood pressure control as well as hypertensive patient management by the anesthesiologist has been the target for continuous discussions in the last 30 years [4]. When treated and well controlled, preoperative hypertension does not seem to be an important risk factor of perioperative myocardial infarction (MI)[5]. However, the importance of immediate preoperative hypertension has not been clearly established.

Prys-Roberts et al.[6] demonstrated that poorly controlled hypertension was associated with greater hemodynamic lability and an increased risk of perioperative myocardial ischemia.

The JNC 7[1] despite recom-

mendations for management of hypertension, it did not mention management of patients with perioperative hypertension. The American College of Cardiology (ACC) and the American Heart Association (AHA) 2002 guidelines also recommended that elective surgery should be postponed in severely hypertensive patients (SBP greater than or equal to 180mmHg and DBP greater than or equal to 110mmHg) in order to gain time to control blood pressure before surgery. No reference, however, is cited for this statement. Moreover, there is little evidence to support this statement in the currently available literature [7].

Weksler et al.[8] carried out a randomized, prospective large sample study in patients with known and treated hypertension and with DBP remaining between 110 and 130 mmHg on arrival at the operating room. Those patients with evidence of target organ damage were excluded from this study. The participants were randomly allocated into two groups. In the control group, surgery was delayed until DBP remained less than 110 mmHg for a minimum of three consecutive days. In the study group, surgery was carried out after DBP was acutely reduced to less than 110 mmHg by intranasal administered nifedipine. The result showed that there was no major difference in the frequency of perioperative cardiac events between these two groups, and the investigators could not demonstrate any benefit of delaying surgery in patients with known and treated hypertension who were in severely hypertensive states prior to surgery.

On the basis of this randomized study[8], it appears that preoperative hypertension per se has limited significance in predicting perioperative risk of major cardiovascular complications, especially when the severity of hypertension is mild to moderate. In addition, it is not clear if delaying surgery in severely hypertensive patients reduces perioperative risk.

The last ACC/AHA 2009 guidelines stated that “stage 1 or 2 hypertension (systolic blood pressure below 180 mm Hg and diastolic blood pressure below 110 mmHg) is not an independent risk factor

for perioperative cardiovascular complications. Based on Weksler et al.[8], stage 3 hypertension (systolic blood pressure greater than or equal to 180 mm Hg and diastolic blood pressure greater than or equal to 110 mm Hg), the potential benefits of delaying surgery to optimize the effects of antihypertensive medications should be weighed against the risk of delaying the surgical procedure. With rapidly acting intravenous agents, blood pressure can usually be controlled within a matter of several hours[9]".

So for stage 1 and stage 2 preoperative hypertension guidelines are quite sharp On the contrary guidelines for stage 3 preoperative hypertension are still vague and not clear enough. For that reason we decided to conduct a prospective, randomized, controlled study among patients scheduled for orthopedic surgery with stage 3 hypertension. So, the aim of this study is to test the hypothesis that rapid control of preoperative high blood pressure (stage 3) is safe as regard perioperative haemodynamic responses, morbidity and mortality and that the conventional control over several days is not feasible.

## Patients and Methods

**Case selection :**

The study protocol was approved by the local ethical committee and written informed consent was obtained from all patients. Patients who were admitted to the Mansoura Emergency Hospital from the 1st of July 2007 to the last of June 2009 for orthopedic operations with stage 3 hypertension on admission, according to the classification of hypertension proposed by European society of hypertension (ESH) / European society of cardiology (ESC) 2007[10] whether poorly controlled under treatment or discovered accidentally before surgery, were subjected to our study.

The operating room is equipped with a waiting room where patients had their vital signs measured and their premedications administered. Upon stabilization of the patients in the waiting room, they received 2 mg of intravenous midazolam, and then rested for 20 minutes. After that Patient's arterial blood pressure was measured

three times with five minutes interval between each measurement. If blood pressure was still elevated the patient was entered into our study. Laboratory investigations including liver function tests, serum creatinine, blood glucose complete blood picture, total CK and MB mass fraction was measured and 12 leads ECG was done.

**Criteria of exclusion:**

It includes 1) Patients with history or ECG evidence of myocardial infarction, left ventricular hypertrophy, angina, previous coronary revascularization, cardiac conduction abnormalities (LBBB, RBBB, A-V block), cardiac rhythm other than sinus rhythm by 12 lead ECG. 2) Patients with history of stroke. 3) Patients with organ failure like chronic renal failure (serum creatinine ≥ 2mg/dl), liver cell failure, heart failure and respiratory failure were excluded.

During the period from the 1st of August 2007 to the last of July 2009, 120 patients fulfilled the protocol criteria.Patients were randomly divided into 2 major groups via sealed envelope assignment:

**Group A:** (study group) It included patients whose blood pressure was going to be managed acutely within 6 hours. These patients were further subdivided into 2 subgroups, group A1 and group A2.

**Group B:** (control group) It included patients whose surgery was postponed until their blood pressure was controlled.

**Preoperative preparation:**

**Group A1:** Patients received atenolol 100 mg tablet orally, then admitted to the postanethesia care unit where nitroglycerin infusion was started at a rate of 0.3μg/kg/minute and was titrated to a target blood pressure of 140/90 over the next 6 hours.

**Group A2:** Patients received nifedipine 20 mg tablet, then admitted to the postanethesia care unit where nitroglycerin infusion was started at a rate of 0.3μg/kg/minute and was titrated to a target blood pressure of 140/90 over the next 6 hours.

**Group B:** Patients were subjected to conventional antihyper-

tensive measures followed by the hospital and they were not allowed to be operated upon until their blood pressure was controlled.

**Anesthesia management:**

A standard anesthetic protocol was used, which consisted of:

**Premedication:**

Two peripheral intravenous lines were inserted one in each limb, for fluid infusion and drug administration. Intravenous midazolam 0.05 mg/kg was given to all patients 10 minutes before the induction. Fentanyl 1 μg/kg was given and additional fentanyl was administered throughout the surgery according to clinical signs. Nitroglycerin infusion was started at a rate of 0.3μg/kg/minute for all patients in all groups and then titrated according to blood pressure response. Lidocaine 1 mg/kg was given 5 min before induction.

**Induction of anesthesia:**

Thiopentone sodium in a sleep dose was given while oxygenation is maintained until at least two of the following were noted; loss of eyelash reflex, eye balls positioned centrally and reduced jaw tone. Muscular relaxation for tracheal intubation and maintenance was achieved with atracurium.

**Maintenance of anesthesia:**

Isoflurane 1 % carried by oxygen-enriched air ($FiO_2$=0.04) was used for maintenance of anesthesia. Hypotension, defined as decreased blood pressure by 20% or less of blood pressure before induction, was treated by stopping nitroglycerine infusion, increasing the rate of fluid administration, ephedrine 5mg was given IV and concentration of inhalational anesthesia was reduced. Hypertension defined as increased blood pressure by 20% or more of blood pressure before induction,was treated by additional fentanyl administration and increasing the concentration of the volatile anesthetic. Bradycardia and tachycardia were defined as a variation of more than 20% of the preinduction rate lasting for at least 120 seconds[11]. Propranolol 0.1 mg IV was given intravenously to antagonize tachycardia (HR>100 bpm). Atropine 0.1mg IV was used to antagonize bradycardia (HR<50 bpm).

**Emergence from anesthesia:**

Lidocaine 1 mg/kg was given 5 min before reversing muscle relaxation. Muscle relaxation was reversed by neostigmine 0.04 mg/kg and atropine 0.01 mg/kg. Patients were extubated while still sedated. Patients were transferred to the postanesthesia care unit where they stayed for 24 hours postoperative then discharged to the ward.

**Monitoring:**

All patients were monitored as follows: continuous ECG with the modified V5 lead, Automatic noninvasive arterial blood pressure was recorded at admission, before induction, immediately after induction, immediately after intubation, every 5 minutes for 30 minutes then every 10 minutes, at the end of operation, before extubation and immediately after extubation, pulse oximetry and continuous capnography. Intraoperative blood losses and fluids administered were calculated and recorded.

**Postoperative follow up:**

IV infusion of nitroglycerine was continued postoperatively as needed according to the blood pressure. Automatic noninvasive arterial blood pressure was recorded every 15 min for one hour then hourly until 24 hours postoperative. Continuous ECG monitoring with the modified V5 lead was done for 24 hours then 12 lead ECG was performed every 8 hour during the following 2 days. Total CK and MB mass fraction was measured daily during the first 3 postoperative days. Liver function tests, serum creatinine, blood glucose and complete blood picture were done in the 2$^{nd}$ postoperative day. Continuation of oral medications as follows: group A1: atenolol 100 mg tablet once daily, group A2: nifedipine 20 mg tablet twice daily, group B: conventional preoperative treatment was continued. Patients were assessed for neurologic complications on the 3$^{rd}$ postoperative day by an independent neurologist who was not involved in the study.

**The main outcome measures in this study were:**

Attacks of hypertension, hypotension, defined as above were recorded. Attacks of dysrhythmia were recorded. Ischemic attacks, defined as reversible ST

depression ≤ 1 mm measured at 60 mseconds from the J point or ST elevations ≥ 2 mm at the J point lasting longer than one minute, were also recorded[12]. Myocardial infarction which was diagnosed by meeting at least two criteria from three; 1) prolonged chest pain and related symptoms of > 20 min duration; 2) the ratio of CK-MB to total CK > 5% of total CK or CK-MB concentration > 15 IU, and 3) presence of Q waves > 0.04 seconds and a Q wave amplitude > one third of the amplitude of R wave [13]. Postoperative renal dysfunction was defined as a postoperative serum creatinine level ≥ 2.1 mg/dl plus an increase in the serum creatinine level 0.9 mg/dl from preoperative to postoperative values[14]. Postoperative neurologic dysfunction assessed by an independent neurologist who was not involved in the study. Perioperative blood losses was estimated by suction losses and weighed sponges during surgery and by the volume of wound drainage for the next 48 hours postoperatively[15]. Mean dose of nitroglycerin was also recorded which was calculated as:

$$\text{Mean dose of Nitroglycerine} = \frac{\text{Total nitroglycerine (μgm)}}{\text{Time (minutes) X weight (Kg)}}$$

Days of hospitalization were also recorded.

**Statistical Analysis :**

The statistical analysis of data was done by using excel program and SPSS (statistical package for social science) (SPSS, Inc, Chicago, IL, USA) version 16. To test the normality of data distribution, K-S (Kolmogorov-Smirnov) test was used. The description of the data was done in the form of mean (+/-) SD (standard deviation) for quantitative data and frequency & percentage for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative data, One Way Anova test was used to compare more than two groups and student t-test was used to compare between two groups. Paired sample t-test to compare one group at different times. Chi square test was used for qualitative data. P value was significant if ≤ 0.05 at confidence interval 95%.

## Results

The three groups were comparable in age, sex, body weight, height, body mass index (BMI) and time of anesthesia. There was no significant difference between the three groups. (Table1)

There was a significant decrease in systolic (p=0.003), diastolic (p=0.008) and mean (p=0.003) blood pressures in each of the three groups after induction when compared to the preinduction values with no significant difference among the three groups. (Tables 2, 3, 4)

Although there was no significant difference in heart rate among the three groups at admission, there was significant decrease (p<0.001) in A1 group compared to the other two groups and significant increase (p<0.001) in A2 group compared to the other two groups at all times from after induction to after extubation. (Table 5).

During the 1st 24 hours postoperative period there was no significant difference among the three groups as regard systolic, diastolic and mean blood pressure at all times. (Tables 6, 7, 8)

At all times during the 1st 24 hours postoperatively there was a significant decrease (p<0.001) in A1 group compared to the other two groups and significant increase (p<0.001) in A2 group compared to the other two groups as regard heart rate. (Table 9)

Intraoperatively, the frequency of hypertension was 10%, 5% and 5% respectively in A1, A2 and B (Control) groups. The frequency of hypotension was 5%, 12.5% and 5%. The frequency of tachycardia was 2.5, 12.5 and 5%. The frequency of bradycardia was 5%, 12.5% and 2.5% in the three groups. Although the frequencies of blood pressure events and dysrhythmias were different among the three groups, however none of them is statistically significant. (Table 10)

In the three groups; total creatine kinase (CK) in the 1st, 2nd and 3rd postoperative days showed significant increase (p<0.001) in comparison to the preoperative values with no signif-

icant difference between groups. On the other hand CKMB mass fraction and CKMB/CK percentage showed no significant difference at different times or between different groups. (Table 11) Based on the previous data, ECG monitoring and according to the criteria of diagnosis mentioned above[12],[13], no patient developed myocardial ischemia or myocardial infarction.

Serum creatinine showed no significant difference at different times or between different groups. According to these data and the criteria of diagnosis mentioned above[14], no patient developed postoperative renal dysfunction. (Table 12).

As regard total blood losses during intraoperative period and 1st 48 hours postoperative as well as the amount of fluids given intraoperatively there was no significant difference among the three groups. On the other hand total (TND) and mean (MND) nitroglycerin doses showed significant decrease (p=0.04 and 0.03) in the control group (B) compared to the other two groups. But the control group (B) showed significant increase (p<0.001) as regard the days of hospitalization compared to the other two groups. (Table 13)

**Table (1):** Patients' characteristics and duration of anesthesia.
Data are in number (percent) or mean ± (SD).

| | | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD |
| Age(yr) | | 49.3 | 4.5 | 51.3 | 3.8 | 49.7 | 4.8 |
| Weight(Kg) | | 76.8 | 4.9 | 74.6 | 3.5 | 75.8 | 4.8 |
| Height(Cm) | | 169.7 | 5.5 | 168.6 | 3.6 | 168.6 | 4.2 |
| BMI(Kg/m2) | | 26.7 | 2.1 | 26.3 | 1.6 | 26.7 | 2.0 |
| Time of anesthesia(m) | | 96.4 | 6.3 | 95.6 | 16.3 | 95.6 | 6.0 |
| sex | M | 25 | 62% | 22 | 55% | 21 | 56% |
| | F | 15 | 38% | 18 | 45% | 19 | 44% |

Significant when P ≤ 0.05

**Table (2):** Systolic arterial blood pressure (mmHg) during the intra-operative period (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Admission | 193.3 | 8.5 | 193.4 | 10.2 | 196.0 | 8.3 |
| Preoperative | 141.1 | 4.5 | 141.4 | 3.2 | 142.3 | 3.6 |
| After induction | 131.3 | 6.6* | 125.3 | 10.2* | 129.6 | 6.3* |
| After intubation | 144.6 | 9.5 | 141.87 | 7.1 | 140.5 | 7.7 |
| 5m | 137.3 | 5.3 | 140.1 | 9.3 | 138.9 | 4.2 |
| 10m | 136.0 | 4.8 | 135.8 | 2.1 | 135.3 | 3.6 |
| 15m | 134.8 | 3.9 | 135.5 | 3.7 | 135.1 | 3.5 |
| 20m | 132.9 | 5.2 | 133.8 | 1.8 | 134.6 | 3.6 |
| 25m | 133.4 | 5.2 | 135.1 | 3.1 | 134.9 | 3.3 |
| 30m | 134.1 | 5.0 | 136.0 | 2.8 | 134.9 | 3.8 |
| 40m | 135.6 | 5.3 | 136.0 | 3.0 | 135.1 | 3.8 |
| 60m | 135.8 | 4.6 | 136.5 | 2.6 | 134.0 | 4.0 |
| 70m | 136.9 | 4.9 | 136.9 | 6.2 | 134.9 | 3.7 |
| 80m | 136.5 | 4.1 | 135.3 | 6.0 | 135.4 | 3.5 |
| 90m | 138.9 | 3.9 | 140.8 | 3.3 | 140.5 | 4.5 |
| 100m | 143.3 | 3.1 | 142.0 | 3.0 | 142.5 | 3.4 |
| End of surgery | 142.8 | 2.8 | 143.4 | 2.4 | 143.1 | 2.9 |
| Before extubation | 148.5 | 8.2 | 147.4 | 5.4 | 145.3 | 7.0 |
| After extubation | 137.8 | 4.8 | 138.8 | 3.7 | 136.6 | 3.0 |

Significant when $P \leq 0.05$

* Significant decrease in comparison to preoperative value.

**Table (3):** Diastolic arterial blood pressure (mmHg) during the intra-operative period (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Admission | 115.0 | 3.4 | 114.1 | 4.1 | 113.4 | 4.1 |
| Preoperative | 88.9 | 3.3 | 88.6 | 2.5 | 89.5 | 3.0 |
| After induction | 81.6 | 6.5* | 76.3 | 8.2* | 79.1 | 7.8* |
| After intubation | 85.9 | 4.5 | 83.1 | 6.1 | 89.7 | 6.1 |
| 5m | 88.9 | 3.1 | 87.8 | 5.1 | 87.0 | 3.0 |
| 10m | 89.9 | 4.6 | 88.9 | 3.1 | 87.3 | 4.7 |
| 15m | 86.1 | 4.3 | 88.9 | 3.7 | 86.9 | 3.9 |
| 20m | 86.1 | 6.3 | 88.4 | 4.1 | 87.4 | 4.8 |
| 25m | 85.9 | 4.7 | 88.6 | 3.4 | 86.5 | 4.3 |
| 30m | 85.3 | 5.1 | 87.8 | 3.6 | 87.4 | 4.1 |
| 40m | 86.4 | 4.1 | 88.3 | 4.0 | 85.6 | 4.1 |
| 60m | 85.4 | 3.3 | 86.8 | 3.4 | 84.9 | 4.9 |
| 70m | 87.3 | 4.2 | 87.9 | 3.9 | 85.3 | 4.9 |
| 80m | 87.0 | 3.7 | 86.8 | 3.3 | 86.5 | 4.6 |
| 90m | 89.0 | 4.7 | 88.3 | 4.2 | 87.1 | 4.4 |
| 100m | 87.8 | 4.1 | 89.6 | 4.1 | 88.2 | 3.6 |
| End of surgery | 92.3 | 2.6 | 92.5 | 3.0 | 92.5 | 2.6 |
| Before extubation | 96.6 | 4.6 | 95.0 | 5.4 | 93.4 | 4.9 |
| After extubation | 87.1 | 4.4 | 85.9 | 4.7 | 87.0 | 4.4 |

Significant when $P \leq 0.05$

* Significant decrease in comparison to preoperative value.

**Table (4):** Mean arterial blood pressure (mmHg) during the intra-operative period (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Admission | 141.2 | 4.7 | 140.5 | 5.9 | 140.9 | 4.9 |
| Preoperative | 106.3 | 3.2 | 106.2 | 2.2 | 107.1 | 2.6 |
| After induction | 98.3 | 6.4* | 92.6 | 8.5* | 95.9 | 6.9* |
| After intubation | 100.5 | 5.8 | 103.9 | 6.1 | 102.6 | 6.3 |
| 5m | 98.1 | 3.2 | 97.3 | 6.2 | 95.3 | 2.1 |
| 10m | 105.3 | 4.1 | 104.6 | 2.1 | 103.3 | 3.4 |
| 15m | 102.4 | 3.5 | 104.4 | 2.3 | 103.0 | 3.0 |
| 20m | 101.8 | 5.5 | 105.4 | 2.6 | 103.2 | 3.7 |
| 25m | 101.1 | 4.6 | 104.2 | 2.8 | 102.7 | 3.2 |
| 30m | 100.2 | 4.4 | 104.0 | 2.6 | 103.2 | 3.3 |
| 40m | 102.9 | 4.1 | 106.1 | 3.3 | 102.2 | 3.3 |
| 60m | 102.0 | 3.2 | 104.3 | 2.6 | 101.8 | 3.4 |
| 70m | 103.6 | 5.5 | 104.0 | 2.4 | 101.6 | 3.7 |
| 80m | 103.6 | 3.4 | 103.5 | 3.4 | 102.7 | 3.4 |
| 90m | 104.9 | 3.9 | 103.8 | 2.9 | 103.3 | 3.4 |
| 100m | 104.9 | 3.8 | 107.6 | 3.5 | 105.5 | 3.3 |
| End of surgery | 105.1 | 3.7 | 108.6 | 4.4 | 107.8 | 4.3 |
| Before extubation | 109.9 | 2.8 | 110.5 | 2.9 | 108.7 | 2.4 |
| After extubation | 104.0 | 5.5 | 109.0 | 4.9 | 108.4 | 4.8 |

Significant when $P \leq 0.05$

* Significant decrease in comparison to preoperative value.

**Table (5):** Heart rate (beat/min) during the intra-operative period (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | | | | | | |
| | Mean | SD | Mean | SD | Mean | SD |
| Admission | 87.1 | 3.5 | 87.4 | 3.9 | 85.9 | 4.0 |
| Preoperative | 79.5 | 3.0* | 86.1 | 3.0§ | 84.4 | 3.9 |
| After induction | 73.4 | 3.5* | 86.1 | 3.6§ | 80.4 | 3.9 |
| After intubation | 80.7 | 6.2* | 90.6 | 7.3§ | 87.1 | 5.7 |
| 5m | 77.7 | 3.6* | 94.4 | 5.8§ | 80.5 | 3.6 |
| 10m | 75.9 | 2.3* | 81.2 | 2.8§ | 77.5 | 3.6 |
| 15m | 74.9 | 2.6* | 77.9 | 5.7§ | 77.2 | 4.4 |
| 20m | 74.5 | 2.7* | 81.8 | 1.7§ | 77.0 | 3.5 |
| 25m | 73.4 | 3.1* | 74.2 | 4.7§ | 77.2 | 3.9 |
| 30m | 73.6 | 3.8* | 75.1 | 3.4§ | 77.6 | 3.0 |
| 40m | 73.7 | 2.5* | 81.5 | 1.4§ | 78.2 | 3.0 |
| 50m | 72.5 | 3.9* | 81.7 | 1.7§ | 78.5 | 3.4 |
| 60m | 74.3 | 4.7* | 80.6 | 2.9§ | 76.7 | 3.5 |
| 70m | 74.1 | 4.6* | 79.1 | 3.7§ | 76.8 | 3.7 |
| 80m | 74.6 | 4.4* | 78.2 | 4.0§ | 77.8 | 2.8 |
| 90m | 74.9 | 3.8* | 81.4 | 1.6§ | 78.3 | 3.3 |
| 100m | 77.3 | 3.0* | 84.4 | 1.6§ | 83.0 | 1.9 |
| End of surgery | 77.9 | 2.7* | 86.8 | 2.6§ | 82.7 | 1.9 |
| Before extubation | 83.2 | 1.6* | 98.9 | 5.8§ | 86.0 | 1.9 |
| After extubation | 76.6 | 2.1* | 93.1 | 2.0§ | 81.0 | 1.7 |

Significant when $P \leq 0.05$

* Significant decrease in comparison to the other two groups.

§ Significant increase in comparison to the other two groups.

**Table (6):** Systolic arterial blood pressure (mmHg) during the 1st 24 hours postoperative (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 15 minutes | 142.5 | 3.9 | 143.5 | 4.6 | 143.0 | 7.2 |
| 30 | 134.1 | 4.5 | 133.1 | 4.6 | 133.9 | 4.2 |
| 45 | 135.9 | 4.8 | 133.0 | 3.7 | 132.8 | 4.2 |
| 1hour | 134.6 | 4.6 | 136.5 | 4.4 | 132.9 | 4.1 |
| 2 | 132.1 | 6.4 | 134.0 | 4.3 | 132.0 | 5.0 |
| 3 | 134.8 | 6.3 | 135.0 | 4.9 | 132.0 | 5.6 |
| 4 | 133.1 | 4.5 | 129.3 | 18.3 | 130.8 | 3.8 |
| 5 | 135.4 | 5.8 | 134.5 | 5.0 | 132.1 | 5.2 |
| 6 | 134.4 | 6.6 | 135.0 | 5.3 | 133.0 | 5.4 |
| 7 | 133.3 | 5.8 | 135.1 | 5.9 | 134.5 | 6.5 |
| 8 | 135.6 | 5.5 | 135.0 | 5.9 | 137.3 | 6.2 |
| 9 | 132.6 | 6.2 | 135.6 | 5.3 | 134.6 | 6.3 |
| 10 | 136.3 | 5.6 | 137.9 | 4.8 | 135.9 | 5.8 |
| 11 | 136.5 | 5.6 | 139.0 | 6.2 | 137.3 | 6.1 |
| 12 | 135.8 | 5.8 | 138.0 | 4.5 | 136.8 | 5.5 |
| 13 | 136.6 | 5.6 | 136.9 | 4.9 | 136.9 | 5.6 |
| 14 | 135.4 | 7.4 | 136.8 | 5.5 | 136.5 | 5.8 |
| 15 | 134.4 | 6.4 | 137.0 | 5.2 | 134.5 | 6.1 |
| 16 | 136.1 | 7.5 | 138.3 | 6.1 | 135.6 | 6.9 |
| 17 | 134.4 | 6.1 | 139.0 | 6.1 | 136.3 | 8.2 |
| 18 | 136.4 | 6.8 | 137.0 | 4.8 | 135.4 | 6.2 |
| 19 | 138.6 | 6.6 | 139.9 | 5.7 | 136.9 | 7.2 |
| 20 | 137.0 | 7.1 | 136.3 | 5.2 | 133.9 | 6.0 |
| 21 | 137.5 | 6.1 | 136.3 | 4.3 | 135.6 | 7.9 |
| 22 | 134.5 | 7.2 | 134.0 | 6.6 | 133.8 | 6.7 |
| 23 | 137.9 | 6.6 | 136.3 | 5.9 | 134.9 | 8.0 |
| 24 | 137.4 | 6.6 | 135.4 | 4.7 | 135.6 | 7.9 |

Significant when $P \leq 0.05$

**Table (7):** Diastolic arterial blood pressure (mmHg) during the 1st 24 hours postoperative (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 15minutes | 90.6 | 2.8 | 92.3 | 3.4 | 91.8 | 4.6 |
| 30 | 84.5 | 4.6 | 85.8 | 3.8 | 84.3 | 3.5 |
| 45 | 84.8 | 3.4 | 83.8 | 3.3 | 85.8 | 2.9 |
| 1hour | 84.1 | 3.4 | 86.8 | 3.5 | 86.5 | 2.8 |
| 2 | 84.0 | 3.8 | 85.9 | 3.0 | 84.6 | 3.8 |
| 3 | 83.9 | 4.0 | 85.8 | 3.1 | 83.9 | 4.2 |
| 4 | 84.4 | 4.0 | 86.5 | 4.0 | 84.9 | 3.5 |
| 5 | 84.3 | 3.7 | 86.0 | 3.0 | 84.6 | 3.8 |
| 6 | 85.4 | 3.1 | 86.6 | 2.9 | 85.1 | 4.3 |
| 7 | 85.4 | 4.1 | 84.6 | 3.5 | 84.3 | 3.1 |
| 8 | 86.6 | 3.3 | 85.6 | 3.0 | 83.9 | 3.5 |
| 9 | 83.3 | 4.0 | 84.9 | 2.7 | 84.1 | 3.2 |
| 10 | 84.1 | 3.4 | 85.5 | 3.4 | 84.6 | 2.9 |
| 11 | 84.4 | 3.6 | 83.4 | 5.5 | 84.3 | 2.9 |
| 12 | 85.5 | 4.4 | 83.4 | 3.8 | 85.4 | 4.9 |
| 13 | 84.6 | 4.3 | 85.3 | 4.2 | 84.3 | 4.3 |
| 14 | 84.9 | 4.6 | 86.1 | 3.7 | 84.0 | 5.4 |
| 15 | 83.9 | 4.7 | 83.6 | 3.4 | 84.0 | 5.0 |
| 16 | 84.8 | 5.1 | 85.3 | 4.4 | 83.8 | 5.6 |
| 17 | 83.8 | 5.4 | 84.5 | 4.5 | 83.0 | 6.1 |
| 18 | 85.8 | 4.2 | 84.9 | 4.0 | 83.5 | 4.7 |
| 19 | 86.0 | 5.0 | 85.1 | 3.5 | 85.0 | 5.4 |
| 20 | 86.3 | 5.5 | 86.0 | 3.4 | 83.6 | 3.9 |
| 21 | 85.3 | 3.6 | 83.0 | 5.2 | 83.0 | 5.8 |
| 22 | 86.6 | 4.1 | 84.4 | 3.6 | 84.1 | 5.5 |
| 23 | 85.9 | 4.7 | 85.1 | 4.3 | 84.6 | 5.8 |
| 24 | 85.4 | 3.6 | 84.3 | 5.1 | 82.9 | 5.8 |

Significant when $P \leq 0.05$

**Table (8):** Mean arterial blood pressure (mmHg) during the 1st 24 hours postoperative (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 15minutes | 108.0 | 2.5 | 109.4 | 3.0 | 108.9 | 5.2 |
| 30 | 101.1 | 4.0 | 101.4 | 2.9 | 100.8 | 2.9 |
| 45 | 101.9 | 3.0 | 100.2 | 2.8 | 101.5 | 2.4 |
| 1hour | 101.0 | 2.4 | 103.5 | 3.3 | 99.6 | 16.3 |
| 2 | 100.1 | 4.1 | 102.0 | 2.9 | 100.5 | 3.5 |
| 3 | 100.9 | 3.4 | 102.9 | 2.7 | 99.6 | 4.0 |
| 4 | 100.7 | 3.7 | 100.8 | 7.1 | 100.2 | 3.2 |
| 5 | 101.2 | 3.7 | 102.2 | 2.7 | 100.5 | 3.6 |
| 6 | 101.7 | 3.2 | 103.4 | 2.6 | 101.2 | 4.1 |
| 7 | 101.4 | 3.2 | 101.5 | 2.5 | 101.1 | 3.2 |
| 8 | 103.0 | 2.8 | 102.1 | 2.9 | 101.8 | 3.3 |
| 9 | 99.6 | 3.8 | 101.9 | 2.5 | 101.1 | 3.2 |
| 10 | 101.4 | 2.8 | 103.0 | 2.3 | 101.9 | 3.0 |
| 11 | 101.1 | 3.0 | 101.8 | 3.7 | 102.1 | 3.1 |
| 12 | 102.3 | 3.9 | 101.7 | 2.5 | 102.6 | 4.2 |
| 13 | 102.0 | 3.1 | 103.7 | 3.4 | 100.8 | 4.1 |
| 14 | 101.8 | 4.6 | 103.0 | 3.1 | 101.0 | 4.3 |
| 15 | 100.4 | 5.3 | 102.0 | 3.0 | 101.0 | 4.5 |
| 16 | 102.0 | 5.0 | 102.9 | 3.9 | 100.5 | 5.5 |
| 17 | 100.6 | 4.9 | 102.6 | 3.7 | 99.5 | 6.4 |
| 18 | 102.5 | 3.3 | 102.3 | 3.1 | 100.2 | 4.6 |
| 19 | 104.9 | 4.2 | 103.3 | 2.6 | 102.0 | 5.3 |
| 20 | 103.2 | 5.4 | 104.1 | 2.1 | 100.4 | 3.9 |
| 21 | 102.5 | 2.2 | 100.8 | 3.2 | 99.3 | 6.0 |
| 22 | 102.5 | 4.2 | 100.8 | 3.6 | 100.8 | 5.3 |
| 23 | 103.3 | 4.0 | 102.1 | 3.2 | 101.4 | 5.8 |
| 24 | 102.6 | 3.0 | 101.2 | 3.4 | 99.2 | 6.0 |

Significant when $P \leq 0.05$

**Table (9):** Heart rate (beat/min) during the 1st 24 hours postoperative (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 15minutes | 84.0 | 2.9* | 91.0 | 2.2§ | 84.1 | 1.7 |
| 30 | 76.2 | 3.4* | 81.9 | 1.7§ | 78.8 | 1.7 |
| 45 | 75.5 | 2.6* | 81.4 | 2.1§ | 78.3 | 2.3 |
| 1hour | 74.9 | 2.6* | 81.5 | 2.1§ | 76.9 | 11.3 |
| 2 | 74.2 | 2.4* | 78.5 | 1.3§ | 76.8 | 4.6 |
| 3 | 75.3 | 2.2* | 78.5 | 2.3§ | 76.9 | 3.0 |
| 4 | 75.2 | 1.9* | 81.7 | 2.3§ | 78.7 | 1.3 |
| 5 | 75.2 | 2.6* | 82.0 | 2.0§ | 77.9 | 1.9 |
| 6 | 74.9 | 1.9* | 80.7 | 3.4§ | 78.4 | 1.5 |
| 7 | 73.8 | 2.7* | 78.7 | 3.8§ | 77.9 | 2.0 |
| 8 | 74.3 | 3.5* | 74.7 | 4.9§ | 78.6 | 1.3 |
| 9 | 75.5 | 3.1* | 82.0 | 2.2§ | 78.4 | 2.3 |
| 10 | 75.3 | 3.1* | 81.6 | 3.1§ | 78.9 | 1.3 |
| 11 | 75.6 | 2.6* | 81.9 | 1.9§ | 78.2 | 1.8 |
| 12 | 75.8 | 2.7* | 77.5 | 5.0§ | 78.6 | 1.4 |
| 13 | 76.0 | 2.7* | 78.9 | 3.4§ | 78.2 | 1.9 |
| 14 | 76.1 | 2.0* | 81.5 | 2.0§ | 78.6 | 1.6 |
| 15 | 75.9 | 2.8* | 82.2 | 2.5§ | 78.3 | 1.8 |
| 16 | 74.3 | 2.4* | 82.3 | 2.7§ | 78.3 | 2.0 |
| 17 | 74.2 | 2.6* | 81.4 | 1.8§ | 78.6 | 1.6 |
| 18 | 74.7 | 2.2* | 78.9 | 4.4§ | 77.4 | 2.1 |
| 19 | 74.6 | 2.1* | 78.4 | 3.5§ | 78.2 | 1.5 |
| 20 | 74.5 | 2.2* | 81.4 | 2.0§ | 78.4 | 2.4 |
| 21 | 75.7 | 1.8* | 81.8 | 2.4§ | 78.5 | 1.6 |
| 22 | 75.7 | 1.5* | 79.3 | 3.9§ | 77.9 | 2.0 |
| 23 | 74.7 | 1.7* | 77.6 | 6.3§ | 78.7 | 1.5 |
| 24 | 74.4 | 2.0* | 77.8 | 3.7§ | 77.6 | 2.1 |

Significant when $P \leq 0.05$

* Significant decrease in comparison to the other two groups.

§ Significant increase in comparison to the other two groups.

**Table (10):** Hypertension, hypotension and dysrhythmia during the intra-operative period (Data are in number of patients and percent).

| | A1 | A2 | B (Control) |
|---|---|---|---|
| **Hypertension** | 4(10%) | 2(5%) | 2(5%) |
| **Hypotension** | 2(5%) | 5(12.5%) | 2(5%) |
| **Tachycardia** | 2(5%) | 5(12.5%) | 1(2.5%) |
| **Bradycardia** | 5(12.5%) | 1(2.5%) | 12(2.5%) |

Significant when $P \leq 0.05$
Hypertension = blood pressure $\geq 20$ % of preinduction value.
Hypotension = blood pressure $\leq 20$ % of preinduction value.
Tachycardia = heart rate $\geq 20$ % of preinduction value.
Bradycardia = heart rate $\leq 20$ % of preinduction value.

**Table (11):** Creatine Kinase (CK) (u/l), CKMB mass fraction (u/l) and percent (CKMB/CK) in the preoperative, 1st, 2nd and 3rd postoperative days (Data are in Mean ± SD).

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **CKpre** | 89.7 | 12.0 | 90.7 | 11.5 | 94.0 | 11.5 |
| **CKpost1** | 171.6 | 12.8* | 156.4 | 7.4* | 159.5 | 7.75* |
| **CKpost2** | 273.5 | 30.5* | 275.6 | 26.6* | 279.1 | 27.3* |
| **CKpost3** | 182.4 | 13.6* | 183.2 | 11.4* | 187.9 | 9.05* |
| **CKMBpre** | 1.6 | 1.2 | 1.6 | 1.3 | 1.9 | 1.4 |
| **CKMBpost1** | 6.2 | 1.1 | 5.8 | .60 | 5.7 | 0.6 |
| **CKMBpost2** | 8.8 | .90 | 8.7 | 0.8 | 8.8 | .80 |
| **CKMBpost3** | 1.6 | 1.1 | 1.8 | 1.1 | 1.5 | 0.9 |
| **CKMB%pre** | 1.7 | 1.2 | 1.7 | 1.3 | 2.0 | 1.5 |
| **CKMB%post1** | 3.6 | .50 | 3.6 | 0.5 | 3.7 | .50 |
| **CKMB%post2** | 3.2 | 0.5 | 3.1 | .40 | 3.2 | .40 |
| **CKMB%post3** | 2.3 | 0.70 | 2.1 | 0.70 | 2.2 | 0.5 |

Significant when $P \leq 0.05$
* Significant increase in comparison to preoperative value.

**Table (12):** serum creatinine (mg/dl) in the preoperative and in the 2nd postoperative day.

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **Creatinine pre** | 0.95 | 0.10 | 0.93 | 0.10 | 0.95 | 0.11 |
| **Creatinine post2** | | 0.10 | 0.96 | 0.11 | 0.94 | 0.11 |

Significant when $P \leq 0.05$

**Table (13):** Total blood losses (ml), intraoperative fluids (ml), total and mean nitroglycerin dose and days of hospitalization.

| | A1 | | A2 | | B (Control) | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **Blood Losses** | 517.5 | 113.8 | 478.7 | 99.3 | 473.7 | 101.9 |
| **Fluids** | 1730 | 223.4 | 1780 | 217.4 | 1682.5 | 219.4 |
| **TND** | 58825 | 6097 | 43650 | 4812.2 | 31650 | 4463.8§ |
| **MND** | 7.95 | 0.9 | 5.6 | 0.3 | 4.3 | 0.5§ |
| **Days of Hospitalization** | 4.7 | 0.8 | 5.2 | 1.0 | 9.5 | 1.8* |

Significant when $P \leq 0.05$
* Significant increase in comparison to the other two groups.
§ Significant decrease in comparison to the other two groups.

## Discussion

In the current study, rapid control was shown to be as safe as its comparator conventional slow control for the managament of stage 3 preoperative hypertension based on absence of significant difference in the primary outcome of the hemodynamic changes, dysrhythmia, myocardial ischemia, MI, renal dysfunction, neurologic dysfunction and perioperative blood losses. However we did observe a significant increase in the period of hospitalization in the control group compared to the two study groups. Also there was a significant decrease in both total (TND) and mean (MND) nitroglycerin dose in the control group compared to the two study groups.

Worldwide hypertension affects as many as 1 billion people and is responsible for 7.1 million deaths per year[1]. Cases presented by immediate preoperative high blood pressure represent a challenge for anesthesia. Approximately 29% of adult patients undergoing noncardiac surgery have a history of arterial hypertension[16]. Adequate preoperative blood pressure control as well as hypertensive patient management by the anesthesiologist has been the target for continuous discussions in the last 30 years[4]. for stage 1 and stage 2 preoperative hypertension, guidelines are quite sharp and there is general agreement that these 2 stages are not an independent risk factor for perioperative cardiovascular complications[6]. On the contrary guidelines for stage 3 preoperative hypertension are still vague and not clear enough[6][9]. It is common practice to postpone surgery when DBP is above 110 mmHg because some anesthesiologists believe that these patients are exposed to an increased risk of perioperative dysrhythmias,[17][18] myocardial ischemia, MI,[19] stroke[20], renal dysfunction and increased blood loss [21].

Most studies of the influence of arterial hypertension in perioperative and postoperative complications have not stratified hypertension according to severity[22]. The National Veterans Administration Surgical Risk Study of 83,000 patients[23] found that hypertension was the second most common risk factor for surgical morbidity. However, in that study, patients were not grouped accord-

ing to the severity of BP, previous treatment, or anesthetic technique. Badrinath et al.[24] found an association between arterial hypertension and mortality in patients undergoing ophthalmic surgeries with local or general anesthesia. In that retrospective study, patients were not allocated to study groups by severity of the arterial hypertension, anesthesia technique, or preoperative treatment. The importance of preoperative control of arterial hypertension was studied by Hartung et al.[25] a 30 % frequency of intraoperative or postoperative cardiovascular complications is found in patients with untreated hypertension, 36% in patients with poorly controlled hypertension, and 16% in patients with well-controlled arterial hypertension. They recommend optimal preoperative treatment of hypertension to reduce perioperative complications. Hartung et al.'s study differs from our study in that it did not address the problem of patients with usually well-controlled hypertension who present immediately before surgery with an acute elevation of BP. So, the problem of patients becoming hypertensive immediately before surgery remains unsolved.

As an original contribution Weksler et al.,[8] 2002 published the results of a large sample, randomized, prospective study in patients with known and treated hypertension and with DBP remaining between 110 and 130 mmHg on arrival at the operating room. Those patients with evidence of target organ damage were excluded from this study. The participants were randomly allocated into two groups. In the control group, surgery was delayed until DBP remained less than 110 mmHg for a minimum of three consecutive days. In the study group, surgery was carried out after DBP was acutely reduced to less than 110 mmHg by intranasal administered nifedipine. The result showed that there was no major difference in the frequency of perioperative cardiac events between these two groups, and the investigators could not demonstrate any benefit of delaying surgery in patients with known and treated hypertension who were in severely hypertensive states prior to surgery. The last American Col-

lege of Cardiology (ACC) and the American Heart Association (AHA) guidelines published in 2009 stated that stage 1 or stage 2 hypertension (systolic blood pressure below 180 mm Hg and diastolic blood pressure below 110 mmHg) is not an independent risk factor for perioperative cardiovascular complications. Based on Weksler et al.[13] for stage 3 hypertension (systolic blood pressure greater than or equal to 180 mm Hg and diastolic blood pressure greater than or equal to 110 mm Hg), the potential benefits of delaying surgery to optimize the effects of antihypertensive medications should be weighed against the risk of delaying the surgical procedure. With rapidly acting intravenous agents, blood pressure can usually be controlled within a matter of several hours [9].

However we do believe that the study of Weksler et al.[8] had some limitations as patients were included in this study based only on diastolic blood pressure (DBP between 110 and 130 mmHg on arrival in the operating room) rather than both systolic and diastolic blood pressures, however, a large number of observational studies has demonstrated that cardiovascular morbidity and mortality bear a continuous relationship with both systolic and diastolic blood pressures[26] which both were independently and similarly predictive of stroke and coronary mortality[27][28]. Also, Weksler et al. did not determine a clear cut point for acute reduction of diastolic blood pressure preoperatively. On the other hand acute reduction of severe diastolic blood pressure over 20 minutes is not desirable as it gives no time for down regulation of autoregulatory mechanisms which have been shifted up [29]. The use of intranasal nifedipine has been criticized by many authors[30][31] as with intranasal administration, the actual dose tends to be unsteady, and the bioavailability is low. Also, the drug is retained in the nasal cavity and is absorbed through the mucosa, but part of it flows into the gastrointestinal tract via the internal nares and pharynx. Therefore, the serum nifedipine concentration shows several peaks, and the whole pharmacokinetics is unclear. The greatest problem with intranasal administration of nifedipine is irritation of the nasal mucosa, which causes

great discomfort to the patients. Another defect in this study is the choice of pancuronium for muscular relaxtion which is known to cause release of catecholarnines at adrenergic nerve endings and increased blood catecholamine levels[32] an effect which is not desired especially in hypertensive patients with well known high sympathetic activity[23]. The study of Weksler et al., did not addressed postoperative renal dysfunction as one of the study outcomes although it is a well known complication of perioperative hypertension[34]. At last Weksler did not assess blood losses, although excessive surgical bleeding is one of the risks of uncontrolled preoperative hypertension [35][21].

In spite of these limitations in Weksler study our results are consistent with their, as regard perioperative hemodynamic events, dysrhythmia, myocardial ischemia, myocardial infarction, postoperative neurologic dysfunction and the period of hospitalization.

Our study showed significant decrease in systolic, diastolic and mean blood pressures in each of the three groups after induction when compared to the preinduction values which is explained by the fact that hypertensive patients shows exaggerated hemodynamic variations to induction of anesthesia than nonhypertensives and has been reported by many authors[36][37].

The current study also showed significant decrease in heart rate in atenolol group at all times of intraoperative and postoperative periods which is consistent with all studies involving β blockers including atenolol[38][39]. Also there was significant increase in heart rate in nifedipine group at all times of intraoperative and postoperative periods which is consisted with many studies that reported that administration of dihydropyridine nifedipine is associated with reflex sympathetic activity and increased plasma level of catecholamines accompanied with increased heart rate[40][41] [42].

The current study also showed that total (TND) and mean (MND) was significantly lower in the control group compared to the other

study groups suggesting that control of blood pressure was easier in that group. A similar approach was used in the ECLIPSE study that compared clevidipine to nitroglycerin, sodium nitroprusside, and nicardipine for acute hypertension treatment in cardiac surgery patients [43].

Our study showed significantly lower hospitalization time in the study groups whose hypertension was managed acutely compared to the slow conventional approach; this shorter hospital stay has psychological, social and economic implications [44].

Finally the results of our study cannot be generalized to all stage 3 hypertensive patients because patients with target organ damage were excluded from our study e.g. patients with coronary heart diseases.

In conclusion, rapid control of preoperative high blood pressure (stage 3) is safe as regard perioperative haemodynamic responses, morbidity and mortality and the conventional control over several days is not feasible.

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# BENHA MEDICAL JOURNAL


## RAPID VERSUS CONVENTIONAL PERIOPERATIVE MANAGEMENT OF HIGH BLOOD PRESSURE IN HYPERTENSIVE PATIENTS FOR ORTHOPEDIC OPERATIONS


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# COMPARATIVE STUDY BETWEEN THE EFFECT OF EPIDURAL AND RECTUS SHEATH BLOCKS USING BUPIVACAINE - FENTANYL MIXTURE IN CHILDREN SUBMITTED FOR ELECTIVE LOWER ABDOMINAL SURGERY

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## Abstract

***Introduction:*** *Postoperative pain management in children has received growing attention for the past two decades. The reason for this was the results from different investigations showing that children did not receive analgesic treatment to the same extent as adults did, and that children often experienced pain postoperative.*

***Aim of the work (Objective):*** *We aimed to study evaluation of the analgesic efficacy, recovery profile, optimum discharge criteria and side effects of single-shot epidural block compared to rectus sheath block in pediatric patients.*

***Patients & Methods:*** *Ninety patients scheduled for elective lower abdominal surgery were randomly allocated into three equal groups according to the technique, each group included 30 patients. Group I (control group) received general anesthesia alone. Group II (Rectus sheath block group) (RSB group) received Rectus sheath block after inhalation induction of general anesthesia using sevoflurane. Group III (epidural group) (EP group) received epidural analgesia after induction of general anesthesia using sevoflurane. Pain was evaluated by Modified objective pain score (MOPS), duration of pain relief (the first request of postoperative analgesia) and total analgesic consumption during the first postoperative 24 hours. Recovery and discharge was assessed using Modified Aldrete Score, Fast-Track score and Post Anesthesia Home*

*Discharge Score (PADS).Post-operative side effects were recorded. A P value <0.05 was regarded as statistically significant.*

***Results:*** *MOPS showed a significant decrease in group II after recovery from anesthesia when compared with group I and group III .The duration of analgesia in hrs showed a significant increase in group II (21±3) when compared with group I (0.2±0.14) and group III (16±7). Also, total analgesic consumption (diclofenac in mg) in the first postoperative 24 hours showed a significant decrease in group II (1.6±13) when compared with group I (34.6±24.9) and group III (9.2±16). Modified Aldrete Score, Fast-Track score and PADS showed a significant increase in group II after recovery from anesthesia when compared with group I and group III. The incidence of PONV immediately after recovery from anesthesia showed a significant increase in group III (9 cases) and group I (7 cases) when compared with group II (3 cases).*

***Conclusion:*** *It could be concluded that the rectus sheath block can be used as an alternative to epidural analgesia in providing adequate analgesia after abdominal surgery.*

***Keywords:*** *Epidural block; rectus sheath block; bupivacaine - fentanyl mixture; Children.*

## Introduction

Enhanced recovery protocols attempt to accelerate patient rehabilitation following surgery. The provision of effective pain relief is an essential pre-requisite for accelerated convalescence as inadequate postoperative pain relief can prolong recovery, increase duration of hospital stay and reduce patient satisfaction. Several analgesic techniques are used in clinical practice but none can achieve complete post-operative pain control [1].

Regional anesthesia is a cornerstone of modern pediatric anesthesia, with a large number of pediatric anesthetists combining general and regional anesthesia to provide superior and long-lasting analgesia without risk of respiratory depression. Furthermore, regional anesthesia techniques have been shown to attenuate the perioperative stress response, to allow early tracheal extubation after major abdominal or thoracic surgery, to decrease the number of days spent in the intensive care unit

and to allow rapid recovery of gastro-intestinal function[2].

Although epidural analgesia is a gold standard technique for postoperative abdominal analgesia, its use is sometimes limited due to perioperative anticoagulant therapy and undesirable complications such as hypotension[3].

Rectus sheath block (RSB) can provide alternative analgesia after midline abdominal surgery. This technique has gained popularity owing to fewer complications and a higher success rate[4]. The technique aims to block the ventral rami of the $7^{th}$ to the 12th intercostal nerves that supply the rectus abdominis muscles and overlying skin[5].

Bupivacaine is a long acting amide local anesthetic agent. It is routinely used for epidural analgesia in children because of its long duration of action and beneficial ratio of sensory to motor block. Maximum safe dose of bupivacaine as a single injection is 2.5mg. $kg^{-1}$ and the preferred concentration for epidural is 0.25%[6]. Opioids addition to local anesthetic for epidural analgesia has been increasingly used in the pediatric population, particularly in lower abdominal surgery. The combinations of these drugs were used in an attempt to reduce local anesthetic requirements and side effects while improving both the quality and duration of the postoperative analgesic effect of the epidural block. Epidural fentanyl, alone or in combination with local anesthetics, is effective and safe with the least likelihood of causing respiratory depression in the pediatric population[7]. This effect is a result of its high lipid solubility, which makes it less likely to migrate rostrally to the brainstem [8].

## Patients & methods

After approval of local ethics committee and obtaining informed consent signed by the patient's parents for regional anesthesia, 90 children of both sexes were randomly enrolled in this study. Eligible patients were: ASA physical status I or II status, Aged 2-8 years & Scheduled for elective lower abdominal v surgery.

Exclusion criteria include: patient's parent refusal, obesity (130% or more than ideal body weight), children with coagulopathy disorders, neuromuscular or spinal diseases, children with endocrinal disorders, a history of seizure disorder, children with mental retardation or delayed development, a hernia sac large enough to prevent insertion of the needle to within 1 cm of the lateral border of the rectus muscles, children with hepatic or renal impairment, known allergy or sensitivity to the trial drugs and infection at site of injection.

All patients were subjected to medical examination, and basal investigations in the form of complete blood picture, blood glucose, coagulation profile, liver and renal function tests. Fasting 6 hours from solid or milk and 2-4 hours from clear fluid. No premedication were administered.

After inhalation induction of general anesthesia using sevoflurane, an I.V line was inserted. When reaching a proper depth of anesthesia, a suitable endotracheal tube (ETT) was inserted. Anesthesia was maintained with 1-1.5% sevoflurane in 100% oxygen. Muscle relaxation was maintained using atracurium at a dose of 0.3-0.6 mg/kg to facilitate controlled ventilation with TV 7-10 ml/kg to maintain end tidal carbon dioxide 30 - 35 mm Hg. Incremental doses of atracurium (1/5 of the initial dose) were given when needed. All children were adequately hydrated by using normal saline or lactated Ringer's at a rate of 4-6 ml/kg/hr.

Patients were randomly allocated into three equal groups according to the technique, each group included 30 patients.

**Group I (control group):** received general anesthesia alone.

**Group II (Rectus sheath block group) (RSB group):** After induction of general anesthesia, a 24-gauge needle was inserted above or below the umbilicus 0.5 cm medial to the linea semilunaris in a perpendicular plane. A “gritty” sensation is encountered upon moving the needle, identifying the anterior rectus sheath and a volume of 0.1 ml/kg of bupivacaine 0.25% with fentanyl 1μg/kg was

injected after a negative aspiration test. As the needle is advanced, the belly of the muscle will be encountered, and the posterior aspect of the rectus sheath can be appreciated by the same "gritty" sensation. The usual depth of needle entry was about 0.5 to 1.5 cm. After a negative aspiration, 0.2 ml/kg of bupivacaine 0.25% with fentanyl 1µg/kg was injected between the posterior aspect of rectus sheath and the rectus abdomenus muscle. Both injections were repeated on the opposite side.

**Group III (epidural group) (EP group):** After induction of general anesthesia, children were positioned in the lateral decubitus position, a midline approach of lumbar epidural needle placement between L4-5 was preferred, identification of the epidural space was achieved by loss of resistance to saline and epidural block was induced with a 24-gauge canula using bupivacaine 0.25% 0.5 ml/kg and fentanyl 1µg/kg.

In all patients skin incision was performed at least 15 min after placement of the block. In all groups blood samples were taken before induction of anesthesia, 20 minutes after skin incision, and 30 minutes postoperatively to determine the level of blood glucose as an index to stress response.

On completion of the surgery, sevoflurane was discontinued and residual effect of the muscle relaxant was reversed with I.V neostigmine 50 µg/kg and atropine 20 µg/ kg and patients were extubated when fully awake .

Intraoperatively all patients were monitored with ECG, Pulse oximetry, non invasive arterial blood pressure and end-tidal CO2 using capnography. These parameters were recorded before induction (basal values) except ETCO2 which was recorded after intubation, then every 15 minutes through the intraoperative period.

Post-operative analgesia was assessed by MOPS[9], physiological pain measures e.g. Heart rate, blood pressure and oxygen saturation, the duration of analgesia (the first request of postoperative analgesia) and the total analgesic consumption during the first 24

hours.

MOPS, heart rate, blood pressure and oxygen saturation was assessed immediately (1 min) after recovery, 30 minutes, 1, 2,6,12 and 24 hours postoperatively. If two consecutive assessments yielded MOPS of ≥ 6, the child was received rectal diclofenac 1mg/kg.

Post-operative recovery and discharge was assessed by Modified Aldrete Score[10] to assess post-operative recovery, Fast-Track score [11] to confirm the PACU bypass (at 1 min interval after discontinuation of anesthesia for 5 min then every 5 min until the patients achieved Fast-Track eligibility) and Post Anesthesia Home Discharge Score (PADS)[12] to confirm the home discharge.

**Statistical Analysis:**

The statistical analysis of data done by using excel program and SPSS program (statistical package for social science version 10). To test the normality of data distribution K-S (Kolmogorov-Smirnov) test was done. The description of the data done in form of mean (+/-) SD for quantitative data and Frequency and proportion for Qualitative data. The analysis of the data was done to test statistical significant difference between groups. ONE WAY ANOVA test to compare more than two groups, followed by Post Hoc test LSD (least significant difference) for inter groups comparisons. 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. P is significant if ≤ 0.05 at confidence interval 95%.

## Results

The demographic data including age, body weight, sex and duration of surgery were comparable with no significant differences among the three groups (table 5).

As regard to hemodynamics, there were no significant changes in heart rate or mean arterial blood pressure in group II and group III when compared to the basal values. These parameters showed significant increase in group I when compared to the basal values (table 6&7).

Arterial oxygen saturation

and End Tidal CO2 showed insignificant differences among the studied groups.

As regard to pain assessment, MOPS showed a significant decrease in group II when compared with group I and group III (table 8). The duration of analgesia in hrs showed a significant increase in group II (21±3) when compared with group I (0.2±0.14) and group III (16±7) (table 9). Also, total analgesic consumption (diclofenac in mg) in the first postoperative 24 hours showed a significant decrease in group II (1.6±13) when compared with group I (34.6±24.9) and group III (9.2±16) (table 10).

Modified Aldrete Score, Fast-Track score and PADS showed a significant increase in group II after recovery from anesthesia when compared with group I and group III (table 11, 12 & 13). The incidence of PONV immediately after recovery from anesthesia showed a significant increase in group III (9 cases) and group I (7 cases) when compared with group II (3 cases) (table 14).

## Discussion

**Table 1:** Modified Objective Pain Score (MOPS).

| Score | 0 | 1 | 2 |
|---|---|---|---|
| **Crying** | none | consolable | not consolable |
| **movement** | none | restless | thrashing |
| **agitation** | asleep<br>calm | Mild can be comforted to lessent the agitation | Hysterical cannot be comforted |
| **posture** | normal | flexed | holds injury site |
| **verbal** | asleep<br>no complaint | Complains but cannot localize | complains and can localize |

- Minimum score: 0
- Maximum score: 10
- The higher the score the greater the pain experience for the child

**Table 2:** Modified Aldrete Score.

| Score | 2 | 1 | 0 |
|---|---|---|---|
| **Activity: Able to move voluntarily or on command** | 4 extremities | 2 extremities | Not able |
| **Respiration** | Able to breathe deep and cough freely | Dyspnea, shallow or limited breathing | Apnea |
| **Circulation** | BP ± 20% of preanesthetic level | BP ± 20-49% of preanesthetic level | BP ± 50% of preanesthetic level |
| **Consciousness** | Fully awake | Arousable on calling | Not responding |
| **Oxygen Saturation** | Able to maintain SaO2 > 92% on room air | Needs oxygen to maintain SaO2 > 90% | Saturation < 90% with oxygen |

Total score =10

The patient can be transported safely from PACU to discharge area if Modified Aldrete Score was ≥ 8.

**Table 3 :** Fast-Track score.

| Score | 2 | 1 | 0 |
|---|---|---|---|
| Level of consciousness | awake and oriented | arousable with minimal stimulation | responsive only to tactile stimulation |
| Physical activity | able to move all extremities on command | some weakness in movement of extremities | unable to voluntarily move extremities |
| Hemodynamic stability | blood pressure < 15 of baseline MAP value | blood pressure between 15-30% of baseline MAP value | blood pressure > 30% below baseline MAP value |
| Respiratory stability | able to breathe deeply | tachypnea with good cough | dyspneic with weak cough |
| Oxygen saturation status | maintains value >90% on room air | requires supplemental oxygen (nasal prongs) | saturation less than 90% with supplemental oxygen |
| Postoperative pain assessment | none or mild discomfort | moderate-to-severe pain controlled with iv analgesics | persistent severe pain |
| Postoperative emetic symptoms | none or mild nausea with no active vomiting | transient vomiting or retching | persistent moderate-severe nausea and vomiting |

Total score =14

Score ≥ 12 required for discharge (with no score <1 for each individual category)

**Table 4**. Post anesthesia Discharge Scoring System (PADS) for Determining Home-Readiness.

| Score | 2 | 1 | 0 |
|---|---|---|---|
| Vital signs<br>Vital signs must be stable and consistent with age and preoperative baseline | BP and pulse within 20% of preoperative baseline | BP and pulse 20%–40% of preoperative baseline | BP and pulse >40% of preoperative baseline |
| Activity level<br>Patient must be able to ambulate at preoperative level | Steady gait, no dizziness, or meets preoperative level | Requires assistance | Unable to ambulate |
| Nausea and vomiting<br>The patient should have minimal nausea and vomiting before discharge | Minimal: successfully treated with PO medication | Moderate: successfully treated with IM medication | Severe: continues after repeated treatment |
| Pain<br>The patient should have minimal or no pain before discharge | Minimal: successfully treated with PO medication | Moderate: successfully treated with IM medication | Severe: continues after repeated treatment |
| Surgical bleeding<br>Postoperative bleeding should be consistent with expected blood loss for the procedure | Minimal: does not require dressing change | Moderate: up to two dressing changes required | Severe: more than three dressing changes required |

Maximal score = 10; patients scoring ≥9 are fit for discharge.

**Table (5):** Patients characteristics of the studied groups, values are in mean ± SD or number

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| Age (year) | 4.3±2.4 | 5.2±1.3 | 6±2 |
| Gender (male/female) | 19/11 | 22/8 | 24/6 |
| Weight (kg) | 14.5±4 | 15±5.4 | 15.2±3.3 |
| Duration of Surgery (min) | 60±14 | 60±15 | 60±18 |

**Table (6):** Peroperative heart rate (bpm) of the studied groups, values are in Mean ± SD

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| Basal | 119±7 | 121± 12 | 120± 11 |
| 15 min | 127 ± 11* | 121± 8# | 116± 10# |
| 30 min | 127± 12* | 120± 9# | 116± 8# |
| 45 min | 126± 10* | 119±9# | 116± 8# |
| 60 min | 126± 11* | 118± 9# | 116± 9# |
| 1 min postop. | 126± 10* | 118 ± 8# | 116± 9# |
| 30 min postop. | 126± 9* | 118± 8# | 115± 8# |
| 1 hour postop. | 125± 10* | 118±8# | 114± 8# |
| 2 hours postop. | 125± 9* | 118± 8# | 114± 9# |
| 6 hours postop. | 124± 8* | 117± 9# | 114± 9# |
| 12 hours postop. | 124± 9* | 117± 9# | 113± 9# |
| 24 hours postop. | 123± 9* | 117± 9# | 113± 9# |

Significant when ($p < 0.05$)

* Significant compared to the basal value in the same group.

\# Significant compared to group I.

**Tables (7):** Peroperative mean arterial blood pressure (mmHg) of the studied groups; values are in Mean ± SD.

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| Basal | 72±12 | 73±13 | 72±10 |
| 15 min | 75±12* | 72±10# | 71±9# |
| 30 min | 76±12* | 72±9# | 71±8# |
| 45 min | 76±11* | 72±10# | 71±8# |
| 60 min | 77±12* | 72±11# | 71±7# |
| 1 min postop. | 77±11* | 71±10# | 71±7# |
| 30 min postop. | 76±11* | 71±11# | 70 ±8# |
| 1 hour postop. | 77±12* | 71±10# | 70±9# |
| 2 hours postop. | 78±12* | 71±9# | 70±10# |
| 6 hours postop. | 76±11* | 71±10# | 70±11# |
| 12 hours postop. | 76±11* | 71±10# | 70±11# |
| 24 hours postop. | 75±11* | 71±11# | 70±10# |

Significant when ($p < 0.05$)

* Significant compared to the basal value in the same group.

\# Significant compared to group I.

**Table (8):** Modified Objective Pain Score (MOPS) for 24 hours postoperative, values are in Mean ± SD.

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| 1 min postop. | 8 ±0.2 | 0.7 ±1.2# | 0.5±0.8# |
| 30 min postop. | 8±0.4* | 0.8±1*# Ø | 0.8 ±1.3*# |
| 1 h postop. | 9±0.3* | 1±1.3*# Ø | 1.5 ±1.6*# |
| 2 h postop. | 9 ±0.5* | 1 ±2*# Ø | 3 ±2*# |
| 6 h postop. | 10 ±0.0* | 2±2*# Ø | 4 ±2*# |
| 12h postop. | 10 ±0.0* | 3±2*# Ø | 5±2*# |
| 24 h postop. | 10 ±0.0* | 4±2*# Ø | 7±2*# |

Significant when ($p < 0.05$)

* Significant compared to the basal value in the same group.

\# Significance compared to group I.

Ø Significant compared to group III.

**Table (9):** Duration of analgesia in hr (first request of analgesia), values are in Mean ± SD.

| Group I | Group II | Group III |
|---|---|---|
| 0.2 ± 0.14 | 21 ± 3# Ø | 16 ± 7 # |

Significant when ($p < 0.05$)

\# Significant compared to group I.

Ø Significant compared to group III.

**Tables (10):** Total analgesic consumption (diclofenac in mg) during the first postoperative 24 hours, values are in Mean ± SD.

| Group I | Group II | Group III |
|---|---|---|
| **34.6 ± 24.9** | **1.6 ± 13# Ø** | **9.2 ± 16 #** |

Significant when ($p < 0.05$)

\# Significant compared to group I.

Ø Significant compared to group III.

**Table (11):** Modified Aldrete Score for (24 hours postoperative), values are in Mean ± SD.

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| 1 min postop. | 8 ±0.2 | 8.2±0.4# Ø | 8.1 ±0.3# |
| 30 min postop | 8.2 ±0.4 * | 9±0.5*# Ø | 8.5 ±0.5*# |
| 1 h postop. | 8.5±0.2 * | 9.3 ±0.4*# Ø | 9.1 ±0.3*# |
| 2 h postop. | 9 ±0.3 * | 9.5 ±0.5*# Ø | 9.2 ±0.4*# |
| 6 h postop. | 10 ±0* | 10 ±0* | 10±0* |
| 12h postop. | 10 ±0* | 10 ±0* | 10 ±0* |
| 24 h postop. | 10 ±0* | 10 ±0* | 10 ±0* |

Significant when ($p < 0.05$)

* Significant compared to the basal value in the same group.

\# Significant compared to group I.

Ø Significant compared to group III.

**Table (12):** Fast Track Score postoperative (for 15 min), values are in Mean ± SD.

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| 1 min postop. | 9.4 ± 1 | 11.7 ±0.5 # Ø | 10.2 ±0.4 # |
| 2 min postop. | 9.4 ±1 | 12.4 ±0.7 *# Ø | 10.3 ±0.5 # |
| 3min postop. | 9.7 ±1 * | 12.4 ±0.6 *# Ø | 10.4 ±0.5 *# |
| 4 min postop. | 10.4 ±1* | 12.9 ±0.8 *# Ø | 10.7±0.6 *# |
| 5 min postop. | 10.7 ±1 * | 13.3 ±0.7 *# Ø | 11 ±0.8 *# |
| 10 min postop. | 11 ±1 * | 13.5 ±0.5 *# Ø | 12 ±0.9 *# |
| 15 min postop. | 11 ±1 * | 13.7 ±0.3 *# Ø | 12 ± 0.9 *# |

Significant when ($p < 0.05$)

* Significant compared to the basal value in the same group.

\# Significant compared to group I.

Ø Significant compared to group III.

**Table (13):** Post anesthesia Discharge Scoring System (PADS) for Determining Home-Readiness for 24 hours postoperative, values are in Mean ± SD.

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| 1 min postop | 5.6 ±1.4 | 7.0 ±0.8# Ø | 5.7±0.9 |
| 30 min postop | 6.0 ±1.3* | 7.8 ±0.4*# Ø | 6.7 ±0.7*# |
| 1 h postop. | 6.9 ±.8* | 8.5 ±0.7*# Ø | 7.7 ±0.7*# |
| 2 h postop. | 7.4 ±1.1* | 8.7 ±0.5*# Ø | 8.2 ±0.7*# |
| 6 h postop. | 7.7 ±1.1* | 9.6±0.7*# Ø | 8.9 ±.7*# |
| 12h postop. | 8.8 ±0.8* | 10 ±0*# Ø | 9.6±0.5*# |
| 24 h postop. | 9.2 ±0.7* | 10 ±0*# Ø | 9.9±0.3*# |

Significant when ($p < 0.05$)

* Significant compared to the basal value in the same group.

\# Significant compared to group I.

Ø Significant compared to group III.

**Table (14):** Post-operative Nausea and Vomiting (for 24 h postoperative), values are number and percentage.

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| **No** | 7* | 3 | 9* |
| **%** | 23.3% | 10.0% | 30.0% |

Significant when ($p < 0.05$)

* Significant compared to group II.

Effective post-operative pain management is an essential requirement for any enhanced recovery programme and it is recognized that the analgesic technique used influences outcome after surgery. Analgesic protocols should aim to reduce pain and improve patient comfort, minimize the side effects of analgesic agents and lessen the deleterious effects resulting from the stress response to surgery[13].

In our present study, the heart rate and mean arterial blood pressure showed a significant decrease in both group II and group III after induction of anesthesia, during intraoperative and postoperative periods compared to group I which show a significant increase in both parameters and this is explained by lack of analgesia in group I.

Our results run parallel to Buggy et al, 2002, who had compared the effect of epidural anesthesia and analgesia versus the effect of intravenous morphine analgesia in patients after major abdominal surgery. They had found that epidural anesthesia and postoperative analgesia attenuate the stress response to surgery, promote systemic vasodilatation and provide superior analgesia compared with parenteral methods of analgesia [14].

Also, our results are in agreement with Ferguson et al, 1996, who conducted a study about rectus sheath block in pediatric anesthesia and found that there were no hemodynamic changes associated with RSB either during instituting the block or with surgery[5].

On the other hand, when we compared hemodynamics between group II and group III, our results suggest that RSB can provide good intraoperative and postoperative analgesia as epidural technique which is reflected by hemodynamic stability as the result of attenuation of sympathetic response to pain and this is explained by direct placing of local anesthetic to the targeted nerves leading to blockade of the anterior cutaneous branches of the thoracic nerves within the rectus sheath producing good intraoperative and postoperative

analgesia [15].

Our result runs parallel to Dolan et al, 2009, who conducted a study about rectus sheath block in which he study the accuracy of local anesthetic placement by trainee anesthesiologists using loss of resistance or ultrasound guidance for diagnostic laparoscopy and suggests that RSB can provides an alternative to epidural analgesia [4].

In this present study, as regards pain assessment, there was a significant increase in the duration of analgesia in group II (21±3 hr) and group III (16±7hr) when compared with group I (0.2±0.14 hr). Also, there was a significant decrease in total analgesic consumption (mg) during the first postoperative 24 hours in group II (1.6±13 mg diclofenac) and group III (9.2±16 mg diclofenac) when compared with group I (35±25 mg diclofenac).

Similar results were reported by Frederick et al 2007, who had compared a rectus sheath block with local infiltration for umbilical herniorraphy. They had concluded that rectus sheath block results in improved postoperative pain control through the more precise delivery of local anesthetic to the intercostal nerves [15].

Also our results are in agreement with those reported by Isaac et al., 2006, who made a pilot study of the rectus sheath block comparing the efficacy of the rectus sheath block with that of our current standard practice local anesthetic infiltration into the surgical wound for pain control after umbilical hernia repair and stated that the use of the rectus sheath block prior to the start of surgery improve pain management in the postoperative period and prolong the duration of analgesia due to better absorption of local anesthetic by intact tissues and placing local anesthetic in close proximity to the intercostal nerves [16].

Moreover, Anwar et al, 2008, made a study about per-operative infiltration of the rectus sheath in abdominoplasty. They had recommended use of RSB as a standard protocol for abdominoplasty patients as the requirements for

postoperative analgesia were reduced significantly in abdominoplasty patients due to relief of rectus spasm[17].

On the other hand, our results are contradictory to those reported by Lisa et al, 2006, who concluded that the rectus sheath block has no advantage over local infiltration into the surgical wound for postoperative pain management in children undergoing umbilical hernia repair[16]. This also explained by the results reported by Charlton et al, 2010, who made a study about perioperative transversus abdominis plane (TAP) blocks for analgesia after abdominal surgery and concluded that," No studies have compared rectus sheath block with other analgesics such as epidural analgesia and there is only limited evidence to suggest use of perioperative rectus sheath block reduce opioid consumption and pain scores after abdominal surgery when compared with no intervention or placebo"[18].

In this present study, MOPS showed a significant decrease in group II and group III at 1 min, 30 min, 1hour, 2 hours, 6 hours, 12 hours and 24 hours after recovery from anesthesia when compared with group I as the result of lack of analgesia in group I. Also, there was a significant decrease in MOPS in group II at 30 min, 1hour, 2 hours, 6 hours, 12 hours and 24 hours after recovery from anesthesia when compared with group III.

Our results are in agreement with those reported by Cornish and Deacon 2007, who conducted a study about rectus sheath catheters for continuous analgesia after upper abdominal surgery and found that RSB used as an analgesic technique for abdominal surgery showed a reduction in visual analogue pain Scores when it was performed as a single shot in Patients undergoing diagnostic laparoscopy and a limited number of studies have since been done in both children and adults and have demonstrated the usefulness of the technique for post-operative pain control [19].

Also, our results are in agreement with those reported with Willschke et al, 2006, who con-

ducted a study about ultrasound guided rectus sheath block in children and concluded that RSB provides sufficient analgesia in all children with no need for additional analgesia in the perioperative period. Objective pain score (OPS) did not exceed 11 from a maximum score of 15 in any of the patients at any time before discharge. Children were usually discharged home after 4 h as per hospital routine. None of the parent or care providers reported any pain management concerns after discharge[20].

In our present study, Fast Track Score and Modified Aldrete Score showed a significant increase in group II after recovery from anesthesia when compared with group I and group III. This can be explained by the analgesic properties of RSB with hemodynmic stability and the significant decreases in pain score (MOPS) which offer better recovery and discharge criteria than the control group and even than the epidural group as there is no motor block.

Same results were reported by Frederick et al, 2007, they had shown a significant decrease in the requirements for postoperative analgesia when a RSB is used compared with local infiltration. They believe that, RSB gets the LA into the correct tissue plane more reliably and RSB also showed a trend towards fewer requirements for any analgesia within hospital and earlier discharge[15].

In our present study, Post anesthesia Discharge Scoring System (PADS) for Determining Home-Readiness showed a significant increase in group II after recovery from anesthesia when compared with group I and group III. These results are parallel with the significant decreases that occurred in group II in MOPS score, the significant increases in the duration of analgesia, Modified Aldrete Score and Fast Track Score and the low incidence of postoperative side effects.

Our results are in agreement with those reported by Parsons et al, 2010, who made a study about use of rectus sheath catheters (RSCs) as an analgesic technique for patients undergoing radical cystectomy and comparing

epidural catheter group (EC) with (RSCs) group and concluded that the potentials for early mobilization, a possible reduction in post-operative ileus rate and a decrease in opioid-mediated side effects mean that RSCs are a regional analgesic technique which may be ideally suited to an enhanced recovery protocol[21].

Also our results are in agreement with those reported by Cornish and Deacon 2007, who conducted a study about rectus sheath catheters for continuous analgesia after upper abdominal surgery and found that RSB is important for several reasons. First, it potentially provides a viable alternative to epidural analgesia. Second, it seems to minimize the use of opioids, with less nausea, ileus and mental confusion. Third, with no motor block of the legs, mobilization can be rapid. Fourth, it facilitates early removal of the urinary catheter. Fifth, it is a simple surgical procedure and the placement of the catheter under direct vision ensures correct placement[19].

In our present study, there was an increase in the incidence of PONV in group III (9 cases) and group I (7 cases) immediately after recovery from anesthesia when compared with group II (3 cases) and this is explained by lack of analgesia in group I.

Our results are in agreement with those reported by Peyton et al., 2003, who made a study about perioperative epidural analgesia and outcome after major abdominal surgery in high-risk patients and found that a combination of local anesthetic and opioid is the most commonly used epidural solution in the UK and Australia however, the use of opioids leads to an increase in unwanted opiate-mediated side-effects such as nausea, vomiting, confusion, pruritus and prolongation of post-operative ileus. Therefore, despite being considered the most effective technique for pain control following major surgery, the role of epidurals in enhanced recovery protocols is put into question by studies that have failed to show a reduction in the length of stay following surgery and this has prompted the search for newer an-

algesic techniques [22].

## Conclusion

Based on the previous results of our study, it could be concluded that the rectus sheath block appears to be safe, easy to learn and perform, and provides the pediatric anesthetist with another method of providing effective and apparently long-lasting analgesia for a common day-case procedure. As the rectus sheath block was associated with intraoperative and postoperative hemodynamic stability, more prolonged analgesic effect, fast emerging and recovery from anesthesia that provides the eligibility of fast tracking process, short stay in PACU, early home discharge and less postoperative side effects. So it can be used as an alternative to epidural analgesia in providing adequate analgesia after abdominal surgery.

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REPRINT


# BENHA MEDICAL JOURNAL


## COMPARATIVE STUDY BETWEEN THE EFFECT OF EPIDURAL AND RECTUS SHEATH BLOCKS USING BUPIVACAINE - FENTANYL MIXTURE IN CHILDREN SUBMITTED FOR ELECTIVE LOWER ABDOMINAL SURGERY


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# EVALUATION OF NITRATE REDUCTASE ASSAY FOR DETECTION OF DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS ISOLATES

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## Abstract

***Background/Aim:*** *After years of decline, tuberculosis (TB) has re-emerged as a serious public health problem worldwide, especially with the increasing rates of multidrug-resistant (MDR) TB and the pandemic of human immunodeficiency virus/AIDS. Early detection of drug resistance in TB is necessary for effective patient treatment and to prevent transmission of the disease in the community. The current methods for drug susceptibility testing (DST) of Mycobacterium tuberculosis (Mtb) are either slow or costly. Therefore, rapid and inexpensive methods are needed for better control of the disease. So, this study was aimed to evaluate the nitrate reductase assay (NRA) as an alternative method for detection of Mtb susceptibility to isoniazid (INH), rifampicin (RIF), streptomycin (STR) and ethambutol (ETH).*

***Patients and Methods:*** *sixty two sputum specimens were subjected to culture on Löwenstein-Jensen (LJ) medium. The revealed 43 isolates of Mtb were subjected to DST by indirect NRA in comparison to LJ proportion method (PM) as a reference standard method.*

***Results:*** *This study revealed that resistance of Mtb isolates to anti-TB drugs by NRA & LJ PM was comparable with insignificant difference ($p>0.05$). There were an excellent agreement between indirect NRA and indirect LJ PM for INH, RIF & STR (kappa=0.85, 0.86 & 0.81 respectively) and a moderate agreement for ETH (kappa=0.53).*

*The sensitivity & specificity of NRA compared to standard method*

*(PM) for INH were 92.9% & 93.3% and for RIF were 89.5% & 95.8%. While sensitivity &specificity of NRA for STR were 81.8% & 96.9% and for ETH were 54.5% & 93.8% respectively. The accuracy of NRA susceptibility test were 93.0% for each of INH, RIF &STR while 84.0% for ETH. The median detection time of NRA was significantly faster (10 days) than LJ PM (28 days).*

***Conclusion:*** *Indirect NRA has been demonstrated to be a promising inexpensive, easy, rapid and accurate alternative tool for detection of anti-TB susceptibility compared to LJ PM.*

## Introduction

Tuberculosis (TB) is today acknowledged as an health catastrophe.[1] Globally, about one-third of the world population is latently infected with the tuberculosis bacillus and new carriers occur at a rate of one per second.[2] In Egypt, the latest surveillance data in 2009 revealed that a total of 18,000 TB cases existed in the country with a prevalence rate of 24/100,000 population and estimated incidence rate of 19 and 8/100,000 for all forms and smear positive TB, respectively.[3]

Conventional drug susceptibility testing (DST) methods for M. tuberculosis (Mtb) on LJ (the proportion method, the absolute concentration method and the resistance ratio method) take about six weeks for the results to be obtained. This leads to loss of time and delayed initiation of proper treatment, resulting in transmission of the drug resistant infection to the community.[4] Liquid automated commercial systems such as the BACTEC-MGIT 960 are rapid but need expensive equipments. Molecular genetic methods such as line probe assay are fast, expensive and not detect all mutations.[5,18]

Therefore, there is obviously a great need for fast, reliable and inexpensive methods for detection of anti-TB drug susceptibility. One of these methods that could potentially satisfy these criteria is the nitrate reductase assay, a colorimetric assay which known also Griess method. It was developed at the Central Tuberculosis Research Institute in Moscow, Russia.[6] This method is based on the ability of Mtb to reduce nitrate to

nitrite by the nitrate reductase enzyme, which is indicated by the development of a dark rose to purple-rose colour after addition of Griess reagent.[7]

The purpose of this study was the evaluation of NRA as an inexpensive, rapid and accurate method for detection of drug susceptibility of Mtb isolates to anti-TB drugs; isoniazid, rifampicin, streptomycine and ethambutol in comparison to the conventional indirect LJ proportion method.

## Patients & Methods

This study was performed on 43 fresh Mycobacterium tuberculosis (Mtb) isolates from Lowenstein-Jensen (LJ) cultures of 62 sputum specimens from pulmonary TB patients attending Mansoura Chest Hospital and thoracic medicine outpatient clinics of Mansoura University Hospitals. All isolates were Mtb complex as identified by NAP (ρ-nitro-α-acetylamino-β-hydroxy-propiophenone) test.

**Media and reagents:**

First-line anti-TB drugs were incorporated into LJ media, for the proportion method (PM) and into LJ media containing 1,000μg/ml of (KNO3) potassium nitrate, for the nitrate reductase assay (NRA) method. The final concentration of isoniazid (INH), rifampicin (RIF), streptomycine (STR) and ethambutol (ETH) were 0.2, 40, 4, and 2μg/ml respectively. Then, the media were steamed at 85°C for 50 minutes. The control media without drugs (LJ for PM & LJ with KNO3 for NRA) were prepared at the same time as the drug containing media. The media were incubated for 48 hours at room temperature for sterility check before use.[8]

Griess reagent was prepared shortly before its use by mixing 1 part of 50% (vo/vol) concentrated hydrochloric acid (HCl), 2 parts of 0.2% (wt/vol) sulfanilamide and 2 parts of 0.1% (wt/vol) N-1-naphthylethylenediamine dihydrochloride.[9]

Mtb suspension was made by scraping about 4 mg of Mtb colonies grown on LJ medium using a wire loop in a tube containing 6-9 sterile glass beads and 3-4 ml of distilled water (DW). Tubes were

vigorously agitated and clumps were allowed to settle 30 min. The supernatant was then adjusted with DW to equal the density of a 1.0 McFarland standard. Serial dilutions of $10^{-1}$ to $10^{-5}$ of the standard Mtb suspension were prepared.

**Indirect NRA assay :**

The NRA was performed as described by Angeby et al.[10] Briefly, for each isolate, 0.2 ml of the undiluted Mtb suspension was added into the tubes containing LJ medium with KNO3 and the anti-TB drugs; and 0.2 ml of $10^{-1}$ diluted Mtb suspension was inoculated into each of 3 drug-free media containing KNO3 (control tubes) and were incubated at 37℃. After 7 days of incubation, 0.5 ml of freshly prepared Griess reagents was added to one of the 3 control tubes; If its colour changed to pink, the drug-containing tubes were tested on the same day but if did not show any colour change and remained the same, this control tube was discarded and the remaining tubes were further incubated, and the procedure repeated on day 10 and 14 respectively.

The isolate was considered resistant to drug if the colour change in the antibiotic tube was the same or greater than the control. While the isolate was considered susceptible if there was no colour change in the drug-containing tube. NRA was considered invalid if the nitrate reaction was negative in the control tube at day 14 inspite of the presence of colonies (figure 1).

**Conventional indirect LJ PM :**

The technique was carried out on normal LJ medium according to the laboratory's standard procedure.[11] For each isolate, 2 drug-free LJ slants were inoculated with 0.1 ml of each dilution[10-3 & 10-5] and the 4 drug-containing LJ slants were inoculated with 0.1 ml of $10^{-3}$ diluted suspension. Slants were incubated at 37℃, and colonies were enumerated on day 28. Drug resistance was defined as the growth of colonies on the drug-containing slant equal to ≥1% of that of colonies on the drug-free slant (figure 1). If the result at the $28^{th}$ day was "sensitive", a second reading was made on the $42^{nd}$ day.

**Statistical analysis :**

Data were analysed using SPSS version 11 (SPSS, Chicago, IL, USA). The Wilcoxon signed-rank test was used to compare the times and the McNemar's test was used for comparing paired proportions, A P value of <0.05 was considered to be statistically significant.[12] The performance of indirect NRA method in comparison with the LJ PM was evaluated in terms of sensitivity (ability to detect true resistance) and specificity (ability to detect true sensitivity). The agreement between the 2 methods was estimated by the Kappa statistic.[13]

## Results

Drug susceptibility testing of 43 Mtb isolates revealed insignificant difference between susceptibility of Mtb to INH, RIF, STR and ETH by indirect NRA and reference standard LJ PM (p>0.05). INH showed the highest resistance by both indirect NRA & PM (62.8% & 65.1% respectively) followed by RIF (41.9% & 44.2%). STR showed a low resistance by indirect NRA & PM (23.3% & 25.6%). ETH revealed the lowest resistance by NRA (18.6%) compared to PM (25.6%), table (1).

Table (2) shows an excellent agreement between indirect NRA and LJ PM for INH, RIF &STR (kappa = 0.85, 0.86 & 0.81) and a moderate agreement for ETH (kappa = 0.53). The highest sensitivity of NRA for detecting resistance was observed with INH (92.9%), followed by RIF (89.5%) & STR (81.8%). On the other hand, NRA showed the lowest sensitivity for detecting resistance with ETH (54.5%). The specificity of indirect NRA for detecting sensitive Mtb isolates was higher for STR & RIF (96.9% & 95.8%) than for ETH &INH (93.8% & 93.3% respectively). The accuracy of NRA susceptibility test was 93.0% for each of INH, RIF & STR while 84.0% for ETH.

Indirect NRA detected drug susceptibility pattern of Mtb isolates within a median time of 10 days (range = 7-14 days) which was significantly faster than indirect LJ PM (p<0.001) which needed a median time of 28 days (range = 28 - 42 days) (p< 0.001), table (3).

**Table 1:** Drug susceptibility pattern of 43 *Mtb* isolates by indirect NRA and PM.

| Drug | indirect NRA | | PM | | P value* |
|---|---|---|---|---|---|
| | Sensitive No (%) | Resistant No (%) | Sensitive No (%) | Resistant No (%) | |
| **INH** | 16 (37.2%) | 27 (62.8%) | 15 (34.9%) | 28 (65.1%) | 1.0 |
| **RIF** | 25 (58.1%) | 18 (41.9%) | 24 (55.8%) | 19 (44.2%) | 1.0 |
| **STR** | 33 (76.7%) | 10 (23.3%) | 32 (74.4%) | 11(25.6%) | 1.0 |
| **ETH** | 35 (81.4%) | 8 (18.6%) | 32 (74.4%) | 11 (25.6%) | 0.453 |

*McNemar's test (statistically significant if p value< 0.05).

**Table 2:** Performance characteristics of indirect NRA compared to PM.

| Drug | LJ | Indirect NRA | | | | | | | | Agreement (Kappa value)* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No | | | % | | | | | |
| | | | R | S | SV | SP | PPV | NPV | accuracy | |
| **INH** | PM | R | 26 | 2 | 92.9 | 93.3 | 96.3 | 87.5 | 93.0 | 0.85 |
| | | S | 1 | 14 | | | | | | |
| **RIF** | PM | R | 17 | 2 | 89.5 | 95.8 | 94.4 | 92.0 | 93.0 | 0.86 |
| | | S | 1 | 23 | | | | | | |
| **STR** | PM | R | 9 | 2 | 81.8 | 96.9 | 90.0 | 93.9 | 93.0 | 0.81 |
| | | S | 1 | 31 | | | | | | |
| **ETH** | PM | R | 6 | 5 | 54.5 | 93.8 | 75.0 | 85.7 | 84.0 | 0.53 |
| | | S | 2 | 30 | | | | | | |

R: Resistant, S: Sensitive, SV: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value

* The kappa statistic describes the strength of agreement as follows: 0.00 to 0.20, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, good ; and 0.81 to 1.00, excellent.

**Table 3:** Detection time of antimycobacterial TB susceptibility tests by indirect NRA and PM.

| DST method | Detection time (days) | | P value* |
|---|---|---|---|
| | Median | Range | |
| **Indirect NRA** | 10 | 7-14 | < 0.001▾ |
| **Indirect PM** | 28 | 28-42 | |

* Wilcoxon Signed Ranks test.
▾ Statistically significant (p value< 0.05).

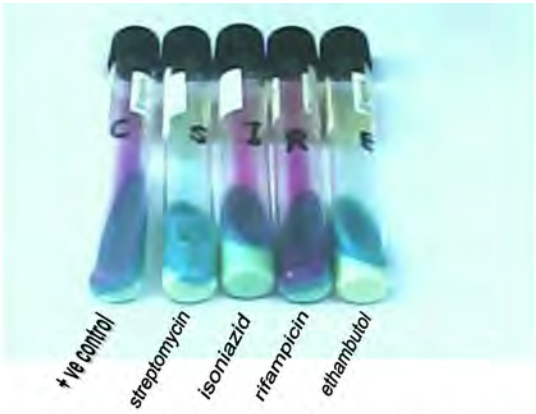


**Figure 1:** Nitrate reductase assay.

- The colour change in isoniazid &rifampicin tubes is the same or greater than control tube ➔ resistant.
- No colour change in both streptomycin &ethambutol tubes ➔ sensitive.

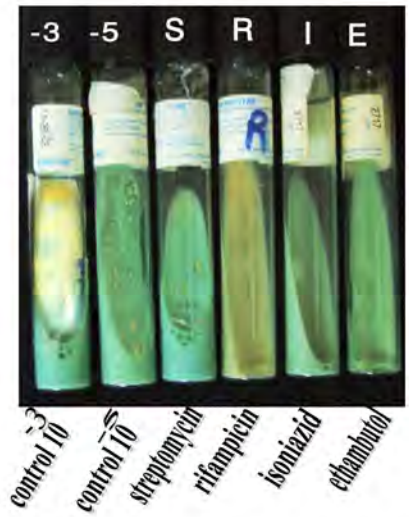


**Figure 2 :** Proportion method on LJ.

**From left to right, the tubes show:**

- Confluent growth on dug free tube inoculated with $10^{-3}$ Mtb suspension.
- Countable colonies on dug free tube inoculated with $10^{-5}$ Mtb suspension ➔ acting as a control for calculating the % of resistant organisms in the isolate.
- Colonies on streptomycin- containing tube less than 1% of that on control ➔ sensitive.
- Colonies on rifampicin -containing tube more than 1% of that on control ➔ rifampicin resistant.
- No colonies on both isoniazid & ethambutol -containing tubes ➔ sensetive.

## Discussion

Tuberculosis (TB), one of the oldest recorded human diseases, is still one of the biggest killers among the infectious diseases, despite the use of a live attenuated vaccine and several antibiotics.[14] The growing problem of drug resistance in Mycobacterium tuberculosis (Mtb) is accompanied by increasing demand for quick, cheap, and easy techniques to detect resistance allowing a better management and treatment of patients and decreasing the risk of disease and possible spread of drug resistance.[15] The methods currently available for rapid drug susceptibility testing (DST) of Mtb are either cheap but slow, or fast but too costly to be applicable in high incidence TB areas.[16]

Therefore, the aim of this study was to evaluate the nitrate reductase assay (NRA) as a rapid method of DST for anti-TB drugs; isoniazid (INH), rifampicin (RIF), streptomycine (STR) and ethambutol (ETH) in comparison with the conventional proportion method (PM).

DST of 43 Mtb isolates by indirect NRA and PM (table 1) revealed comparable values for all tested anti-TB drugs with insignificant difference ($p>0.05$). INH showed the highest resistance (62.8% by NRA vs. 65.1% by PM), followed by RIF (41.9% by NRA vs. 44.2% by PM). The lowest resistance was encountered with STR (23.3%). ETH showed a lower resistance by NRA (18.6%) than PM (25.6%). In the same locality, using radiometric BACTEC TB 460, Zaghloul,[17] reported that the drug resistance in pulmonary TB were; 36%, 21%, 29% and 86% for INH, RIF, STR and ETH but in the study of Abdelaal et al.[18] the drug resistance in TB cases were; 52%, 46%, 36% and 44% for INH, RIF, STR and ETH respectively which is consistent with our results. The significant increase in INH and RIF resistance in the last 10 years could be attributed to poor compliance and wide use of these drugs for non-TB conditions. While, the high sensitivity of STR & ETH (74.4% for each) was attributed to the restriction of its use in the management of TB patients.[19] In clinical microbiology unit, Mansoura University, genotypic analysis done for RIF and INH resistant

strains revealed that 87% of RIF resistant cases were having rpoB gene mutation. The point mutation in Kat G gene was found in 92% of INH resistance.[18]

The sensitivity and specificity of indirect NRA compared to PM were 92.9% & 93.3% for INH. These results are in agreement with Lemus et al.[20] who obtained sensitivity and specificity of 91.7% & 99.0% respectively. Also, with Angeby et al.[10] who obtained sensitivity and specificity of 97% &96% respectively. On the other hand, it is slightly lower than those obtained by Ani et al.[21] (100% sensitivity & 100% specificity).

The sensitivity and specificity of the NRA method for RIF were 89.5% & 95.8% respectively. Ani et al.[21] reported similar sensitivity & specificity (90% & 96%) by NRA. Also, Lemus et al.[20] obtained 93% sensitivity &99% specificity. However, Angeby et al.[10] and Montoro et al.[22] reported 100% sensitivity & 100% specificity.

The accuracy of NRA, compared to PM, was 93.0% for each of RIF and INH susceptibility testing. These results are close to those of Lemus et al.[20] (99.1% for RIF & 98.4% for INH).

The high accuracy of indirect NRA for RIF and INH is important because RIF & INH are the most important anti-TB drugs and RIF resistance has been identified as a useful surrogate marker for estimation of multidrug resistance.[23] Thus, NRA could be used to screen the resistance to INH &RIF for assessment of MDR prevalence particularly in highly endemic regions.

Sensitivity and specificity of NRA for STR were 81.8% & 96.9% respectively. These results are higher than those obtained by Ani et al.[21] (66.6% sensitivity & 91.8% specificity). However, studies by Lemus et al.[20] and Angeby et al.[10] showed a higher sensitivity of 96.5 % & 95% with a lower specificity of 88.0 % & 83%. On the other hand, the sensitivity and specificity of NRA for STR are in accordance with Cohn et al.[24] who reported sensitivity and specificity of 82.1% & 92.3% and

suggested a review on drug concentrations. Martin et al.[25] revealed similar findings for STR and explained this discrepancy by a possible interaction between the drugs and the potassium nitrate.

This study of indirect NRA in comparison to the reference standard LJ PM revealed low sensitivity (54.5%) & high specificity (93.8%) for ETH susceptibility testing. These results are consistent to some extent with that obtained by Ani et al.[21], Mengatto et al.[26] and Angeby et al.[10] (75% & 100%, 75% & 95% and 75 & 98% respectively). On the contrary, Lemus et al.[20] revealed a higher sensitivity and specificity (88% & 99.1% respectively).

This low sensitivity of NRA for detecting ETH resistance could be explained by the presence of borderline isolates; 5 out of 11 (45.4%) with a low proportion of ETH-resistant organisms (from 1 to 10%).[27] In these borderline isolates, less than 10% of the organisms could grow in the ETH-containing tube (inoculated with undiluted suspension) and exhibit a color change lower than that obtained in the control tube (inoculated with 1:10 diluted suspension) and misidentified as ETH-sensitive Mtb by NRA. Therefore, these borderline isolates are frequently associated with problems in the interpretation of ETH DST that affect the performance of NRA[26],[27] and the conventional standard methods.[28]

In this study, the median time needed to complete the indirect NRA DST was 10 days (range, 7 to 14 days) which is faster than indirect LJ PM (median, 28 days and range, 28 to 42 days). This is attributed to the fact that the NRA depends on detection of nitrate reduction as an indication of growth which could be obtained much faster than visual detection of colonies.[7] The results of the majority of our cases (74.4%) were available in 10 days. These findings are similar to those of Mengatto et al.[26] and Angeby et al.[10] who detected the majority of their cases within 7-10 days.

Although results that obtained by radiometric BACTEC 460 or mycobacterial growth indicator tube (MGIT) were within 7-10

days, they are expensive and require instruments.[29] Genetic methods such as line probe assay, are fast but are too expensive to be used in resource poor settings and have been developed mainly for rifampicin resistance testing.[30] Traditional DST on solid medium (LJ) is slow requiring 4-6 weeks after obtaining initial isolates.[11] On the other hand, the NRA method is inexpensive as it utilizes standard solid LJ medium with addition of KNO3. It is also easy and rapid as it requires 7-14 days.

More than 99 % of Mtb strains possess nitrate reductase enzyme and are capable of reducing nitrate to nitrite. Other Mycobacterium species like M. kansasii & M.smegmatis possess this enzyme. However, these strains are not frequently encountered in human infections. However, M. bovis does not reduce nitrate, therefore the NRA technique is not applicable.[9]

## Conclusion

This study shows an excellent agreement between indirect NRA and LJ PM for rapid detection of isoniazid, rifampicin and streptomycin resistance and a moderate level of agreement for detection of ethambutol resistance. Therefore, indirect NRA (inexpensive, easy, rapid & accurate) could be used to screen the resistance to INH & RIF for assessment of MDR-TB prevalence particularly in highly endemic regions.

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REPRINT


# BENHA MEDICAL JOURNAL


## EVALUATION OF NITRATE REDUCTASE ASSAY FOR DETECTION OF DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS ISOLATES


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# THE EFFECT OF ADDING NITROGLYCERINE AS AN ADJUVANT TO LIDOCAINE ON THE QUALITY OF INTRAVENOUS REGIONAL ANESTHESIA

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## Abstract

***Introduction:*** *Intravenous regional anesthesia (IVRA) of the upper limb is a simple and effective technique that can be used at all age groups, and its cost is low. One of its limitations is lack of postoperative pain relief after tourniquet deflation. Several pharmacological adjuvants (drugs) have been added to lidocaine to improve postoperative analgesia after IVRA, such as opioids, tramadol, nonsteriodal anti-inflammatory drugs, clonidine, dexmedetomidine and other drugs.*

***Methods:*** *We used nitroglycerine (NTG) as an adjuvant to lidocaine to evaluate its effect on the quality on IVRA and postoperative pain relief. After informed written consent and ethical committee approval, forty patients undergoing short hand and forearm surgery under IVRA were randomly assigned into two equal groups. Group I (control group) received 3 mg/kg of lidocaine 2% diluted with saline to a total volume of 40 ml for IVRA. Group II (NTG group) received the same dose of lidocaine to which we added 100 µg nitroglycerine diluted to the same volume of solution. Tourniquet pain as well as hemodynamic parameters were measured and recorded 2, 5, 10,15,20,30 and 40 minutes after tourniquet inflation. Onset of sensory and motor block was recorded in each patient. At the end of surgery and after tourniquet deflation, sensory and motor recovery times were tested at 5, 10, 15, 20 and 30 minutes then every 1 hour till full recovery. Visual analogue scale (VAS) was used for assessment of postoperative pain at 15; 30 minutes, 1 hour, and then every 2 hour for 24 hours. Time for the first analgesic requirement was observed and recorded. Postoperative analgesia was achieved*

*by using diclofenac 75mg. i.m, oral diclofenac 50mg. was given if needed in the first 24 hours (if VAS>3). Side effects were noted and recorded.*

***Results:*** *There was significant shortening in VAS score of tourniquet pain in NTG group compared to control group (P<0.05). Sensory and motor block onset times were less in NTG in comparison with control group (3.95 ± .82 vs 5.60 ± 1.0 min ) for sensory, (6.35 ± .98 vs 7.65 ± 1.20 min) for motor block. Sensory and motor recovery times were prolonged in NTG group compared to control group (7.75 ± 2.7 vs 3.8 ± 1.2 min) for sensory, (12.60 ± 3.76 vs 5.95 ± 1.7min) for motor recovery. Postoperative analgesia after tourniquet deflation was prolonged with statistically significant difference in VAS scores in all reading of first four hours postoperatively. Time (in minutes) to the first analgesic requirement time was prolonged in NTG group compared to control group (206 ± 33 vs 62 ± 20 min). Diclofenac consumption was much less in NTG group than in control group (127 ± 27 vs 165± 27 mg). No side effects were observed in any patients of either group.*

***Conclusion:*** *addition of nitroglycerine to lidocaine in intravenous regional anesthesia improves sensory and motor block and decreases tourniquet pain and prolongs postoperative analgesia with no side effects.*

## Introduction

Intravenous regional anesthesia (IVRA) is one of the simplest forms of regional anesthesia and has the most frequent success[1]. Lidocaine 0.5%-1% is one of the commonly used local anesthetic for IVRA[2].

Intravenous regional anesthesia has their limitations which are tourniquet pain and its inability to provide postoperative analgesia [3]. To improve block quality, prolong postoperative analgesia, and decrease tourniquet pain, different additives been added to local anesthetics, there are tramadol[4], clonidine[5], dexmedetomidin[6] and dexamethasone[2,4] added to the local anesthetics. Also various nonsteroidal anti-inflammatory drugs have been demonstrated to enhance analgesia such as ketorolac[7] and opioids[8]. Literatures on the effect of adding nitroglycerine as an adjuvant to lidocaine for IVRA are few.

Nitroglycerine is metabolized to

nitric oxide (NO) in the cell. NO causes an increase in the intracellular concentration of cyclic guanosine monophosphate, which produces pain modulation in the central and peripheral nervous system [9].

The aim of this study is to evaluate the effect of adding nitroglycerine to lidocaine for IVRA on tourniquet pain, sensory and motor block onset and recovery times and postoperative pain.

**Methods:**

after obtaining institutional ethical committee approval and written informed consent, 40 patients between 20-50 years; undergoing hand and forearm surgery (carpal tunnel, trigger finger, and tendon release or repair, simple ganglion removal, fracture of ulna or radius fixation) under IVRA; were the subject of the study. Patients having sickle cell anemia, history of drug allergy and Reynaud's or other vascular disease were excluded from the study. Pre-operative assessment (history, examination and investigations) were done and VAS is explained to all patients, routine monitoring intraoperatively in the form of electrocardiography (ECG), automated noninvasive blood pressure measurement and pulse oximetry to detect heart rate (HR), mean arterial blood pressure (MAP) and oxygen saturation ($SpO_2$%) were applied to every patient. IV cannulae were placed; one was in the dorsum of operative hand and the other in the opposite hand for fluid infusion or drugs administration. The operative arm was elevated then squeezed with bandage; a tourniquet was then placed around the upper arm. Absence of radial pulse and loss of pulse oximetry in the index finger of the operative hand were checked before giving IVRA. In control group (group I) received IVRA with 3mg/kg of lidocaine 2% diluted with saline to a total volume of 40 ml, study group (group II) received IVRA with (3mg/kg) of lidocaine 2% with 100 µg nitroglycerine diluted with same volume. Sensory block was assessed by a pinprick with needle. Motor block was assessed by asking the patient to flex and extend wrist and fingers, complete motor block was determined as voluntary movement lost. The op-

erative tourniquet was applied after complete sensory and motor block, and the proximal tourniquet was released and surgery was started. The operative tourniquet was released not before 30 min after its application. Intraoperative Tourniquet pain as well as hemodynamic parameters (MAP, HR, and $SpO_2$%) were measured and recorded 2, 5,10,15,20,30 and 40 minutes after tourniquet inflation. Onset of sensory and motor block was recorded in each patient. At the end of surgery and after tourniquet deflation, sensory and motor recovery times were tested at 5, 10, 15, 20 and 30 minutes then every 1 hour till full recovery, sensory recovery time was evaluated by pinprick and motor block recovery time was evaluated by movement of fingers. VAS was used for assessment of postoperative pain at 15; 30 minutes, 1 hour, and then every 2 hour for 24 hours. Time for the first analgesic requirement was observed and recorded. Postoperative analgesia was achieved by using intramuscular diclofenac 75mg, oral diclofenac 50mg was given if needed in the first 24 hours (if VAS>3). The total diclofenac consumption was recorded in the first 24 hours postoperatively. Side effects of local anesthetic toxicity or nitroglycerine such as tinnitus, tachycardia, hypotension, headache, nausea, or other side effects were noted and recorded.

The data were analyzed to detect the significant statistical difference using SPSS statistical package version[16]. Descriptive statistics, qualitative data were expressed in number and percent while quantitative data were expressed in mean (X), standard deviation (SD). The analytical statistics, following tests were done; student t test and Chi square test ($X^2$)[10].

## Results

There were no significant differences in age, sex, body weight ASA classification and the tourniquet time between the groups as shown in table (1).

There were no significant differences in preoperative, intraoperative, postoperative MAP; HR and $SpO_2$% in NTG group compared to control group (P>0.05); as shown in table (2).

The onset of tourniquet pain was prolonged (35.70± 3.90 in NTG group vs 28.90 ± 5.80 min control group) and there was significant shortening in VAS score of tourniquet pain in NTG group compared to control group (P<0.05). There is no incisional pain, ten patients in control group and five patients in nitroglycerine group received pethedine (50 mg) for tourniquet pain. (Table 3).

Sensory block onset times were shorter (3.95 ± .82 vs 5.60 ± 1.046 min) with statistically significant difference (P<0.001) in NTG group when compared to control group, Motor block onset times were shorter and statistically significant in NTG group (6.35 ± .988 min in NTG group vs 7.65 ± 1.2 min in control group, P<0.05), sensory and motor block recovery times were prolonged and statistically significant in NTG group (7.75 ± 2.712 min in NTG group vs 3.80 ± 1.240 min in control group for sensory recovery times and 12.60 ± 3.761min in NTG group vs 5.95 ± 1.701 min in control group for motor, P<0.001). (Table 3).

Postoperative VAS scores were shorter and statistically significant (P<0.001) in NTG group compared with control group in the first four hour after tourniquet release as VAS scores at 15 min (.75 ± .639 cm in NTG group vs 1.90 ± .641 cm in control group), after thirty minutes (1.45 ± .510 cm in NTG group vs 2.60 ± .940 cm in control group), after two hours (2.30 ± .657 cm in NTG group vs 3.90 ± .940 cm in control group) and after four hours (3.30± .813 cm in NTG group vs 4.35 ± .801 cm in control group). There was no significant difference at 8, 16, 24 hour (P>0.05). (Table 4).

The first postoperative analgesic requirement time was prolonged and statistically significant in NTG group (206.84 ± 33.00 min in NTG group vs 62.50 ± 20.03 min in control group, P<0.001). (Table 5).

Diclofenac consumption was smaller and statistically significant in NTG group in first postoperative twenty-four hours difference between the two groups (127.50 ± 27.980 mg in NTG group vs 165.00 ± 27.386

mg in control group, P<0.001). (Table 5).

There was no significant difference between the two groups in side effects (10% in control group vs 30 % in nitroglycerine group, P>0.05). Two patients in control group developed transient tinnitus while two patients developed transient tachycardia at tourniquet release, one patient developed transient slight headache, and three patients develop venous congestion in operative side in nitroglycerine group.

**Table (1) :** Patient's demographic data, ASA class and tourniquet time given as mean (SD).

| | Control group | Nitroglycerine group | P value |
|---|---|---|---|
| Age (years) | 36.95± 7.756 | 38.20±8.464 | >0.05 |
| Sex (male: female) | 10:10 | 11:9 | >0.05 |
| body weight kg | 74.25±6.544 | 75.20±7.098 | >0.05 |
| ASA I:II | 15:5 | 15:5 | |
| tourniquet time | 47.50±3.441 | 47.75±6.340 | >0.05 |

**Table (2) :** Comparison between Control group & Nitroglycerine group as regards preoperative, intraoperative, postoperative MAP; HR and $SpO_2$ %.

| | | MAP (mmhg) | | HR (beat/min) | | $SpO_2$ % | | *P value* |
|---|---|---|---|---|---|---|---|---|
| | | Control group | NTG group | Control group | NTG group | Control group | NTG group | |
| | | Mean ±SD | Mean ±SD | Mean ±SD | Mean ±SD | Mean ±SD | Mean ±SD | |
| Preoperative | | 94.35±9.778 | 92.05±7.850 | 77.25±6.248 | 76.75±5.486 | 96.45±1.146 | 96.75±.910 | >0.05 |
| Intraoperative | 2 min | 94.60±9.428 | 89.85±10.505 | 78.40±6.082 | 77.35±5.019 | 97.35±.745 | 97.60±.681 | >0.05 |
| | 5 min | 94.80±8.989 | 89.75±6.805 | 78.60±6.269 | 76.50±5.206 | 97.45±.510 | 97.55±.686 | >0.05 |
| | 10 min | 94.75±8.441 | 92.05±8.300 | 78.70±5.921 | 76.85±4.626 | 97.45±.605 | 97.55±.605 | >0.05 |
| | 20 min | 95.45±9.528 | 90.55±7.571 | 78.35±6.418 | 78.85±4.671 | 97.55±.686 | 97.45±.686 | >0.05 |
| | 30 min | 95.65±9.783 | 91.45±7.193 | 79.20±6.363 | 79.50±4.174 | 97.20±.616 | 97.50±.688 | >0.05 |
| | 40 min | 102.25±6.840 | 95.42±7.525 | 82.22±8.333 | 82.82±4.309 | 97.00±.535 | 97.24±.809 | >0.05 |
| Postoperative at 30 min | | 96.00±10.270 | 92.70±7.616 | 80.75±7.203 | 82.35±6.089 | 96.85±.813 | 96.55±.759 | >0.05 |

**Table (3) :** Comparison between control group & nitroglycerine group as regards onset of tourniquet pain sensory and motor block onset and recovery times.

| | Control group | | NTG group | | t | p |
|---|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | | |
| Onset of tourniquet pain | 28.90 | 5.80 | 35.70 | 3.90 | 2.3 | <0.05 |
| Sensory block onset times | 5.60 | 1.046 | 3.95 | .826 | 5.5 | <0.001 |
| Motor block onset times | 7.65 | 1.226 | 6.35 | .988 | 3.7 | <0.05 |
| Motor block recovery times | 5.95 | 1.701 | 12.60 | 3.761 | 7.2 | <0.001 |
| Sensory block recovery times | 3.80 | 1.240 | 7.75 | 2.712 | 5.9 | <0.001 |

**Table (4) :** Comparison between Control group & Nitroglycerine group as regards Postoperative visual analog scale (VAS) scores.

| | | Control group | | NTG group | | t | p |
|---|---|---|---|---|---|---|---|
| | | Mean | ±SD | Mean | ±SD | | |
| visual analog scale (VAS) | 15 min | 1.90 | .641 | .75 | .639 | 5.7 | <0.001 |
| | 30 min | 2.60 | .940 | 1.45 | .510 | 4.8 | <0.001 |
| | 2 hour | 3.90 | .641 | 2.30 | .657 | 7.8 | <0.001 |
| | 4 hour | 4.35 | .801 | 3.30 | .813 | 4.1 | <0.001 |
| | 8 hour | 4.15 | .707 | 2.75 | .967 | 1.7 | >0.05 |
| | 16 hour | 4.30 | .733 | 3.95 | .887 | 1.4 | >0.05 |
| | 24 hour | 4.25 | .550 | 4.05 | .826 | 0.8 | >0.05 |

**Table (5) :** Comparison between Control group & Nitroglycerine group as regards first analgesic requirement time (min) and diclofenac consumption (mg).

| | Control group | | NTG group | | t | p |
|---|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | | |
| First analgesic requirement time (min.) | 62.50 | 20.033 | 206.84 | 33.00 | 16.6 | <0.001 |
| Diclofenac consumption (mg) | 165.00 | 27.38 | 127.50 | 27.98 | 4.3 | <0.001 |

## Discussion

The results of this study revealed that addition of nitroglycerine to lidocaine in intravenous regional anesthesia improves sensory and motor block and decreases tourniquet pain and prolongs postoperative analgesia with no significant side effects.

Hemodynamic parameters in this study show no significant difference, this is in agreement with Selda Sen et al[11] and Abbasivash et al.[12] who studied the effect of adding NTG (200 µg) to lidocaine (3mg/kg) for IVRA and they found that MAP, HR, and SpO2% were show no significant difference between the groups at intraoperative or postoperative period (P > 0.05).

There was significant shortening in VAS score and prolonged onset of tourniquet pain in NTG group compared to control group (P<0.05), which agree with Selda Sen et al who reported that NTG shortened VAS scores of tourniquet pain (P = 0.023), Abbasivash et al found that onset of tourniquet pain was prolonged in NTG group compared with control group (25 vs. 16.65min., respectively, P <0.05). This effect may be due to anti-inflammatory effects and analgesia induced by nitric oxide generators which block hyperalgesia and the neurogenic component of inflammatory edema [13] or through direct stimulation of peripheral fibers similar the actions of locally applied acetylcholine [14].

Sensory and motor block onset times were less and recovery times were prolonged in NTG group compared to control group in this study, Selda Sen et al reported that NTG shortened sensory and motor block onset time (3.2 ± 1.1 versus 4.5 ± 1.2 min; and 3.3 ± 1.6 versus 5.2 ± 1.8; P = 0.009 in group NTG and control group, respectively), prolonged sensory and motor block recovery times (6.8 ± 1.6 versus 3.1 ± 1.2 min and 7.3 ± 1.3 versus 3.6 ± 0.8 P < 0.0001 in group NTG and control group, respectively) and Abbasivash et al. found that sensory and motor block onset time were shortened in NTG group (2.61 vs.5.09 and 4.22 vs. 7.04 min, respectively; P <0.05), prolonged sensory and motor block recovery times (7.26 vs. 3.43, 9.70 vs. 3.74 min., respec-

tively; P <0.05). The rapid onset of sensory and motor block explained by the strong vasodilator effect of NTG that causes rapid distribution of lidocaine to nerves [11].

Elmetwaly et al.[15] compared the effect of adding ketamine or nitroglycerine (NTG) as adjuvants to lidocaine for IVRA; they reported that both ketamine and nitroglycerine shorten sensory and motor blocks. NTG has a rapid onset of sensory and motor blocks than ketamine which could be also explained by the its strong vasodilator effect while ketamine produced better tolerance to tourniquet than NTG due to antagonism of NMDA receptors in peripheral nerves[16].

Turan A et al.[17] investigated the effects of transdermal NTG 10 mg which applied 2 hours before giving IVRA using 2% prilocaine (3 mg/kg), they found that transdermal NTG shorten onset of sensory block and motor block recovery time was significantly longer (p<0.05), and this agrees with the result of this study but they differ in using prilocaine and transdermal patch instead of lidocaine and intravenous NTG, also they did not evaluate postoperative pain.

Marashi et al.[18] disagree with the results of previous investigators or this study as they studied the analgesic effect of intravenous neostigmine (0.5 mg) added to lidocaine and transdermal NTG (5mg) as adjuvants to IVRA, transdermal NTG was applied at the proximal forearm above surgical site. Ten minutes was allowed after injection of local anesthetic for block onset. After 15 min of injection in all patients, distal tourniquet was inflated. They reported that there were no significant differences in severity of tourniquet pain, tourniquet pain onset when transdermal NTG used as an adjuvant to lidocaine with or without neostigmine. This may be due to operation started after 15 min of injection while transdermal NTG patch vasodilator effect takes 20-40 minutes to start which is not strong or rapid as intravenous NTG[19].

Postoperative analgesia after tourniquet deflation was prolonged with statistically significant

difference in VAS scores in all reading of first four hours postoperatively in NTG group (P<0.001), this is in agreement with Selda Sen et al. who reported that VAS scores were lower in group NTG after tourniquet release and in the postoperative period (P = 0.001), also Abbasivash et al. reported that pain intensity at 4, 6, 12 and 24 hr postoperatively were lower in the study group (p <0.05).

The first analgesic requirement time was prolonged and diclofenac consumption was much less in NTG group than in control group in our study, this is in agreement with Selda Sen et al. who found first analgesic requirement time was longer and postoperative analgesic requirements were significantly smaller in group NTG than in control group (P < 0.0001). Also Abbasivash et al, reported that analgesia time after tourniquet deflation was prolonged NTG group (p <0.05). This may be due to pain modulation in the central or peripheral nervous system [9].

Nitroglycerine may cause dose-dependent side effects such as hypotension, tachycardia, or headache[20] and has a very short half-life[9]. In this study, there was statistically no significant difference between the two groups in side effects due to the use of small dose of NTG and tourniquet release not done before 30 min.

## Conclusion

The addition of nitroglycerine to lidocaine in intravenous regional anesthesia improves sensory and motor block, decreases tourniquet pain, prolongs sensory and motor block recovery times and postoperative analgesia and decreases analgesic consumption with no significant side effect.

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# BENHA MEDICAL JOURNAL


## THE EFFECT OF ADDING NITROGLYCERINE AS AN ADJUVANT TO LIDOCAINE ON THE QUALITY OF INTRAVENOUS REGIONAL ANESTHESIA


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# TERATOGENIC EFFECTS AND HISTOPATHOLOGICAL CHANGES OF DOXORUBICIN (ADRIAMYCIN) ON THE ABDOMINOPELVIC REGION OF THE FETAL RATS

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## Abstract

***Background:*** *The adriamycin is an antineoplastic drug used for treatment of various types of cancer. The drug is embryotoxic and teratogenic in rats.*

***The aim of this work :*** *was to confirm the teratogenic effects and the histopathological changes in the abdominopelvic region of the fetal rats following the administration of adriamycin in the pregnant rats.*

***Material and Methods:*** *Eight pregnant rats were divided into two equal groups :*

*The control group and the adriamycin group. In the control group, the pregnant rats were injected intraperitoneally with a saline at a dose of 2ml /kg B.W. once daily from the $6^{th}$ to the $9^{th}$ day of gestation. In the adriamycin group, the pregnant rats were injected intraperitoneally with adriamycin at a dose of 4mg /kg B.W. once daily from the $6^{th}$ to the $9^{th}$ day of gestation. On the $20^{th}$ day of gestation, the pregnant rats underwent cesarean sections. The number of fetuses and their weights were recorded. The abdominopelvic region of the fetuses were examined by the dissecting microscope to discover any congenital anomalies in the gastrointestinal and the genitourinary systems. Two fetuses from each pregnant rats were selected randomly for serial sections and histo-pathological examination.*

***Results:*** *There was a significant reduction of the fetal weight ($p<0.002$) in the adriamycin group when compared with the control*

*group. The recorded congenital malformation in the adriamycin group were in the form of: fibrous cord which extended between the umbilicus and the ileal loop in 16.7%,umbilical hernias in 12.5%, rectal atersia in 8.3% and abnormal shape of umbilical cord in 4.2% of cases. In the adriamycin group, there was severe degeneration of the fetal hepatocytes which were replaced by many vacuoles. In the fetal kidney, most of the glomeruli and the tubules were degenerated. The lumens of most the tubules contained esinophilic materials. In the fetal testis, there was a partial degeneration of the seminiferous tubules.*

***Conclusion:*** *Adriamycin has a strong teratogenic activity producing numerous congenital defects during pregnancy. It has a toxic effects on the fetal rat liver ,kidney and testis.*

***Keywords:*** *Adriamycin, teratogenic effects, histopathological changes, fetal rat, liver, kidney, testis.*

## Introduction

Doxorubicin (adriamycin) is an anticancer drug widely used against leukemia and solid tumors such as cancer of the breast, bladder, lung, bronchus, thyroid gland and ovaries[1, 2] .

The mechanism of adriamycin (ADR) activity is explained by DNA binding and inhibition of nucleic acid synthesis. The metabolic conversion of ADR generates free radicals, which are responsible for the cytotoxic activity of the drug [3].

The previous studies on experimental animals showed that the ADR has a teratogenic activity and animals develop numerous congenital defects during pregnancy[4,5,6,7,8,9,10]. When ADR is given to rats on day 6-9 of pregnancy, congenital anomalies are similar to the human VATER syndrome (vertebral defects ,anal atersia, tracheo-esophageal fistula, esophageal atersia and radial dysplasia)[10].

The side effects of ADR include cardiomyopathy,nephropathy, and gastrointestinal disturbances. It is embryolethal in rabbits and teratogenic in rats. The observed abnormalities are dose related which ranges from 2mg/kg/day up to 8mg/kg /day. The most susceptible period has been identified on day 6-9 of gestation[11].

So, the aim of this work was to confirm the teratogenic effects and the histopathological changes in the abdominopelvic region of the fetal rats following the administration of adriamycin in pregnant rats.

## Material and Methods

**Materials:**

**- Animals:** Eight female white rats were used in this study. Their ages ranged from 2.5 to 3 months old, with an initial body weight of 250-300 gms.The rats received standard food and water during the experiment .

**B- Drug :** Doxorubicin (adriamycin) is one of the most effective chemotherapeutic drugs. It is used to treat a wide range of cancers. It is produced by EBEWE pharmacy Ges. m. b. H. Nfg. KG, A-48866 Unterach, Austria. Each vial contains 10 mg which was diluted in 5 ml saline. Each 1 ml =2 mg of adriamycin. The dose of adriamycin used in this study was 4 mg /kg body weight.

**Methods:**

**A-Experimental design :** The female rats were placed overnight with males. If vaginal smear in the following morning contained sperms, that day was considered as day zero of gestation. The eight pregnant rats were divided into two equal groups :

**1- Control group:** Four pregnant rats were used as a control group. They were injected intraperitoneally with saline at a dose of 2ml/ kg body weight once daily from the $6^{th}$ to the $9^{th}$ day of gestation.

**2- Adriamycin (ADR) group:** Four pregnant rats were injected intraperitoneally with ADR at dose of 4mg/kg body weight once daily from the $6^{th}$ to the $9^{th}$ day of gestation. On the $20^{th}$ day of gestation (24 hours before the expected day of delivery), the pregnant rat underwent cesarean sections to prevent the mothers from devouring any damage to the offspring. The pregnant rats were anaesthetized by inhalation of ether, then the anterior abdominal wall was incised. The uterine horns were carefully inspected for the number of fetuses and post implantation death. It is appeared as a dark brown blood clot attached to the uterine wall at the site of implan-

tation. the uterine horns were cut longitudinally to deliver each fetus inside its amniotic sac. Each sac was incised at the dorsal aspect of the fetus and cautiously pealed from it. The umbilical cord was cut at its placental attachment. The number of fetuses (whether alive or dead )and their weights were recorded .The fetuses were collected and fixed in Bouin's solution for 3 days .

**B- Inspection for any abnormalities:** The fetuses were cut transversely at the level of diaphragm. The abdominopelvic regions of the fetuses of the two groups were collected and examined for any external malformations by using a magnifying lens and a dissecting microscope. A dissecting microscope was used to examine any abnormalities in the gastrointestinal tract and the genitourinary system. These abnormalities were photographed by a camera which was connected to the dissecting microscope. Statistical analysis of the quantitative data was reported .

**C- Histopathological examination :** Two fetuses from each mother were selected randomly for microscopic examination. The abdominopelvic specimens were fixed in Bouin's solution for 48 hours and embedded in paraffin wax. The sections were cut serially at 5µm.Some specimens were cut transversely. Other specimens were cut sagittally or obliquely. The sections were stained with Hematoxylin and Eosin. The sections of ADR group were compared with the sections of control group by light microscope and then photographed.

## Results

In the control group, the four pregnant rats gave 32 fetuses which were alive. The dead fetuses were not found. In adriamycin (ADR) group, the four pregnant rats gave 31 fetuses of which 24 (77.4%) were alive and 7 (22.6%) were dead (3 were reabsorbed and 4 were hydropic).

In control group, the mean fetal body weight was 4.1±0.4gm (3.7-4.5gm). In ADR group, the mean fetal body weight was 2.7±0.3gm (2.4-3.0gm). Statistically, the difference between these two groups was significant ($p<0.002$).

The external malformations in the abdominopelvic region were observed in relation to the umbilicus. In one case (4.2%) of ADR group, the umbilical cord had abnormal shape with a stem which was divided into two trunks. Each trunk subdivided into two branches. The histological examination of one branch of umbilical cord showed that it contained one umbilical artery (not two), one umbilical vein, remnant of vitellointestinal duct and three very thin walled blood vessel. Mostly, these are tributaries of umbilical vein (Figs. 1a, 1b). The umbilical hernia was observed in 3/24 (12.5%) of alive fetuses of ADR group. The cross section of this hernia contained the cut sections of jejunum which had thick wall and highly folded mucosa (Figs. 2a, 2b).

The visceral malformations in the abdominopelvic region were inspected and examined by histological sections. In ADR group, there were four cases of fibrous cords (4/24-16.7%). This fibrous cord extended from the inner aspect of umbilicus to be attached to the loop of ileum. This cord may be a remnant of vitellointestinal duct. In control group, this cord was absent (Figs. 3a, 3b). The sagittal sections of anorectal region of control group showed that the lumen of the rectum was patent and its mucosa was folded (Fig. 4a). In ADR group, two cases of rectal atersia were recorded (2/24-8.3%). In these cases, the lower part of the rectum was obliterated with absence of the mucosal folds (Fig.4b).

The histological examination of the fetal liver in control group showed that the hepatocytes were arranged in cords toward the central vein. These cords were separated from each other by liver sinusoids. The hepatocytes had deeply stained nuclei with visible nucleoli (Fig. 5a). In ADR group, the fetal liver showed severe degeneration of the hepatocytes with wide spread of the vacuoles. The hepatic cords lost its architecture. The liver sinusoids and the central veins contained dark stained blood cells (Fig. 5b).

The histology of the fetal kidney in the control group showed different levels of glomeruli in the cortex. The outer glomeruli were

immature with absence of the urinary space. The inner glomeruli were mature. Each mature glomerulus had a tuft of capillaries which were covered by visceral layer of the Bowman's capsule. This visceral layer was separated from the parietal layer by urinary space. Most of the tubules were properly formed with a regular lumen, while some tubules were still obliterated (Fig. 6a, 6b). In ADR group, the fetal kidney showed severe degeneration of the glomeruli and tubules. The vacuolations were observed in the glomerular tufts and in the tubules. The lumens of the degenerated tubules contained esinophilic materials. Few tubules were still intact (7a, 7b).

The fetal testis in the control group contained intact seminiferous tubules which were arranged close to each other. The lumen of the seminiferous tubules were still obliterated (Fig. 8a). In ADR group, the fetal testis contained some degenerated seminiferous tubules. These degenerated tubules were located in the central area of the testis in some cases .In other cases, the degenerated tubules were located in the upper and lower poles of the testis (Figs.8b,8c).

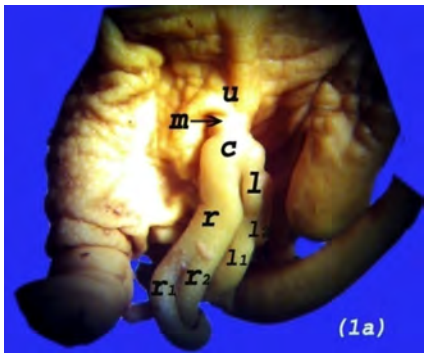


**Fig. 1a :** A photograph of rat fetus of adriamycin group showing: Abnormal shape of the umbilical cord (c) having one stem (m) attached to the umbilicus (u). This stem divides into right (r) and left(l) trunks. Each trunk subdivides into two branches (r1,r2) (l1,l2). (Magnification x5)

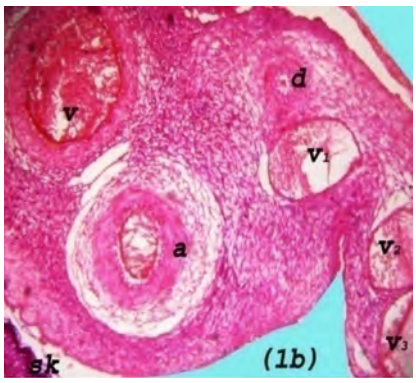


**Fig.1b :** A photomicrograph of the cross section of one branch of the umbilical cord of the previous fetus showing: The contents of this branch which are one umbilical artery(a), one umbilical vein(v), remnant of vitellointestinal duct (d) and abnormal thin wall of small umbilical veins (v1, v2, v3). Notice the skin (sk) of anterior abdominal wall. (HX&E.X100)

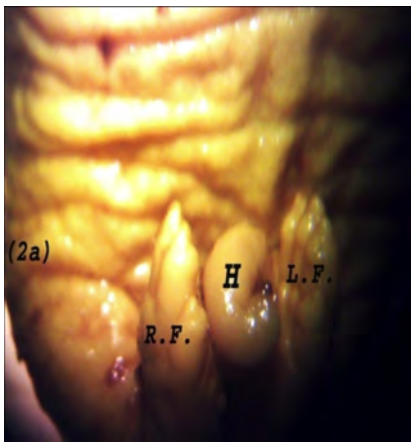


**Fig. 2a :** Aphotograph of rat fetus of adriamycin group showing :congenital umbilical hernia (H) which is surrounded by two feet. (Magnification x5)

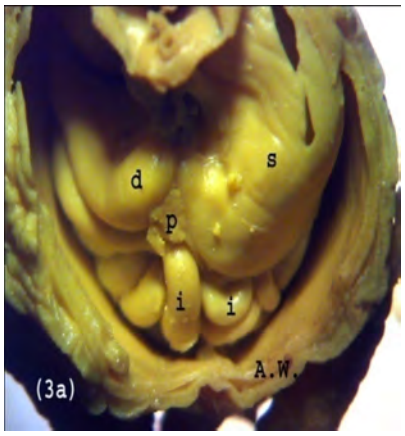


**Fig.3a :** A photograph of a control rat fetus showing: Normal abdominal organs ;stomach(s), duodenum (d), pancreas (p),loops of small intestine(i).Notice the anterior abdominal wall(AW). (Magnification x5)

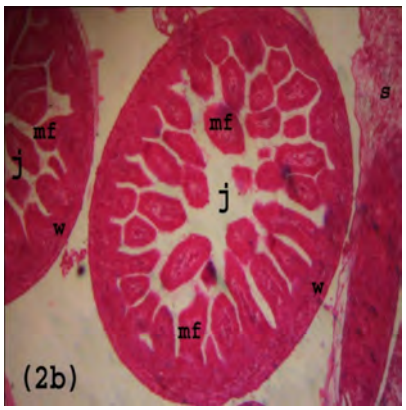


**Fig. 2b :** A photomicrograph the cross section of the previous fetus showing: The cut sections of the jejunum(j) inside the hernial sac (s).The jejunum has many mucosal folds (mf) and thick wall(w). (HX &E. x100).

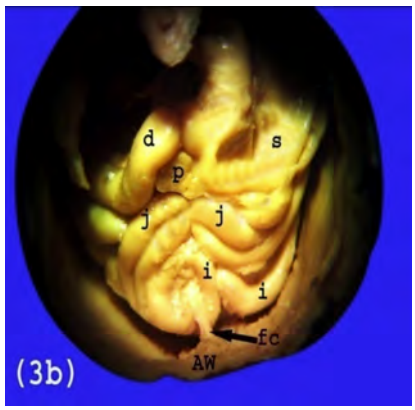


**Fig. 3b :** A photograph of rat fetus of adriamycin group showing : A fibrous cord (fc) extending from the anterior abdominal wall (AW) at the level of umbilicus to the loop of ileum (i) . Notice the collapsed stomach (s) , duodenum(d), loops of jejunum(j) and pancreas(p). (Magnification x3)

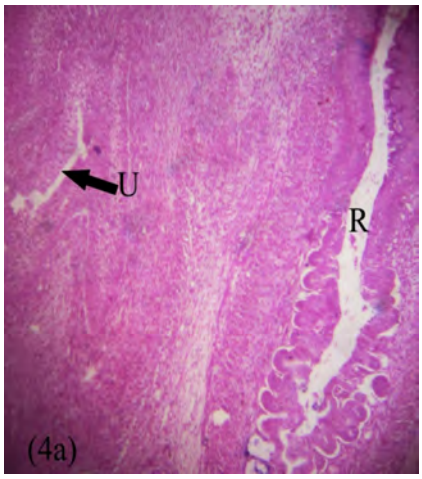


**Fig. 4a :** A photomicrograph of sagittal section of control rat fetus showing : The rectum (R) with patent lumen and folding of its mucosa. Notice the part of the urethra (u)is seen anteriorly. (HX&E.X100)

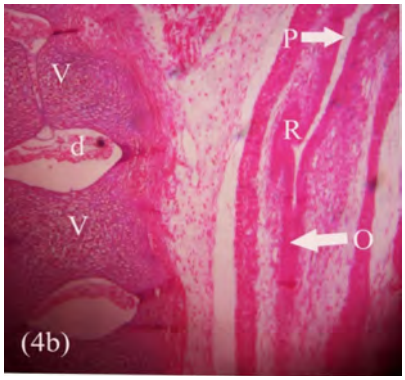


**Fig. 4b :** A photomicrograph of sagittal section of the rat fetus of adriamycin group showing :Obliteration(o) of The lower part of the rectum (R),while its upper part is patent (p).Notice the absence of the rectal mucosal folds .Notice also the sacral vertebrae (v) with intervertebral discs (d) in between. (HX &E. X100)

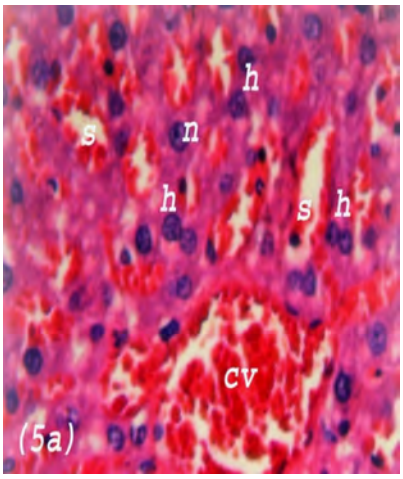


**Fig. 5a :** A photomicrograph of control fetal liver showing: The hepatocytes (h) arranging into cords toward the central vein(cv)which contains blood cells . the hepatic cords are separated from each other by liver sinusoids (s). Notice the hepatocytes contain large deeply stained nuclei(n) with prominent nucleoli. (HX &E. X400)

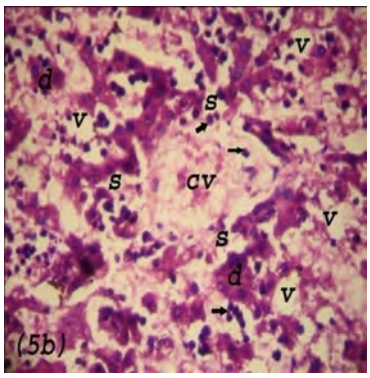


**Fig. 5b :** A photomicrograph of the fetal liver of adriamycin group showing : Severe degeneration of the hepatocytes (d) with loss of its architecture. Many vacuoles(v) are seen in the whole section .Notice the liver sinusoids(s) and the central veins (cv) contain dark stained blood cells(arrow). (HX &E. X400)

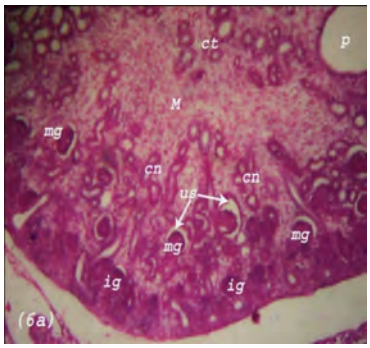


**Fig. 6a :** A photomicrograph of transverse section of control fetal kidney showing: Different level of the glomeruli in the cortex . The outer glomeruli are immature (ig) with absence of urinary space. The inner glomeruli are mature(mg)with urinary space (us).The cortex contains the cut sections of the convoluted tubules(cn).The medulla (M)contains the cut sections of the collecting tubules(ct).Notice a part of the renal pelvis (p) is seen in the section. (HX E. X100)

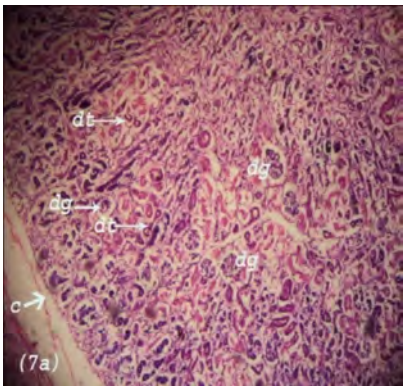


**Fig. 7a :** A photomicrograph of the fetal kidney of adriamycin group showing : Severe degeneration of the glomeruli(dg)and the tubules (dt). Notice the renal capsule(c).
(HX &E. X100)

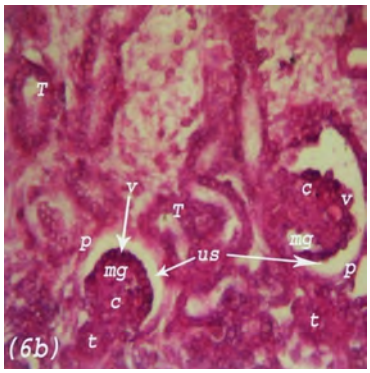


**Fig. 6b :** A higher magnification of the previous section showing: The mature glomeruli(mg) has tufts of capillaries (c) which is covered by visceral layer of Bowman's capsule(v).This visceral layer is separated from the parietal layer (p) by urinary space (us). Most of the tubules (T)are properly formed with a regular lumen .Some tubules(t) are still obliterated. (HX&E. X400)

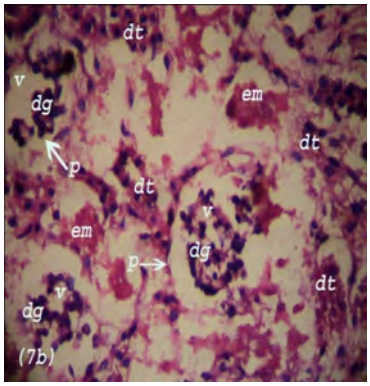


**Fig. 7b :** A higher magnification of the previous section showing : Degeneration of the glomeruli(dg). The vacuolations(v) in the capillary tufts are seen. The parietal layer (p) of the Bowman´s capsule is disrupted. Most of the convoluted tubules (dt) are degenerated with accumulation of esinophilic materials (em)in their lumens. Notice, few tubules (T) are still intact.
(HX &E. X400)

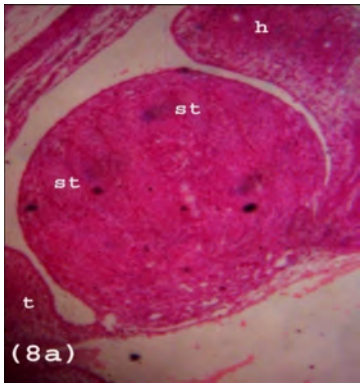


**Fig. 8a :** A photomicrograph of the control fetal testis showing: The seminiferous tubules (st) are intact with obliteration of their lumens. They are arranged close to each other. Notice the head (h) and the tail(t) of epididymis.
(HX &E. X100)


**Fig. 8b :** A photomicrograph of the fetal testis of adriamycin group showing : Degeneration of the central seminiferous tubules (ds). The peripheral seminiferous tubules (s) are intact. Notice the head of the epididymis (h). (HX &E. X100).

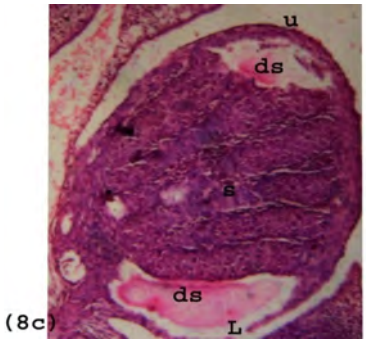


**Fig. 8c :** A photomicrograph of the fetal testis of adriamycin group showing: Degeneration of the seminiferous tubules(ds)in the upper(u) and lower(l) poles of the testis, while the central seminiferous tubules (s) are intact.
(HX & E. X100)

## Discussion

The embryotoxic effects of doxorubicin were investigated by the present study to confirm the results of the previous studies. In the present study, there was a significant reduction of the fetal body weight ($p<0.002$) in adriamycin group in relation to the control group. Also, the percentage of the fetal death in adriamycin group was 22.6%. These results are in agreement with the results of[2,12]. Menegola et al. explained the reduction of the fetal body weight in adriamycin group that was due to the reduction of water and food consumption during and after the treatment period. They also reported that the administration of adriamycin in a dose of 8mg /kg/day to the pregnant rats at the 6th to the 9th day of gestation produced one hundred percent of the fetal death at term[2].

In the present study, the umbilicus of the fetuses in the adriamycin group showed congenital malformations in the form of fibrous cord which extended between the umbilicus and the loops of ileum in 4/24 (16.7%), umbilical hernias in 3/24 (12.5%) and abnormal shape of the umbilical cord in 1/24 (4.2%). These results are nearly similar to the results of [2] who reported that the umbilical hernia was observed in 13% of adriamycin group at dose level of 4mg /kg/day.

In the present study ,two cases of rectal atersia (8.3%) were recorded in the adriamycin group. Dawrant et al. reported that the anorectal malformation occurred in all of the fetuses of the pregnant mices injected with 6mg/kg of adriamycin on day 7 and 8 of gestation[13].

In the present study, the fetal liver in the adriamycin group showed severe degeneration of the hepatocytes with loss of their arrangements. The degenerated hepatocytes were replaced by wide spread of vacuoles. Barranco et al. reported that the adriamycin interferes with DNA and RNA synthesis and kills the embryonic cells in S-phase[14]. Haerr and Pratt reported that the adriamycin is embryotoxic and teratogenic in rats and embryotoxic and abortifacient in rabbits[15]. Gillick et al.

reported that the adriamycin produces increasing in the cell death of the embryo [16].

In the present study , the fetal kidney in the adriamycin group showed severe degeneration of the glomeruli and the tubules. The lumen of the degenerated tubules contained esinophilic materials. Few tubules were still intact. These results are similar to the results of[12,13,17,18]. Goncalves et al. reported atrophy of the cortex and the medulla of the fetal kidney after adriamycin injection in pregnant rats. This atrophy was not accompanied by any inflammatory or developmental changes [12]. Pedrycz et al. reported focal damage of the epithelial cells of the tubules with vacuoles in the cytoplasm. The lumens of some tubules contained esinophilic materials [17].

In the present study, the fetal testis of adriamycin group showed partial degeneration of the seminiferous tubules which affect the central area, upper or lower poles of the testis. This result is similar to the result of[19,20] who reported atrophy of the genital organs after adriamycin treatment.Also, the number of the spermatogonia. were decreased. Speth et al. reported that the adriamycin is metabolized in the microsomes by cytochrome p-450. As a result of conversion by cytochrom p-450, semichinon is generated which is the free radical responsible for the cytotoxic activity of adriamycin. Additionally, adriamycin inhibits the enzymatic complex that liberates the free radicals that intensify the cell damage[21]. Therefore, it is likely that the free radicals are responsible for the changes in the fetal liver, kidney and testis as observed in the present study.

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# BENHA MEDICAL JOURNAL


## TERATOGENIC EFFECTS AND HISTOPATHOLOGICAL CHANGES OF DOXORUBICIN (ADRIAMYCIN) ON THE ABDOMINOPELVIC REGION OF THE FETAL RATS


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# FREE VASCULARISED ILIAC CREST FLAP FOR RECONSTRUCTION AFTER SEGMENTAL MANDIBULECTOMY, A CASE REPORT

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## Abstract

***Introduction:*** *Today, free tissue transfers have become an accepted tool in head and neck reconstruction and the standard for reconstruction of many defects within the head and neck.*

***Aim of the work:*** *In this study we aimed to present and show feasibility of free vascularized iliac crest flap for reconstruction in a case of segmental mandibular defect after excision of squamous cell carcinoma.*

***Case Study:*** *A 54 old years male patient with sqamous cell carcinoma of gingiva infiltrating the mandible planned for segmental mandibular resection and reconstruction utilizing microvascular free iliac crest flap. The flap survival was evidenced by Doppler examination on the site of microvascular anastmosis. Success of the flap was ensured with panorama X-Ray postoperative.*

***Conclusion:*** *We'll continue to offer this technique of free iliac crest flap for patients with mandibular defects after ablative cancer surgery. We believe that there is much needs to be done in the future in relation to the correct flap choice and better functional recovery.*

***Key Words:*** *Free Flap; mandible reconstruction; iliac crest.*

## Introduction

Free tissue transfer is frequently incorporated into the reconstructive algorithm for a multitude of defects in the head and neck [1].

Because of the wide variety of options available for transfer, the high flap survival rates, and the improved outcomes associated with free tissue transfer, this technique has modified the classically described "reconstructive ladder" in which free tissue transfer would typically be the flap choice for

complex defects, which are not amenable to other reconstructive options[2].

With the popularization of microvascular reconstruction techniques, osseous free flaps have become methods of great value in reconstruction of segmental mandibular defects following oncological resective surgery[3].

The vascularized iliac crest provides a great amount of corticocancellous bone, good in terms of quantity and quality. Also, the height of the bone, in the iliac crest flap is higher than the fibular flap.

Squamous cell carcinoma is the most common malignant tumor of the oral cavity. It accounts for about 90% of all oral malignancies[4].

10% of all malignant tumors of the oral cavity occur on the gingiva, and that tumors arise more commonly in edentulous areas, although they may develop at sites where teeth are present. It is generally agreed that carcinomas of the mandibular gingiva are more common than those of the maxillary gingiva[5].

**Case Report :**

A 54 old years male patient presented to the surgical oncology department, Mansoura Oncology Centre with ulcerating mass on the right side of mandibular ginigiva without pain or inflammatory signs. Local examination showed ulcerating lesion fixed to the mandible with local bulging, figure (1). Neck examination showed enlargement of cervical lymph nodes. Panoramic X-ray was performed and showed erosion of mandible. figure (2).

Incisional biopsy under general anaethesia was performed and concluded Sqamous Cell Carcinoma infiltrating the mandible.

We planned segmental mandibular resection with excision of the ulcerating gingival lesion en block with one cm safety margin, Fgure (3), and modified radical neck block dissection to be performed on the right side. Preparation of the recipient neck vesseles was then performed.

We planned reconstruction by vascularized iliac crest free flap taken from the ipsilateral hip.

The incision is outlined 2cm superior to the connection of the pubic tubercule and the ASIS, starting just lateral to the pulse of the femoral artery, Figure (4). Skin and subcutaneous fatty tissue are incised between the femoral artery and the ASIS. Keeping dissection unitl identefication of the vascular pedice (Deep Circumflex Iliac Artery and Vena Commitants).

The abdominal muscles are transected and the pulse of the DCIA is palpated at the inner surface of the pelvic curvature 1-3 cm inferior to the inner rim and therefore can easily be preserved during transection of the muscles.

Muscular detachment is continued at the ASIS, where the sartorius muscle is transected directly at its origin from the bone.

After measuring the required length, osteotomy begins distally at the iliac crest after transection of the abdominal muscles covering the bone. The vascular pedicle is transected and ligated at the distal osteotomy.

The osteotomized bone segment is elevated, and residual muscle fibers are transected, Figure (5).

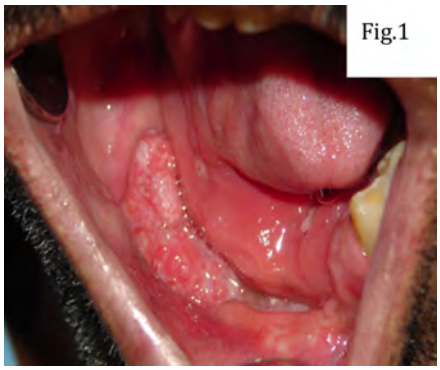
Once the recipient vessels are ready for anastomosis, the pedicle is separated, Figure (6). Then the flap is taken to the recipient site. At that time 5000 unite heparin sulphate is injected IV. Trimming of the adventitia of the vascular pedicle was then performed. We started by fixation the flap to the mandible using 2.4mm mini plates, Figure (7). Microvascular anastmosis is then done using 5.5 X magnification (Keeler surgical loop) and suturing using Prolene 7/0. After completion of vascular anastmosis, repair of the floor of mouth and closure of the neck wound with corrugated suction drain.

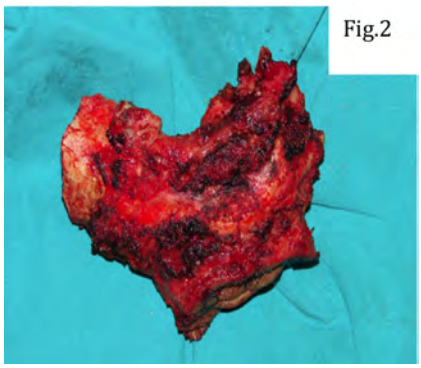
Wound closure of the donor site was performed carefully with the use of mesh reinforcement after insertion of a deep drain and accurate hemostasis. Bone wax was used at the cutting surfaces of the pelvic bone. The patient is

immobilized for 3-4 days, and ambulation is then begun.

The patient was kept in the ICU unit at Oncology Centre-Mansoura University for two days. Monitoring of the flap was performed using Doppler on the marked site of anastmosis. Monitoring was done every 2 hours for the first 2 days then every 4 hours for the next 3 days. Patient is put on heparin sulphate infusion 5000 units/12 hours for five days.

Patient is discharged ten days postoperative with imaging by digital panorama X-Ray before discharge, Figure (8).



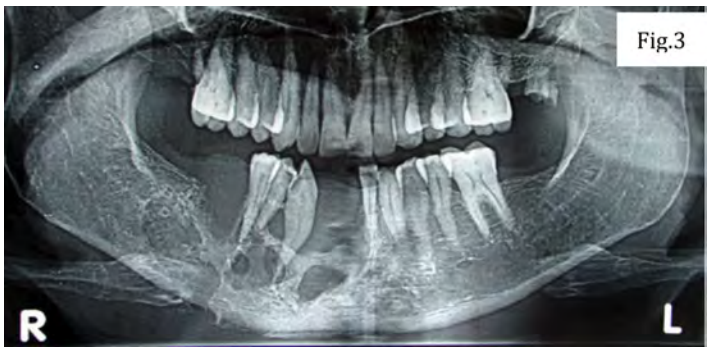
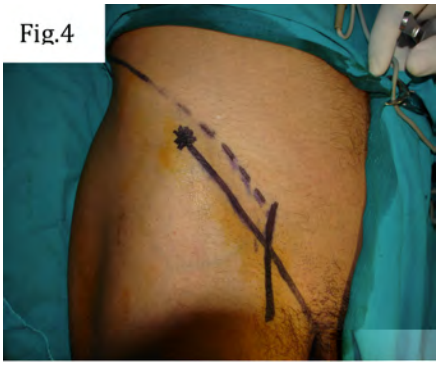
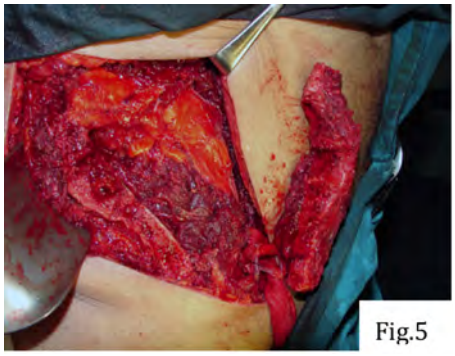
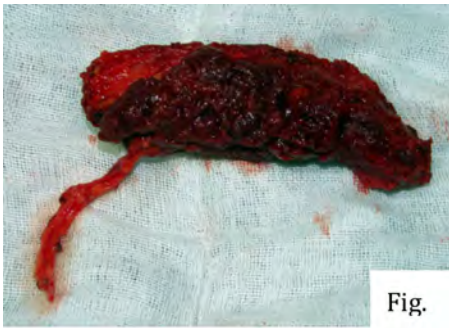




**Figure (1)** Preoperative SCC mandibular gingiva, **Figure (2)** Preoperative Panorama X-Ray showing destruction of the mandible at the site of the lesion, **Figure (3)** After segmental mandibulectomy,





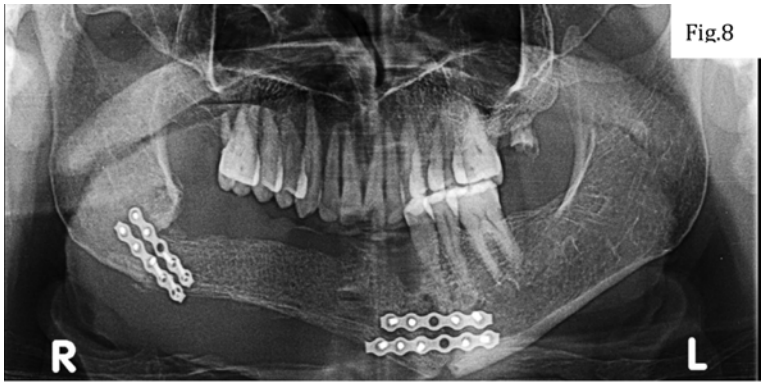



**Figure (4)** Iliac crest flap design, **Figure (5)** Iliac crest flap harvest, **Figure (6)** After separation of the iliac crest flap, **Figure (7)** After fixation of the iliac crest flap and microvascular anastmosis, **Figure (8)** Postoperative panorama X-Ray showing good bone density.

## Discussion

Microsurgical free flaps are today considered state of the art in head and neck reconstruction after composite tumor resections. Free flaps provide superior functional and aesthetic restoration with less donorsite morbidity [6].

We presented this case of free iliac crest flap reconstruction of mandibular defect after oncological resection, to show that it's a feasible and reliable technique in reconstruction with very good outcome.

The vascularized iliac crest provides a great amount of corticocancellous bone, good in terms of quantity and quality. Also, the height of the bone, in the iliac crest flap is higher than the fibular flap [7].

Other types of free osseous flaps for mandibular defects exist, of which fibular flap are very common. A study comparing the two most frequently used osseous donor sites (the fibula and the iliac crest) suggests that there is little difference between the two[8].

One of the most common complications of iliac crest free flap is postoperative hernia. Careful closure of the muscular layers coupled with mesh reinforcement is commonplace, and a variety of techniques have been described to minimize the risk of postoperative hernia[9].

In our presented case we have used mesh reinforcement for donor site closure, which resulted in a good wound healing and without postoperative hernia.

## Conclusion

We conclude that iliac crest free tissue transfer is a feasible technique for head and neck reconstruction in a tertiary referral and teaching center (Oncology Centre - Mansoura University). We'll continue to offer this technique for patients with mandibular defects after ablative cancer surgery. Enhanced attention to patient selection, preparation of patients for surgery, fluid balance, and coordination between experienced members of the team should increase effectiveness and safety even further.

We believe that there is much needs to be done in the future in relation to the correct flap choice and better functional recovery.

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## FREE VASCULARISED ILIAC CREST FLAP FOR RECONSTRUCTION AFTER SEGMENTAL MANDIBULECTOMY, A CASE REPORT


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# SURGERY ALONE VERSUS PERIOPERATIVE CHEMOTHERAPY FOR LOCALLY ADVANCED GASTRIC CANCER

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## Abstract

***Purpose:*** *to determine whether chemotherapy before and after radical surgery improves the outcomes of locally advanced gastric cancer compared with surgery alone.*

***Patients and Methods:*** *seventy two patients with locally advanced gastric adenocarcinoma were randomly assigned to either perioperative chemotherapy and gastrectomy (Group I) or to upfront gastrectomy (Group II). Group I included 36 patients received 3 cycles of preoperative chemotherapy protocol. Each 4-week cycle consisted of cisplatin (100 mg/m$^2$) was given intravenously on day 1 over a period of 2 hours with adequate hydration, and fluorouracil (800 mg/m$^2$) daily for 5days by continuous intravenous infusion.*

***Results:*** *Curative resection (R0) was documented in (87.5%) of patients in the group I, while in the group II curative resection was done in (58.3%), the difference was statistically significant (p=0.013). With a median follow up of 20 months progression free survival was significantly higher in the group I than in the group II. Two- year progression free survival rates were 44% and 23% in group I and in group II, respectively. The overall survival in the group I was significantly higher than in the group II. Two - year survival rates were 48% and 27% in the group I and in the group II, respectively. The chemotherapy was generally well tolerated and all observed toxicities were manageable.*

***Conclusion:*** *Chemotherapy before and after radical surgery is preferred compared with surgery alone in treatment of locally advanced gastric cancer as regard response, survival and tolerance.*

***Key Words:*** *gastric cancer, preoperative chemotherapy, gastrectomy.*

## Introduction

Gastric cancer remains a major health issue and a leading cause of death worldwide. More new cases of gastric cancer are diagnosed in China each year than in any other country, an estimated 21,130 new cases of gastric cancer and10,620 new deaths occurred in United States in 2009[1].

Surgery is the only curative treatment modality for gastric cancer and the overall survival rate of early-stage gastric cancer patients is up to 90%[2]. Complete surgical resection with radical lymphadenectomy provides information for accurate staging, which is very important in prognosis prediction and further therapy decisions[3]. However, as the majority of gastric cancer patients are at the advanced stage at the time of diagnosis, the prognosis of locally advanced tumor is poorer, due to a high unresectability rate at presentation, and a much higher relapse rate after radical surgery, thus demanding further studies regarding adjuvant and neoadjuvant treatment[4].

Chemotherapy delivery may be more efficient if given prior to surgical disruption of vasculature, tumor down-staging may substantially facilitate surgical resection, and preoperative chemotherapy can be used to evaluate tumor chemosensitivity to cytotoxic medications[5]. Furthermore, gastric cancer patients may tolerate preoperative cytotoxic treatment better than postoperative treatment, as performance status is usually negatively impacted by surgery[6]. However, lack of response to neoadjuvant chemotherapy may delay curative surgery and chemotherapy-induced toxicity may increase surgical complications[7].

Cisplatin-5-FU (CF) based regimen have been proposed as reference regimen in advanced gastric cancer for clinical use because they have been widely investigated in clinical studies and have demonstrated favorable survival outcomes[8].

The present study was designed to determine whether chemotherapy before and after radical surgery improves the outcomes of locally advanced gastric cancer compared with surgery alone.

## Patients and Methods

From January 2006 to December 2009, 72 patients with locally advanced gastric or gastroesophageal junction adenocarcinoma presented to Clinical Oncology and Nuclear Medicine Department, Surgical Gastroenterology Center and Oncology Center, Mansoura University Hospital.

**Inclusion criteria:**

Patients were eligible for the study if they had pathologically confirmed locally advanced gastric or gastroesophageal junction adenocarcinoma with no evidence of distant metastases, and with an Eastern Co-operative Oncology Group (ECOG) performance status of 0 to 1. Adequate hematologic, renal and hepatic functions were required. All patients were fit for surgery and gave informed consent.

**Pretreatment assessment:**

History taking and physical examination were performed at baseline. Echocardiography, complete blood count, liver and kidney function tests and tumor markers (CEA and CA 19-90) were done. The lesions were evaluated as regard its site and extensions by upper esophago-gastric endoscopy, endoscopic ultrasound and multiple biopsies were taken for histopathological examination. Staging procedures included computed tomography of the chest, abdomen and pelvis. Bone survey or scan (if indicated) were performed to exclude bone metastases.

Patients were randomly assigned to either preoperative chemotherapy followed by surgical resection and postoperative chemotherapy (Group I) or to upfront surgery only (Group II). Group I included 36 patients received 3 cycles of pre-operative chemotherapy protocol. Each 4-week cycle consisted of cisplatin (100 mg/$m^2$) was given intravenously on day 1 over a period of 2 hours with adequate hydration, mannitol diuresis and anti-emetic coverage, and fluorouracil (800 mg/$m^2$) daily for 5 days by continuous intravenous infusion. Randomization was done using the block randomization method. Severity of adverse effects, defined according to the National Cancer Institute Common Toxicity Criteria

(version 3), were assessed after each cycle.

Three weeks after the third cycle, response to treatment was assessed using CT scan for abdomen and chest (if gastroesophageal junction involved). Criteria from the Response Evaluation Criteria in Solid Tumor (RECIST) were used, depending on one-dimensional measurements[9].

Surgery was scheduled to take place after randomization for group II (36 patients) and to 32 patients out of 36 patients in group I (2 patients had progressive disease and 2 patients refused surgery in spite of no progression, they continued on chemotherapy). The surgery was done within three to six weeks after completion of the 3 cycles of preoperative chemotherapy.

The extent of resection was determined by the surgeon according to extent of the disease in each patient. In radical total gastrectomy, the whole stomach was removed, along with the greater and lesser omenta and any other organs involved by extension of the primary growth. It was done in 28 patients, while subtotal gastrectomy was done in 30 patients. The procedure for a radical subtotal distal gastrectomy was the same, but a small, variable gastric remnant was left intact. In both procedures, the resection lines had to be at least 3 cm from the edge of the macroscopic tumor. For proximal tumors and tumors of the gastroesophageal junction (GEJ), proximal gastrectomy was done in 10 patients with frozen-section-guided resection of the distal esophagus.

The surgeon decided the extent of the lymph node dissection. In D1 lymphadenectomy (lymph nodes along the lesser and greater curvatures were included). While in D2 type the omental bursa along the frontal leaf of the transverse mesocolon is removed and corresponding arteries are cleared completely (dissection extended to celiac group). Surgeons stated whether the procedure was likely to be curative or not. All resected specimens were pathologically examined. The R classification takes into account the pathological findings. A reliable classification

requires the pathological examination of resection margins. Reports on gastric resection specimens should include, as a minimum, type of tumor, depth of invasion, involvement of the resection margins, nodal disease (including number of involved lymph nodes), and metastatic spread.

The R classification denotes absence or presence of residual tumor after treatment. Residual tumor may be localized in the area of the primary tumor and/or as distant metastases. R0 corresponds to resection for cure, there is no macroscopic and microscopic residual tumor cells detected at safety margin or tumor bed. R1 corresponds to microscopic residual tumor, while R2 indicates macroscopic residual tumor[10].

The patients were staged according to a standard protocol that used the tumor- node- metastasis (TNM) classification system for staging of gastric carcinoma. If the primary tumor invades lamina propria or submucosa, it considers T1. If the tumor invades muscularis propria or subserosa, it considers T2a; while in T2b the tumor invades subserosa. The tumor penetrates serosa without invasion of adjacent structures in T3; if they are infiltrated it denotes T4. As regard N1, the metastses were found in one to six regional lymph nodes. If the number of positive lymph nodes were from 7 to 15, it considered N2. N3 represents more than 15 positive lymph nodes in the examined specimen. M1 denotes distant metastases[11].

Three to six weeks after surgical resection, the responder patients in group I (32 patients out of 36 patients) received the same preoperative chemotherapy protocol to complete a total of six cycles. After the end of therapy, all patients were subjected to follow up schedule including: all investigation done for pretreatment evaluation (monthly for one year and every 3 months thereafter, CT scan and upper esophagogastric endoscopy every six months, and diet regimen for patients suffering from postgastrectomy feeding complications.

The main end points were response rate to preoperative chem-

otherapy and rate of curative resections. The progression free survival, defined as the time from randomization to the date of first events (local recurrence or progression, distant metastases, death or lost follow up) and overall survival, defined as the time from randomization to death (including deaths with or without recurrence) or lost follow up for those who were still alive.

**Statistical analysis**

Data was analyzed using SPSS (statistical package for social science. Version 15). The qualitative data were presented in the form of number and percentage, Chi-square or Fisher's exact test was used for comparison between groups, as appropriate. Quantitative data were presented as mean ± standard deviation (SD). Kaplan-Meier survival analysis with log-rank test was used for comparison of overall survival and progression free survival, in months between treatment groups.

## Results

From January 2006 to December 2009, 72 eligible patients with locally advanced gastric carcinoma were enrolled into this study. Thirty six patients were randomly assigned to receive preoperative chemotherapy, surgery followed by adjuvant chemotherapy (group I) and 36 patients assigned to upfront surgical resection (group II). The baseline characteristics were listed in Table 1, and there was cross matching between the two groups.

All patients received the preoperative chemotherapy as planned. Response to chemotherapy was assessed for all patients in group I after receiving preoperative chemotherapy, 20 patients (55.6%) had partial response, 14 patients (38.9%) had stationary disease and 2 patients had progression of the disease. No patient reached clinical complete response.

As shown in table (2), 32 patients in group I referred to surgery within 3-4 weeks from the third cycle of preoperative chemotherapy. Six patients had proximal gastrectomy, 10 patients had subtotal gastrectomy, and 16 patients had total gastrectomy. Regarding lymph node dissection, 14 patients had D1 and 18 patients had

D2 lymphadenectomy. In group II, four patients underwent proximal esophagogastrectomy; 20 patients underwent subtotal gastrectomy and 12 patients underwent total gastrectomy. Lymph node dissection (D1) was done for 25 patients and 11 patients had D2.

Curative resection (R0) was documented in 28 out of 32 patients in group I (87.5%), while in group II curative resection was done in 21 out of 36 patients who underwent resection (58.3%), the difference was statistically significant (p=0.013). In group I, 3 patients (9.4%) had positive microscopic residual (R1 resection) in spite of negative frozen section of the margins and the reported curative resection by the surgeon and only one patient left with macroscopic disease; while in group II, positive microscopic residual found in 11 patients (30.6%) and 4 patients (11.1%) left with macroscopic disease.

The pathological results reported that the depth of tumor invasion (pT) was in group I less than the depth tumor invasion in group II (p=0.014). In group I, 3 patients (9.4%) had no evidence of tumor invasion (pT0) and 4 patients (12.5%) had pT1 and 20 patients (62.5%) had pT2; while in group II, 2 patients (5.6%) had pT1 and 15 patients (41.7%) had pT2. Also, regarding lymph node involvement, there was a trend to less nodal infiltration in preoperative chemotherapy group than in surgery group (p=0.039). The surgical cut margin was negative in 29 patients (90.6%) in group I and 24 patients (66.7%) in group II.

The mean maximum diameter of the resected tumor was smaller in the preoperative chemotherapy group than in upfront surgery group (6.0 ± 2.69 cm versus 7.8± 2.58 cm; p=0.007); this finding is consistent with tumor shrinkage by preoperative chemotherapy (table 2).

Postoperative hospital stay was equal in both groups (ranged from 6 to 10 days). One patient in group I had postoperative anastomosic leakage. Preoperative chemotherapy effects as wound infection and delayed wound healing occurred in 3 patients while infection occurred in one patient in

group II. One patient in both groups developed dumping syndrome. One patient developed biliary reflux in group I, persistent vomiting was observed in 2 patients in group I. In group I one patient died from electrolyte imbalance and adhesive intestinal obstruction was documented in one patient (table 3).

As regard preoperative chemotherapy toxicities, grade 1 and 2 nausea and vomiting were observed in 72.2% of patients, followed by grade 1 and 2 mucositis (33.3%) and diarrhea (22.2%). As regard hematological toxicity, grade 1 and 2 anemia occurred in 44.4% of treated patients and grade 1 thrombocytopenia occurred in two patients only (5.6%). The treatment was generally well tolerated and all observed toxicities were manageable. Grade 3 or 4 neutropenia occurred in one patient. Regarding impaired serum creatinine, 3 patients had grade 1 & 2 impaired levels (table 4).

Although treatment was well tolerated in preoperative settings, some patients experienced adverse effect of postoperative chemotherapy; details of toxicity of postoperative chemotherapy are shown in table (4). Grade 1 & 2 anemia were reported in 20 patients (66.7%), while thrombocytopenia was reported in 4 patients (grade 1 & 2 in 2 patients and grade 3 & 4 in another 2 patients. Grade 1&2 nausea and vomiting were reported in 20 patients (66.7%); grade 3 vomiting was reported in 2 patients (6.7%). Ten patients (33.3%) had both grade 1 & 2 mucositis and diarrhea. Regarding impaired serum creatinine, 3 patients had grade 1 & 2 impaired levels

During the followed up period with a median of 20 months ranged from 2 to 55 months, disease recurrence was documented in 14 patients in group I versus 22 patients in group II. Progression free survival was significantly higher in group I than in group II (hazard ratio for progression, 0.40; 95% confidence Interval, 0.14-1.11; p=0.004). Two-year progression free survival rates were 44% and 23% in group I and in group II, respectively (figure 1).

The overall survival in group I was significantly higher than in group II (hazard ratio for death, 0.48; 95% confidence Interval, 0.18-1.28; p=0.041). Two - year survival rates were 48% and 27% in group I and in group II, respectively (figure 2).

**Table (1) :** Patients' characteristics .

| | Chemotherapy plus surgery Group I (N=36) No (%) | Surgery alone Group II (N=36) No (%) | P value |
|---|---|---|---|
| **Age** (mean ± SD) | 46.9 ± 13.38 | 50.2 ± 9.29 | 0.231 |
| **Gender** | | | |
| Male | 25(69.4) | 28 (77.8) | 0.422 |
| Female | 11(30.6) | 8(22.2) | |
| **ECOG performance status** | | | |
| 0 | 27 (75.0) | 24 (66.7) | 0.437 |
| 1 | 9 (25.0) | 12 (33.3) | |
| **Presenting symptoms** | | | |
| Pain | 15(41.7) | 11 (30.6) | |
| Vomiting | 9(25.0) | 14 (38.9) | |
| Dyspepsia | 8(22.2) | 6(16.7) | 0.729 |
| Dysphagia | 7 (19.4) | 2 (5.6) | |
| Hematemsis | 2(5.6) | 4(11.1) | |
| Melena | 2(5.6) | 4(11.1) | |
| **Site of tumor** | | | |
| Antrum | 12(33.3) | 14(38.9) | |
| Body | 16 (44.5) | 18 (50.0) | 0.652 |
| Cardia | 4 (11.1) | 2 (5.6) | |
| Diffuse | 4 (11.1) | 2 (5.6) | |
| **Pathology** | | | |
| Adenocarcinoma | 20 (55.6) | 18 (50.0) | |
| Signet ring cell | 8(22.2) | 10 (27.7) | 0.703 |
| Mucinous | 4 (11.1) | 2 (5.6) | |
| Undifferentiated | 4(11.1) | 6(16.7) | |
| **Pretreatment tumor diameter** | | | |
| Mean ± SD (cm) | 9.03±2.26 | 8.06±2.33 | 0.077 |

The patients may have more than one symptom.

**Table (2) :** Surgical and pathological results.

| | Chemotherapy plus surgery Group I (No. =32) | Surgery alone Group II (No. =36) | P value |
|---|---|---|---|
| **Type of surgery** | | | |
| Proximal resection | 6(18.8) | 4(11.1) | 0.126 |
| Subtotal resection | 10(31.3) | 20(55.6) | |
| Total resection | 16(50.0) | 12(33.3) | |
| **Extent of nodal dissection** | | | |
| D1 | 14(43.8) | 25(69.4) | 0.032* |
| D2 | 18(56.2) | 11(30.6) | |
| **Extent of resection** | | | |
| R0 | 28(87.5) | 21 (58.3) | 0.013* |
| R1 | 3(9.4) | 11 (30.6) | |
| R2 | 1(3.1) | 4 (11.1 | |
| **Tumor stage** | | | |
| pT0 | 3(9.4) | 0(0.0) | |
| pT1 | 4(12.5) | 2(5.6) | |
| pT2a | 8(25.0) | 5(13.9) | 0.014* |
| pT2b | 12(37.5) | 10(27.8) | |
| pT3 | 4(12.5) | 16(44.4) | |
| pT4 | 1(3.1) | 3(8.3) | |
| **Nodal stage** | | | |
| pN0 | 8(25.0) | 4(11.1) | |
| pN1 | 22(68.8) | 21(58.3) | 0.039* |
| pN2 | 1(3.1) | 8(22.3) | |
| pN3 | 1(3.1) | 3(8.3) | |
| **Surgical cut margin** | | | |
| Negative | 29(90.6) | 24(66.7) | 0.017* |
| Positive | 3(9.4) | 12(33.3) | |
| **Pretreatment tumor maximum diameter** Mean ± SD (cm) | 6.0 ± 2.69 | 7.8± 2.58 | 0.007* |

* Significant difference ($p \leq 0.05$).

**Table (3):** Postoperative complications.

| Postoperative complication | Chemotherapy plus surgery Group I (No. =32) | Surgery alone Group II (No. =36) |
|---|---|---|
| Anastomotic leakage | 1 | 0 |
| Delayed wound healing | 1 | 0 |
| Infection | 2 | 1 |
| Biliary reflux | 1 | 0 |
| Persistent vomiting | 2 | 0 |
| Dumping | 1 | 1 |
| Adhesive intestinal obstruction | 1 | 0 |
| Death | 1 | 0 |

**Table (4):** Adverse effects with preoperative and postoperative chemotherapy.

| Adverse effect | Preoperative chemotherapy (No. =36) | | Postoperative chemotherapy (No. =30) | |
|---|---|---|---|---|
| | Grade 1&2 *No. (%)* | Grade 3&4 *No. (%)* | Grade 1&2 *No. (%)* | Grade 3&4 *No. (%)* |
| **Non- hematological** | | | | |
| Nausea & vomiting | 26(72.2) | 0 | 20(66.7) | 2 (6.7) |
| Diarrhea | 8(22.3) | 0 | 10 (33.3) | 0 |
| Mucositis | 12(33.3) | 0 | 10 (33.3) | 0 |
| Renal | 2(5.6) | 0 | 3 (10.0) | 0 |
| **Hematological** Anemia | 16(44.4) | 0 | 20(66.7) | 0 |
| Thrombocytopenia | 2(5.6) | 0 | 2 (6.7) | 2 (6.7) |
| Neutropenia | 25(69.4) | 1(2.8) | 1(3.3) | 0 |

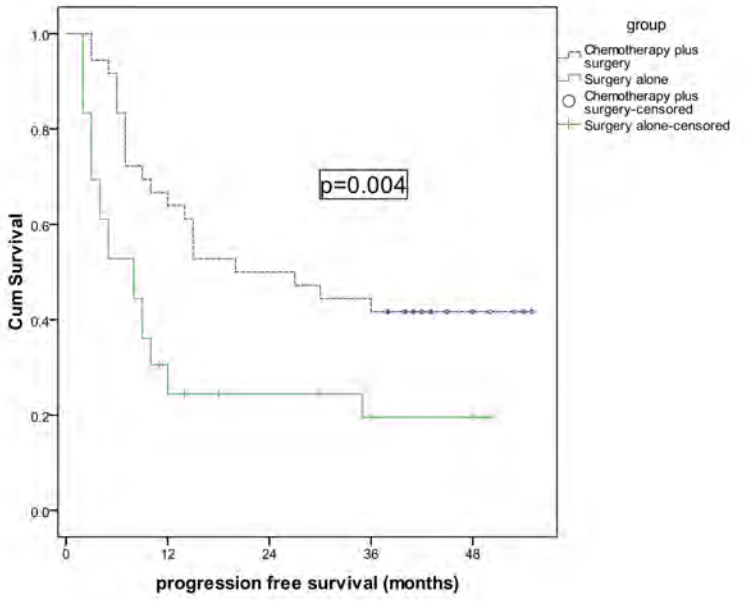


**Figure (1) :** Progression free survival in both groups.

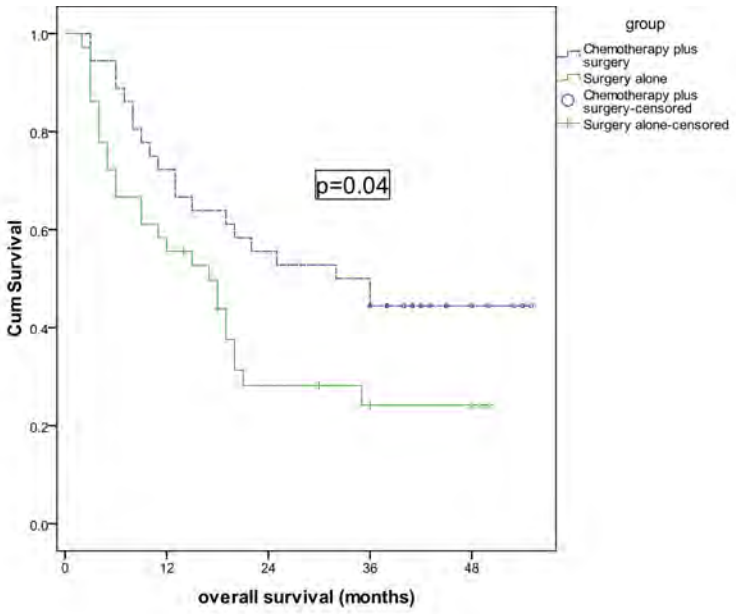


**Figure (2) :** Overall survival in both groups.

## Discussion

In spite of a declining incidence in the Western world, gastric cancer is still the second leading cause for cancer mortality world wide[12]. While localized disease, limited to the submucosa, can be best treated surgically, with a long-term survival of 70%-95%, the prognosis of locally advanced tumor is poorer, due to a high unresectability rate at presentation, and a much higher relapse rate after radical surgery[13]. To date, most therapeutic efforts are directed toward an individualization of therapeutic protocols, tailoring the extent of surgery and integrating it with the administration of preoperative and/or postoperative treatment[14].

Persiani et al reported that R0 resection is the most important prognostic factor for resectable locally advanced gastric cancer, they encouraged to consider neoadjuvant chemotherapy a promising modality for increasing the R0 percentage of gastric carcinoma patients who could benefit from a curative surgery. The main goals of neoadjuvant chemotherapy are to improve the likelihood of achieving a complete surgical resection, and to destroy tumor deposits outside the surgical field that could otherwise result in tumor recurrence or distant metastases[15].

The present study was designed to determine the effect of chemotherapy before and after radical surgery in locally advanced gastric cancer. In our study, the rate of curative resection was 87.5% in preoperative chemotherapy group versus 58.3 % in upfront surgery group; which was comparable to results reported by Boige et al.[16] who reported higher rate of curative resection (87%). Also, it was in agreement with D'Ugo et al.[17]; in their phase II study of perioperative chemotherapy using different protocols (EEP or ECF). In the European MAGIC Trial, the rate of R0 resections was 79% after 3 cycles of ECF[18]. However, the Dutch Gastric Cancer Group reported a disappointing result after 4 cycles of FAMTX, as the curative resection was 56% only[19].

In our study, the mean maximum diameter of the resected tu-

mor was smaller in the preoperative chemotherapy group than in upfront surgery group (6.0 ± 2.69 cm versus 7.8± 2.58 cm; p=0.007); this finding is consistent with tumor shrinkage by preoperative chemotherapy that matched with the finding of MAGIC trial[18]. In contrast, Hartgrink et al.[19] presented the final results of the first randomized controlled trial of exclusively preoperative chemotherapy for gastric cancer. The regimen used was FAMTX (fluorouracil, doxorubicin, and methotrexate), which was, at that time, the gold standard of treatment for adenocarcinoma of the stomach. The investigators reported a high rate of tumor progression during treatment (36%).

In Egypt gastric cancer usually presents late in advanced stage, the estimated 2 year overall survival rate after radical gastrectomy and systematic lymph node dissection was 43.6%[20]. In the present study, perioperative chemotherapy in locally advanced and potentially operable gastric carcinoma demonstrated a survival benefit as compared with upfront surgery group. Two - year overall survival rates were 48% and 27% in group I and in group II, respectively and progression free survival in group I and in group II were 44% and 23%, respectively. Similar results were confirmed by both Boige et al.[16] and Cunningham et al.,[18].

Postoperative adjuvant chemotherapy was associated with reduced risk of death in gastric cancer compared with surgery alone, and is recommended for patients who have not received perioperative treatments after complete resection of their gastric cancer [21]. Although, postoperative chemotherapy in upfront surgery group did not add a survival benefit to surgery that was consistent with the results of meta-analysis by Hermans et al who analyzed 11 randomized trials, with a total of over 2,000 patients, that compared postoperative chemotherapy to surgery alone. Most of these trials used older 5-FU-based regimens, including FAM (5-FU/ doxorubicin/mitomycin [Mutamycin]), 5-FU/mitomycin, and 5-FU/ semustine (methyl-CCNU) [22].

A Swedish meta-analysis found

a small overall survival benefit for adjuvant chemotherapy; however, when the authors analyzed Western and Asian trials separately, no survival benefit was found in the subgroup of Western patients, whereas the benefit seen in treated Asian patients remained significant [23].

A North American Intergroup randomized trial demonstrated that five cycles of post-operative chemotherapy with 5-FU/ leucovorin (LV) before, during and after radiotherapy (45 Gy in 25 fractions over 5 weeks) resulted in an 15% improvement in 5-year overall survival [24].

Although postoperative chemoradiotherapy is considered to be standard therapy in the USA, it has not gained wide acceptance in Europe because of concerns about toxicity with abdominal chemoradiation and the type of surgery used. Distant relapse was the most common pattern of recurrence in the adjuvant group (33% vs 18%), whereas local recurrence was more common in the surgery-only group (29% vs 19%). In that trial, < 10% of patients underwent formal D2 dissection, whereas 54% underwent D0 dissection. A common interpretation of that result is that adjuvant therapy may be useful in high-risk patients treated with inadequate lymph node dissection, because, through radiotherapy, it can eliminate residual lymph node metastasis, which would have been removed by D2 resection [14].

As regard tolerability, in accordance with the MAGIC trial[18] and Boige et al[16], our study found that postoperative chemotherapy is tolerated worse than preoperative therapy without increase in postoperative mortality and morbidity, Moreover, symptomatic improvement induced by preoperative chemotherapy before major surgery like gastrectomy encouraged these patients to complete postoperative chemotherapy as planned.

## Conclusion

Chemotherapy before and after radical surgery is preferred compared with surgery alone in treatment of locally advanced gastric cancer as regard response, survival and tolerance.

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# BENHA MEDICAL JOURNAL


## SURGERY ALONE VERSUS PERIOPERATIVE CHEMOTHERAPY FOR LOCALLY ADVANCED GASTRIC CANCER


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# INFLUENCE OF AGE ON PAW PRESSURE AND FORMALIN-INDUCED PAIN RESPONSE IN RAT: A ROLE OF NITRIC OXIDE

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## Abstract

***Aim of Work:*** *the aim of the present study is to elucidate the effect of age on pain response to paw pressure and formalin injection, and to investigate how age affects the role of nitric oxide (NO) in these nociceptive responses.*

***Methods:*** *pain response evoked by mechanical pressure on hind paw and intraplanter injection of formalin (5%) into the hind paw was evaluated in 2 groups of adult male Sprague Dawley rats; 2 months old (young) and 24 months old (aged). Pain responses were evaluated after intraperitoneal (ip) injection of L-arginine (200 mg/kg), L-NAME (50 mg/kg) and after intracerebroventricular (icv) injection of L-arginine (1µg/rat) or L-NAME (1µg/rat). NADPH-diaphorase staining was done to compare neuronal activity of nitric oxide synthase in hippocampal CA1 region and at lumbar segments of spinal cord in both age groups.*

***Results:*** *pain response was significantly reduced in the aged rats; greater mechanical pressure (pressure test) and shorter duration of licking (formalin test). L-arginine (i.p) had no effect on pain response induced by formalin in both age groups but enhanced pain response to paw pressure in aged rats only. L-arginine (i.c.v) had no effect on pain response evoked by paw pressure in both groups but enhanced biphasic pain response to formalin. L-NAME (i.p & i.c.v) suppressed pain response to paw pressure in both age groups. L-NAME (i.c.v) suppressed pain response to formalin during the acute phase only, but enhanced it during the late phase in both age groups. NADPH-d activity was signif-*

*cantly greater in hippocampal CA1 and in the dorsal horns of spinal lumbar regions in young rats than in aged rats.*

***Conclusions:*** *the current results indicate that pain response evoked by paw pressure and formalin is blunted in the old rats. Peripheral loading with L-arginine improves nociception evoked by pressure in the old rats only, while central L-arginine enhances formalin-evoked pain response in both age groups. Peripheral inhibition of NO with L-NAME has an antinociceptive effect on pain response evoked by pressure and formalin. However, central inhibition of NO with L-NAME has pronociceptive effect on response during the late phase of formalin, suggesting a dual role of centrally synthesized NO in pain response evoked by formalin.*

## Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage[1]. Nociceptive sensitization (hyperalgesia) that results from a persistent nerve or tissue damage is perceived as a decreased threshold to noxious stimuli with the value of protecting the injured site and avoiding further damage[2]. It is unclear whether age influences the sensory processes involved in pain perception as it does in most sensory systems[3]. Thus, some studies in rat have indicated that reactivity to pain decreased in 25 month old rats compared to 3 month old either in response to thermal and mechanical stimuli4 or to thermal and electrical stimuli[5]. By contrast, Islam et al.[6] reported an enhanced nociception in 12, 18 and 24 month old rats compared to 6 month old rats; while Crisp et al.[7] have shown that pain reactivity is enhanced in 25 month old rats when compared to 15 month old rats but not to 6 month old rats. Meanwhile, Goicoechea et al.[8], using the tail-shock test, found no difference in nociception between 25 and 10 month old rats.

The mechanism of nociceptive sensitization is widely studied and it has been attributed to peripheral and central pathways. Peripheral sensitization is shown to be mediated by inflammatory mediators such as prostaglandin E2[9], bradykinin, nerve growth factor, and

nitric oxide (NO) in the injured site[10]. These mediators increase the sensitivity and excitability of primary afferent of nociceptors. On the other hand, there is still much debate about the mediators involved in central sensitization but there is accumulating evidence of the important role of NO[11,12]. NO is synthesized from L-arginine, in presence of different cofactors, by nitric oxide synthases (NOS) enzymes[13]. Of the three identified NOS isoenzymes, the neuronal NOS (nNOS) has been associated with nitrergic signalling in the brain. However, many evidences have shown that endothelial NOS (eNOS) also contributes to the physiological actions of NO in the brain[14]. As indicated above, there is accumulating evidence that NO has a role in nociception. However, there is still controversy whether NO has a pronociceptive or antinociceptive roles. Thus, some studies have shown that treatment with inhibitors of NOS enzyme inhibited hyperalgesia in the rat in response to either thermal or mechanical noxious stimuli suggesting a role of NO in nociception[15]. Further, intracutaneous or intravenous injection of NO solution has been found to evoke pain in humans indicating a role of NO in peripheral nociception[16]. By contrast, other studies have demonstrated that stimulation of the arginine-NO-cGMP pathway exerts antinociceptive effect[17,18]. Further, some studies have demonstrated that the antinociceptive properties of some drugs are mediated through NO as pretreatment with NOS-inhibitors has been shown to interfere with the effects of these drugs, including clonidine[19], morphine[20], D-Pen 2, D-Pen 5-enkephalin[21], sildenafil[22], and muscarinic agonists in the rostral ventral medulla of rats[23].

Thus, the main aim of the present study is to further elucidate the effect of age on pain sensitivity in rats in response to chemical and mechanical stimuli, and also to investigate how age affects the role of NO, either peripheral or central, in these nociceptive responses.

## Materials and Methods

### Experimental animals :

Sprague-Dawley male rats were kept in the animal care facility at

Physiology Department, Mansoura Faculty of Medicine under a 12-h light/dark cycle and had free access to food and water. All experimental protocols were approved by Mansoura Faculty of Medicine Animal Care and Use Committee.

**Experimental design :**

**Rats were divided into 2 main groups:** young adult rats (2 month old, number = 12, weight = 180-200 gm) & old rats (24 month old, number = 12, weight = 350-380 gm). The two groups were subjected to pain sensitivity test in response to chemical and mechanical stimuli to compare the effect of age on nociception. Then, to further evaluate the role of NO in nociception in the old rats, the NOS inhibitor, L-NAME and L-arginine (NO precursor) were injected intraperitoneal (i.p) and intracerebroventricular (i.c.v). According to the method of administration of the agent rats were further divided into 2 subgroups:

**1. L-NAME pretreated group, which is further subdivided into 2 subgroups:** a (received L-NAME intraperitoneal 50mg/Kg 30 min before test[24] & b (received L-NAME intracerebroventricular at a dose of 1.0 ug, 15 min before testing[25].

**2. L-arginine pretreated group, which is further subdivided into 2 subgroups:** a (received L-arginine intraperitoneal 200 mg/Kg 45 min before test[24] & b (received L-arginine intracerebroventricular at a dose of 1.0 ug, 15 min before testing[25].

**Surgical procedure of stereotaxis :**

A mixture of diazepam 5mg/Kg and ketamine HCL (75mg/Kg) was used to anesthetize the experimental animals. Then, the rat was fixed into stereotaxis table (Stoelting stereotaxic unit, model 51600, USA) and a short incision was made along the midline of the scalp using a scalpel, and the skin was pulled back to expose the calvarium. A small hole was made on the calvarium, at stereotaxic coordinates: 0.95mm posterior to bregma, 1.4mm lateral to the midline. Then, a stainless steel needle of outer diameter 0.5mm was fixed at this point, by dental cement to a depth 3.2mm below the surface

of the brain[26]. Rats were allowed to recover their activities for 10 days in separate cages before pain threshold testing.

**Central microinjection procedure:**

Intracerebroventricular injection (i.c.v.) was made using the microinjection technique of Haley and McCormick[27], using a 10-µl microsyringe (Hamilton, Reno, NV). A volume of 5 µl (containing 1 ug) of drug solution was delivered directly into the lateral cerebral ventricle over 30 s.

**Pain threshold testing :**

**Mechanical Stimulus:** (Paw withdrawal threshold (PWT)

The paw pressure test consisted of gently holding the body of the rat while the hind paw was exposed to increasing mechanical pressure. The analgesy-meter (Ugo-Basile, Varese, Italy, cat. # 7200) is designed to exert a force on the paw that increases at a constant rate, similar to the Randall and Sellito[28] test of mechanical nociception. Force was applied to the right hind paw that was placed under a small plinth under a cone-shaped plunger with a rounded tip. The operator depressed a pedal-switch to start the mechanism that exerted force. The force in grams at which the rat withdrew its paw was defined as the paw pressure threshold. The baseline paw pressure was measured before injecting vehicle or drug. Five determinations per animal (with interval between sequential measurements greater than 10 min) were collected in each test session, averaged and expressed in grams.

**Formalin test (Chemical stimulus) :**

The formalin test was performed as previously described29. In brief, the rat was acclimated to the observation chamber (transparent glass box permitting an unobstructed view of rat's paws to the observer) for ~20 min. Then, the rat was restrained and formalin (5% in 10 µl) was injected subcutaneously into the right hind paw using a Hamilton microsyringe with a 30 G needle and replaced again in the observation chamber. Then, the duration (in seconds), the animal spent licking, lifting and / or favoring the injected paw was recorded between 0-5

min (early phase), and 15-60 min (late phase) after the formalin injection.

**NADPH-diaphorase Histochemistry**

In order to identify the effect of aging on the neuronal NOS distribution in the rat brain and spinal cord, NADPH diaphorase (NADPH-d) technique was used.

**Specimens collection and sectioning**

Animals were anaesthetized with ether and perfused intracardially with 10% neutral buffered formalin. Then, their brains and the lumbosacral region of the spinal cords were carefully dissected out. The brains were sliced into small pieces by removing the anterior part of the forebrain by a coronal section at the rostral end of corpus callosum. The hind brain was separated by a transverse section at the lower end of the midbrain. The tissue was then stored in the same fixative for 4-5 days in order to provide an adequate NADPH staining. After being fixed, the tissues were stored in 15% sucrose in 0.1 M phosphate buffer, pH 7.4, overnight at 4°C to minimize the formation of ice crystals. The tissues were sectioned with a cryostat at (8 and 40 µm) thick and collected on coated slides and stained with gallocyanin chrom alum stain for Nissle substance and NADPH-d histochemistry.

**NADPH-d histochemistry :**

The sections were rinsed in the buffer, floated in a diluent of 0.3% Triton-X 100 in phosphate buffer while being shaken for 1-2 min. The staining was then performed by incubating the tissue in a solution containing equal parts of nitro-blue tetrazolium (0.4 mg/ml in buffer) and NADPH (1 mg/ml in buffer) for about 20-24 h at 37°C, sections were then rinsed using distilled water and mounted using glycerol gel[30].

**Gallocyanin chrom alum stain:**

8 µm thick sections were stained with a mixture of gallocyanin and chromium potassium sulphate (chrom alum) overnight. The sections then were rinsed in running tap water, dehydrated using ascending grades of alcohol and cleared in xylene and mounted using DPX[31].

**Drugs :**

L-arginine and NG-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma fine chemical Co., Egypt. The drugs were prepared freshly on the day of the experiment by dissolving in sterile 0.9% NaCl solution.

**Statistical analysis :**

All values are expressed as means ± SD (standard deviation). Comparison between the two age groups was done using unpaired student t-test. Comparison within the same group was done using paired-t-test. P< 0.05 was considered significant.

## Results

**Effect of aging on basal pain threshold :**

Fig. 1 shows that there is a significant increase in pain threshold in aged rats (24 months) when compared with young rats (2 months) ($p < 0.01$) by using both mechanical and chemical stimuli.

**Effect of systemic administration of L-arginine and L-NAME on paw pressure threshold:**

Fig. 2 shows a significant decrease in paw pressure threshold in aged rats only after systemic administration of l-arginine when compared with basal value ($p < 0.01$). Pain threshold is decreased in aged rats by 35.06%, but increased in young rats by 9.49% after systemic l-arginine. On the other hand, systemic administration of L-NAME caused significant increase in pain threshold in both young and aged rats ($p < 0.01$). However, the effect was marked in the young rats (106.48%) when compared to the aged rats (21.80%).

**Effect of i.c.v injection of L-arginine and L-NAME on paw pressure threshold:**

Fig. 3 shows i.c.v administration of l-arginine caused non significant decrease in paw pressure threshold in young rats by 26.07% and in aged rats by 9.29% when compared with basal value. On the other hand, i.c.v administration of L-NAME caused significant increase in pain threshold in both young rats (by 160.84%) and aged rats (by 44.39%) when compared with basal value ($p < 0.01$).

**Effect of systemic administration of L-arginine and L-NAME on formalin-induced pain threshold:**

As shown in Fig. 4a, systemic administration of l-arginine caused non-significant difference in time of paw licking induced by formalin during early phase in both young and aged rats when compared with basal value. By contrast, systemic administration of L-NAME caused significant decrease in the time of paw licking & lifting during early phase in both young and aged rats (by 41.9% & 57% respectively) when compared to basal value ($p < 0.01$). In late phase, L-NAME also caused significant decrease in pain threshold in young (by 32.53%) and aged (by 36.42%) rats ($p < 0.01$), while, L-arginine caused no significant change in time of paw licking in both age groups (fig.4b).

**Effect of i.c.v administration of L-arginine and L-NAME on formalin-induced pain threshold:**

During early phase of formalin test, there was significant increase in pain threshold in the young rats (by 28.53%) and aged rats (by 79.48 %) after i.c.v administration of l-arginine when compared with basal value ($p < 0.01$, 0.001 respectively; fig. 5a). By contrast, during the same phase, i.c.v administration of L-NAME caused significant decrease in pain threshold in both young (by 47.25%) and aged rats (by 37.91%) ($P< 0.01$). In late phase, i.c.v administration of l-arginine caused significant increase in pain threshold in the young and aged rats (by 192.81% & 66.45% respectively) ($p < 0.001$; fig. 5b). In contrast to its effect during early phase, i.c.v administration of L-NAME caused significant increase in formalin-induced pain threshold during late phase in both young (30.76%) and aged rats (49.84%) when compared with basal value ($p<0.001$; fig.5b).

**NADPH-diaphorase activity:**

Examination of the CA1 region of the hippocampus showed increase in the activity of NOS of the young rats (fig 6b) in comparison to the aged rats (fig. 6e). Moreover, NADPH-d reaction product was also localized to the vascular endothelial lining and choroidal

epithelial cells of both young and aged rats but the reaction was more intense in the young rats (figs. 6c and d).

The NOS activity was examined in the coronal sections of the lumbar segments of the spinal cord of the rats and it was found that the number of NADPH-d cells was greater in the dorsal horn (lamina I-IV) in the young rats in comparison to that of the aged rats (figs 7a and b). On the other hand, few positive cells were found around the central canal (lamina X) in the aged rats (figs. 7c and d).

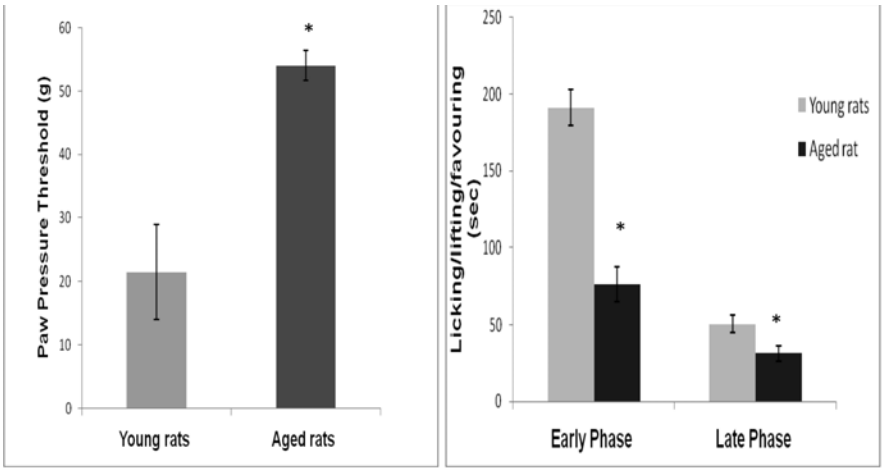


**Fig. 1 :** Basal pain threshold in the young (2 months, n=12) and aged rats (24 months, n=12) in response to paw pressure test (a) and formalin injection test (b). Values are expressed as mean ± SD. * $p \leq 0.01$ aged rats vs. the young rats (student's unpaired t-test).

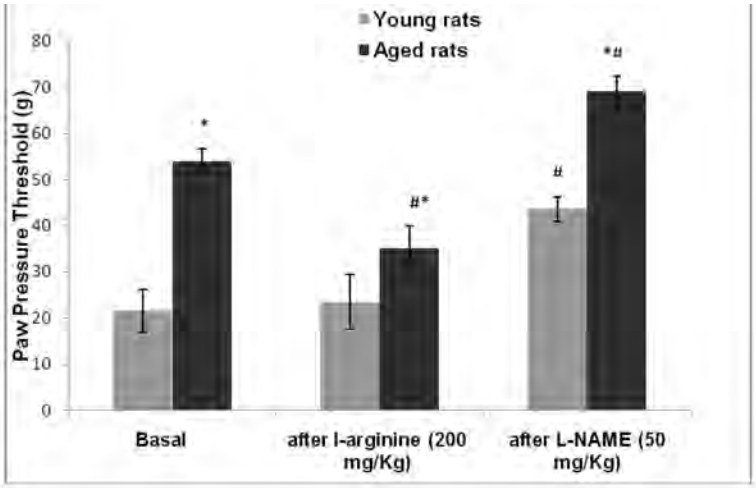


**Fig. 2 :** Effect of systemic adminstration of L-arginine and L-NAME on paw pressure threshold in young (2months) and aged rats (24 months). Values are expressed as mean ± SD. * $p < 0.01$; signficant vs. the young rats (unpaired t-test).# $p < 0.01$; significant vs. the basal value (paired t-test).

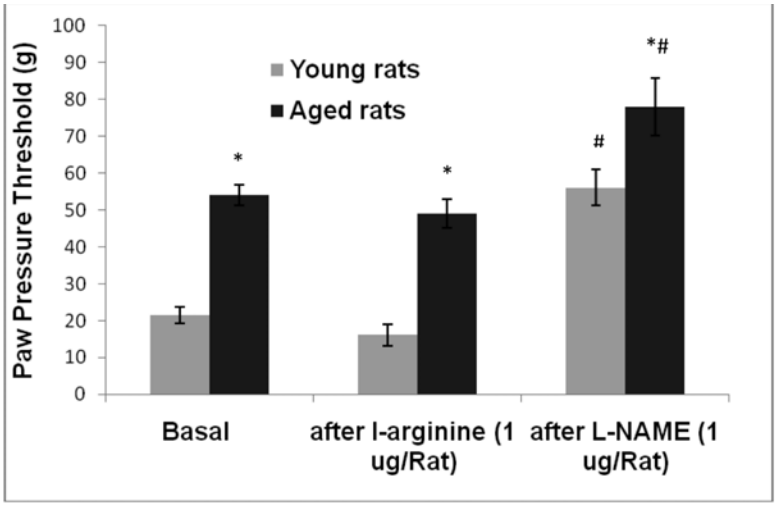


**Fig. 3 :** Effect of icv adminstration of L-arginine and L-NAME on paw pressure threshold in young and aged rats. Values are expressed as mean ± SD. * $p < 0.01$; signficant vs. the young rats (unpaired t-test).# $p < 0.01$; significant vs. the basal value (paired t-test).

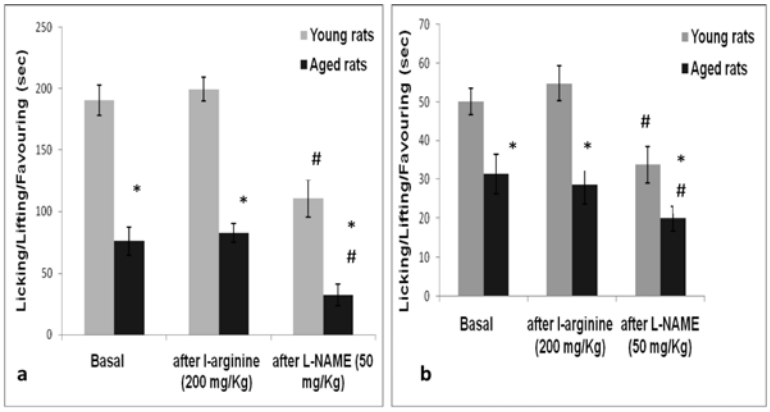


**Fig. 4 :** Effect of sysetmic L-arginine and L-NAME adminstration on time of paw licking & lifting (sec) induced by formalin injection in young (2 months) and aged rats (24 months) during early phase (a) & late phase (b). Values are expressed as mean ± SD. * $p < 0.01$; signficant vs. the young rats (unpaired t-test).# $p < 0.01$; significant vs. the basal value (paired t-test).

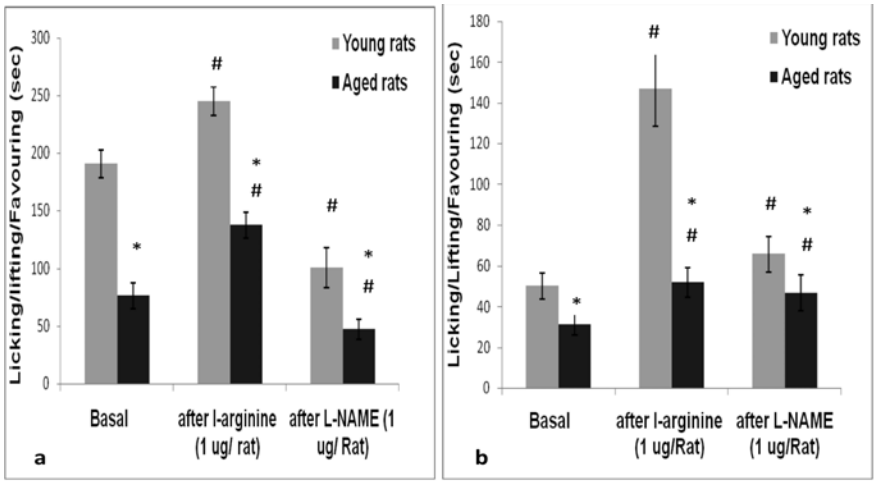


**Fig. 5 :** Effect of i.c.v adminstration of L-arginine and L-NAME on time of paw licking & lifting (sec) induced by formalin injection in young (2 months) and aged rats (24 months) during early phase (a) & late phase (b). Values are expressed as mean ± SD. * $p < 0.01$; signficant vs. the young rats (unpaired t-test).# $p<0.01$ significant vs. the basal value (paired t-test).

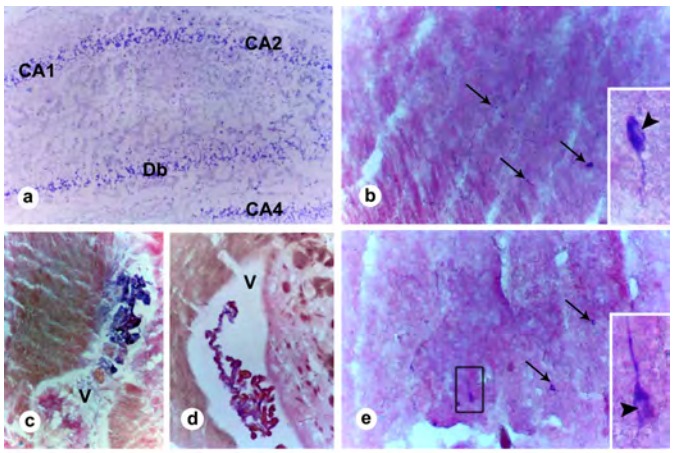


**Fig. 6 :** Photomicrographs of the rat brain. **(a)**-section of the hippocampus of adult rat showing its different regions. The CA1, CA2 and CA4 fields of cornu Ammonis and the buried (Db) blade of the dentate gyrus. (Gallocyanin chrom alum stain; X40). (**b**)-section of the hippocampus at CA1 area of young adult rat showing positive NADPH-d cells (arrows). Inset shows a higher magnification of one of these cells, note the unstained nucleus (arrowhead). (NADPH-d histochemistry; X100, inset; X400). **(c)**- section of the brain of the ventricle of the adult young rat and **(d)**- in old rat showing positively stained NADPH-d endothelial cells lining the choroid plexus. (NADPH-d histochemistry; X40). **(e)**- section of the hippocampus at CA1 area of young adult rat showing few positive NADPH-d cells (arrows). Inset shows a higher magnification of one of these cells (rectangle), note the unstained nucleus (arrowhead).(NADPH-d histochemistry; X100, inset; X400).

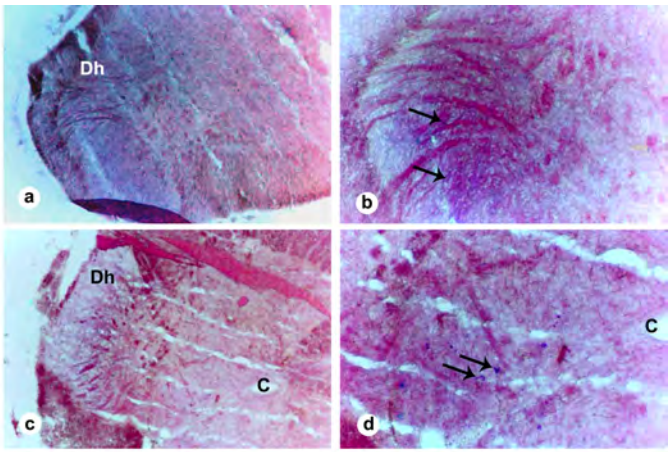


**Fig. 6 :** Photomicrographs of the rat spinal cord. **(a)**-Section of the spinal cord of adult young rat and **(c)**- of the old rat showing the dorsal horn (Dh) and the central canal (C), note the increase of the intensity of the stain in lamina (I-IV) of the dorsal horn of the young rats in comparison to old rats.(NADPH-d histochemistry; X40). **(b)**-Higher magnification of (a) showing positive NADPH-d cells (arrows) in the dorsal horn. NADPH-d histochemistry; X100) (**d**)- Higher magnification of (**c**) showing positive NADPH-d cells (arrows) in the grey matter surrounding the central canal. (NADPH-d histochemistry; X100).

## Discussion

So far, few studies have investigated the effect of ageing on the nociceptive pathway. Moreover, the results are conflicting and this might be explained by differences between species and/or types of nociceptive stimuli used. In the present experiments, two nociceptive stimuli were used; mechanical (paw pressure test) and chemical (formalin test). In paw pressure test an increasing force (16 g/s) was applied to the right hind paw and the pressure that forced the rat to withdraw the paw is taken as an index of nociceptive mechanical withdrawal threshold. The pressure applied to the hind limb activates certain slowly adapting mechanoreceptors that send signals via C fibers to spinal cord[32]. On the other hand, the nociceptive response to formalin consists of 2 phases; an early phase (0-5 min) which is considered to be produced by a direct activation of peripheral afferent C fibers by formalin. The second late phase of the response (15-60 min) is mediated by ongoing stimulation of nociceptors by inflammatory mediators from injured tissues and/or central sensitization as a response to first early phase[33]. The use of 2 different nociceptive stimuli and the fact that formalin test with its 2 phases of nociception is reflecting different pathophysiological processes was helpful to augment the outcome of the experiments.

The results of the present study have shown that the nociceptive threshold of aged rats (24 month-old) is significantly higher than that of the mature rats (2 month-old) to both mechanical and chemical stimuli, indicating a decrease in the reactivity to pain in aged rats. This finding of the decrease in pain sensitivity to mechanical nociceptive stimuli are in accord with that of Akunne & Soliman4 who found less sensitivity of 24 month old Fischer-344 rats to paw pressure test when compared with 3 month old rats. By contrast, Jourdan et al.34 have shown decreased mechanical withdrawal threshold in response to paw pressure test in 24 month old Lou/c rats than in middle aged and mature rats. On the other hand, the present finding of the increase in pain threshold induced by formalin in old rats is different from the

study by Gagliese & Melzacka[35] who showed that there was no difference between 3 and 24 month old Long-Evans rats. A recent study by Hagiwara et al.[36] has found hypersensitivity to formalin injection in middle aged 11 month old female Sprague Dawely rats.

Another important aim of the present study was to further elucidate the role of NO in nociception. In response to pressure nociceptive stimulus, the present results shows that with either i.p or i.c.v administration of L-NAME, the sensitivity to pain decreased in both the young and aged rats suggesting a pronociceptive role of NO that is mediated both peripherally and centrally. Further, this antinociceptive effect of L-NAME was more pronounced in the young rats than in the aged rats suggesting that the pronociceptive role of NO in response to paw pressure is decreasing with age. Moreover, the decrease in pain sensitivity in the young rats was more marked with i.c.v administration than with i.p L-NAME administration (160%) that may reflect a greater central role of NO in pain perception in the young rats than in the aged rats. Indeed, the results of the histochemistry study revealed a greater activity of NADPH-d cells in hippocampus sections from the young rats relative to the old rats indicating greater activity of neuronal NO; a finding that is consistent with the above assumption that the central pronociceptive role of NO is decreasing with age. There is evidence from previous studies about a possible role of hippocampus CA1 region in nociception[37,38] and that is most probably through a mechanism related to NOS activation[25]. Thus, this study extends this to its involvement in blunted pain sensitivity in aged rats.

On the other hand, with the use of the NO precursor, L-arginine, the sensitivity to pressure-induced pain increased after systemic administration in the aged rats only, while there was no change in the pain reactivity after i.c.v L-arginine in both groups (see Fig. 2 & 3). This finding suggests that with augmenting the synthesis of NO with L-arginine, the pronociceptive effect of NO is mediated peripherally in the aged rats only while it has no central role in

nociception in both groups. The fact that the aged rats showed less reactivity to paw pressure when compared with the young rats (Fig. 1) and that their reactivity to pain was enhanced by systemic L-arginine supports again the above suggestion that the pronociceptive role of NO in response to paw pressure decreases with age. What is interesting here is that i.c.v L-arginine had no effect on pain reactivity in both groups. This might be related to the dose used in the present experiments as in the study by Sousa & Prado[39] a higher dose of the NO donor (3-morpholinosydnonimine (SIN-1) had no effect or increased the pain produced by chronic ligature of sciatic nerve, but had antinociceptive effect in rat tail-flick test. The present findings regarding the role of NO in nociception induced by mechanical stimuli are in agreement with previous studies that used also the non selective NOS inhibitor L-NAME although they have used different mechanical stimuli as tail flick test[23] or von frey hair test[39], 40. However, studies using other NO inhibitors have not produced consistent results as intraplantar or i.c.v L-NOARG was ineffective in paw pressure test[41,42] while intrathecal 7-nitroindazole (7-NI) did not affect pain induced by rat tail-flick test[43].

Regarding the response to formalin injection, i.p L-arginine did not affect the pain threshold in both age groups either during the acute or the tonic phase (fig. 4a&b). By contrast, i.c.v administration of l-arginine enhanced pain sensitivity in both groups during acute and tonic phases of the nociceptive response, but with greater influence in the young rats during the late phase. This suggests a central pronociceptive role of L-arginine-NO pathway in response to formalin that is more evident for the late chronic phase in the young rats, indicating that L-arginine-NO pronociceptive pathway is decreasing with age. What is unexplained here why i.c.v L-arginine did not change the nociceptive response to paw pressure while enhanced it in response to formalin; whether this is related to the dose or it is related to the modality of pain tested warrants further investigation.

On the other hand, i.p L-NAME inhibited the acute and tonic nociceptive response to formalin injection in both groups of rats as indicated by the shorter time spent licking or lifting the paw, with the young rats still more sensitive to pain than the old rats. This suggests a pronociceptive role of NO that is mediated peripherally. However, with i.c.v L-NAME, the decrease in pain reactivity was noticed only during early phase while the sensitivity to pain increased during late phase indicating a dual role of NO with central inhibition of its synthesis; exerting a pronociceptive effect in response to acute pain and an antinociceptive effect during the tonic or chronic phase of pain. Indeed, previous studies on formalin-induced nociceptive response after NOS inhibitors have shown a complex role of NO. Thus, Malmberg & Yaksh,[44] and Sakurada et al.[45] reported that i.p, intrathecal or i.c.v L-NAME inhibited the nociceptive response during the late phase with little or no effect on the acute phase. However, other studies have reported that intrathecal injection of L-NAME greatly reduced the acute phase[46] and the tonic phase of nociceptive response to formalin[47]. Recently, Kolesnikov et al 48 demonstrated that local application of the NOS inhibitor, NOArg, did not affect either phase of the formalin test, while supraspinal blockade of nNOS with NOArg reduced both phases, suggesting the presence of opposing nNOS systems in pain modulation.

In conclusion, the present experiments show that the pain response evoked by either paw pressure or formalin injection is decreased (blunted) in the old rats as compared to the young ones. It seems that augmenting the synthesis of peripheral NO with L-arginine has a pronociceptive effect on response to paw pressure in the old rats only, while central L-arginine enhances pain response induced by formalin in both groups. Further, either peripheral or central inhibition of NO synthase with L-NAME has antinociceptive effect on pain response induced by paw pressure and during the acute phase induced by formalin injection in both groups. This antinociceptive effect of L-NAME seems to be more

manifest in the young rats than in the aged rats. On the other hand, L-NAME (i.c.v) enhanced pain response evoked during the late phase of formalin test in both groups, suggesting that centrally synthesized NO has an antinociceptive effect during the chronic stage of pain evoked by formalin.

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# BENHA MEDICAL JOURNAL


## INFLUENCE OF AGE ON PAW PRESSURE AND FORMALIN-INDUCED PAIN RESPONSE IN RAT : A ROLE OF NITRIC OXIDE


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# STUDY OF SERUM ANGIOPOIETIN-2 IN EGYPTIAN PATIENTS WITH LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

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## Abstract

***Background/Aims:*** *Cirrhosis is characterized by disruption of liver vascular architecture and formation of new microvessels. Angiogenesis plays a key role in the growth of tumors. We aimed to evaluate angiopoietin-2 in cirrhosis and hepatocellular carcinoma (HCC).* ***Methods:*** *Serum angiopoietin-2 levels were measured in 50 patients with HCC; 30 cirrhotics and 30 healthy controls. Underlying aetiology of cirrhosis in all patients was hepatitis C. Alfafetoprotein (AFP) was determined in the HCC group. Characteristics of both markers were compared.* ***Results:*** *Angiopoietin-2 levels were elevated in patients with cirrhosis and HCC compared to controls (p=<0.0001). Levels were higher in cirrhosis compared to HCC (p=<0.0003). Angiopoietin-2 correlated inversely with serum albumin, platelet count and prothrombin concentration and positively with serum bilirubin. Angiopoietin was significantly higher in patients having ascites (p= 0.017) and was significantly higher in Child A compared to control. It was significantly higher in Child B and C patients compared to Child A (p=0.01 and p=0.024). Among HCC cases, serum angiopoietin did not differ with the following: size exceeding 5 cm, multiplicity of nodule, portal vein thrombosis, porta hepatis lymphadenopathy, increasing Okuda stage and advancing BCLC stage. Angiopoietin levels were not different among HCC cases with AFP>200 ng/ml and those with lower AFP levels.* ***Conclusion:*** *Serum angiopoietin-2 is a good marker of increasing severity of liver cirrhosis but does not represent an additive tool to diagnose HCC in HCV-related liver cirrhosis.*

***Key words:*** *Angiogenesis, angiopoietin-2, hepatocellular carcinoma.*

## Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. However, its early detection is still difficult due to the lack of adequate biomarkers that show high sensitivity and specificity. Alpha-fetoprotein (AFP) serum level is commonly used for the screening for HCC, but given its low positive predictive value, its value is limited in a population at average risk. Therefore new biomarkers are needed.[1]

HCC is characteristically a highly vascular tumor with a propensity for vascular invasion and intrahepatic metastasis. Hypervascularity is evident on angiography, computed tomography (CT) and magnetic resonance imaging (MRI). [2] In the early stage of hepatocarcinogenesis, the portal blood supply to HCC is predominant; thereafter, the arterial blood supply increases as these tumors develop. Angiogenesis, the formation of new blood vessels, plays an important role in the invasiveness and progression of this tumour. [3] Angiogenesis is regulated by a balance between pro and antiangiogenic molecules produced by tumors and host component cells. Among these factors, is vascular endothelial growth factor (VEGF) which promotes the growth, proliferation and migration of endothelial cells, and inhibits apoptosis of endothelial cells in pathological angiogenesis.[4]

A new family of endothelial growth factors, angiopoietins (Ang), has been identified, which comprises ligands for the vascular endothelium specific tyrosine kinase receptor (Tie2). Angiopoietin-1 (Ang-1), which is an agonist of Tie2 and induces its phosphorylation, helps to maintain and stabilize vascular networks. [5] Angiopoietin-2 (Ang-2) is a biological antagonist of Ang-1 and reduces vascular stability to block the stabilizing action of Ang-1. However, in the presence of VEGF, Ang-2 induces vascular sprouting and angiogenesis.[6,7]

Ang-2 was markedly expressed in organs that undergo vascular remodeling, such as the ovaries and placenta, and also in several malignancies including HCC.[8] In addition, overexpression of Ang-2 protein and/or mRNA correlates with poor prognosis in some cancers as non-small cell lung, breast

and colorectal cancers. [9,10,11]

To date, there are only a few studies on the relationship between the expression of Ang-2 and angiogenesis in HCC. In addition, the expression pattern of Ang-2 in cirrhotic liver has not been analyzed and little is known about its role in chronic liver disease or cirrhosis. It is true that elevated Ang-2 levels are observed in patients with chronic hepatitis C, but this finding may relate to the inflammatory condition per se and not chronic liver disease, since elevated levels are also observed in inflammatory bowel diseases and sepsis. [12]

Hence the aim of the present study was to evaluate angiopoietin-2 in Egyptian patients with viral-related liver cirrhosis as well as those with hepatocellular carcinoma and to study the relationship of this marker to tumor characteristics.

**Methods:**

This study was conducted at the National liver institute, Menoufyia University wherein 80 patients with liver cirrhosis, attending the hepatology outpatient clinics and admitted in the inpatient unit, were recruited after receiving informed consent. Fifty of these patients suffered from hepatocellular carcinoma. Hepatocellular carcinoma was diagnosed by the demonstration of arterial hypervascular focal lesions with rapid wash out on top of liver cirrhosis using a single imaging modality (triphasic spiral CT, MRI, angiography) or 2 imaging modalities demonstrating the aforementioned feature for lesions <2cm. [13]

Patients with hepatocellular carcinoma were further divided into: a) Patients with solitary focal lesion ≤5cm and b) patients with any tumor area >5cm, whether uni or multifocal, with or without portal vein thrombosis or extrahepatic metastasis. A group of 30 age and gender matched healthy individuals was included as a control group. Patients diagnosed with malignancy other than HCC, sepsis, inflammatory bowel disease were excluded.

All patients were subjected to thorough history taking, detailed physical examination. The follow-

ing laboratory investigations including complete blood picture, routine liver function tests, serum α-fetoprotein and serum angiopoietin-2 were done for all subjects. Whole blood concentrations of ANG-2 were measured by ELISA (R&D Systems, Minneapolis MN) according to the manufacturers' instructions. Concentrations were interpolated from standard curves The lower and upper limits of detection for the assay were as follows ANG-2 (54.69 - 3,500 pg/mL). Radiological investigations included abdominal ultrasonography with doppler assessment of portal vein, triphasic CT abdomen or MRI abdomen. Chest X- ray, bone scan and CT chest were performed when indicated.

For all patients, Child-Pugh score was calculated. For the HCC group: patients were classified according to Okuda score and Barcelona clinic for liver cancer (BCLC).

**Statistical Analysis:**

Data were statistically analyzed using SPSS (statistical package for social science) program version 13. Data are shown as mean, range or value and 95% confidence interval (95% CI), frequency and percent. Chi square test was done for qualitative variable analysis. Fischer exact test for 2 x 2 tables when expected cell count of more than 25% of cases was less than 5. Student t- test was done for normally distributed quantitative variables. Mann-Whitney test was done for quantitative variables which were not normally distributed. ANOVA test was done to compare three variables: one qualitative variable and the other two are quantitative variables of normally distributed variables. Kruskal-Wallis test was done to compare three or more variables; one qualitative variable and the other variables are quantitative variables of non-normally distributed variables. LSD test is a post hoc test which was done for variables of significant difference of more than two groups of normally distributed data after ANOVA test to detect the significant difference between either groups. Tamhane test is another post hoc test which was done for variables of significant difference of more than two groups of non-normally distributed data after Kruskal Wallis test

to detect the significant difference between either groups. Pearson's correlation test was done to study correlation between two normally distributed quantitative variables. A p value < 0.05 was considered statistically significant. ROC curve (Receiver operating characteristic curve) was done to detect cut-off level of the tested variable where at this level there was the best sensitivity and specificity.

## Results

The present study included 80 patients (50 HCC and 30 cirrhotics) as well as 30 controls. Table I demonstrates the demographic and clinical characteristics of the studied groups and table II shows the tumour characteristics and staging of HCC cases.

Measurement of the level of serum Ang-2 revealed that there was a statistically significant difference ($p<0.001$) among the three groups. The level was highest among the liver cirrhosis group followed by the HCC group then the healthy control group. Serum Angiopoietin-2 level was significantly higher in liver cirrhosis compared to HCC cases ($p<0.0003$) {Fig. 1}.

Among HCC cases, serum angiopoietin did not differ with the presence of the following adverse tumor characteristics: size exceeding 5 cm ($p= 0.83$); multiplicity of nodules ($p=0.23$), presence of portal vein thrombosis ($p=0.49$), or the presence of porta hepatis lymphadenopathy ($p=0.18$). Serum Angiopoietin levels were not different among HCC cases who had AFP more than 200 ng/ml and those who had AFP levels less than 200 ($p=0.059$) {Table III}. Furthermore, angiopoietin did not change significantly with either increasing Okuda stage ($p=0.32$) or advancing BCLC stage ($P=0.97$).

There was no statistically significant difference in serum Ang-2 level among the different Child classes of liver cirrhosis ($P=0.22$). However, when both groups (HCC and cirrhosis) were combined to increase the sample size, Child Pugh B and C class had significantly higher angiopoietin levels compared to Child A patients with no significant difference between the 2 groups {Table IV}. Angiopoietin was significantly higher in patients having ascites ($p= 0.017$).

Correlation analysis in both cirrhosis and HCC groups revealed a strong negative correlation between serum angiopoietin and serum albumin (p=0.001), platelet count (p=0.04) and prothrombin concentration (p=0.02). A positive correlation was detected between angiopoietin and bilirubin (p=0.04). No significant correlation between angiopoietin and other studied variables was detected {Table V}

Fig. 2 and Fig. 3 show the ROC curves demonstrating the sensitivity and specificity of angiopoietin and AFP respectively. At a cut-off level of 1105.7 µg/L the sensitivity of angiopoietin was 70% but the specificity was poor (30%). On the other hand, at a cut-off level of 10ng/ml, the sensitivity of AFP was 90% and specificity 92%. Adding angiopoietin to AFP did not augment either the sensitivity or the specificty of AFP in the diagnosis of HCC {Table VI}.

**Table (I):** Demographic and clinical characteristics of studied groups

| **Parameter** | **HCC (n=50)** | **Cirrhosis (n=30)** | **Control (n=30)** |
|---|---|---|---|
| **Age** | | | |
| Range | 30-75 | 40-65 | 35-60 |
| $\bar{X}$±SD | 56.44±9.8 | 46.9±7.3 | 45.9±4 |
| **Gender** | | | |
| Male/ Female | 42/8 (84%/16%) | 26/4 (86.7%/13.3%) | 7/3 (70%/30%) |
| **Rural/Urban** | 40/10 | 24/6 | 7/3 |
| **Splenomegaly:** +ve/-ve | 41/6 | 24/5 | - |
| **Ascites:** +ve/-ve | 29/21 | 26/4 | - |
| **Child-Pugh class:** A/B/C | 16/25/9 | 4/9/17 | Not applicable |
| • 4 cases had splenectomy and were excluded from analysis in HCC group. | | | |
| •A single case had splenectomy and was excluded from analysis in LC group | | | |

**Table (II):** Tumor characteristics and staging of HCC cases.

| Parameter | Frequency (n=50) | Percent |
|---|---|---|
| **No. of nodules:** Single / Multiple | 35/15 | 70%/30% |
| **Site:** Rt.Lobe/ Lt.Lobe/ Bilobar | 36/7/7 | 72%/14%/14% |
| **Size:** <5 cm/≥5 cm | 24/26 | 48%/52% |
| **Portal vein thrombosis:** +ve/-ve | 7/33 | 34%/66% |
| **Porta hepatis L N:** +ve/-ve | 4/46 | 8%/92% |
| **AFP** > 200/ AFP< 200 | 17/33 | 34%/66% |
| **Child-Pugh class:** A/B/C | 16/25/9 | 32%/50%/18% |
| **Okuda grade:**1/2/3 | 18/24/8 | 36%/48%/16% |
| **BCLC stage:** A/B/C/D | 22/7/12/9 | 44%/14%/24%/18% |

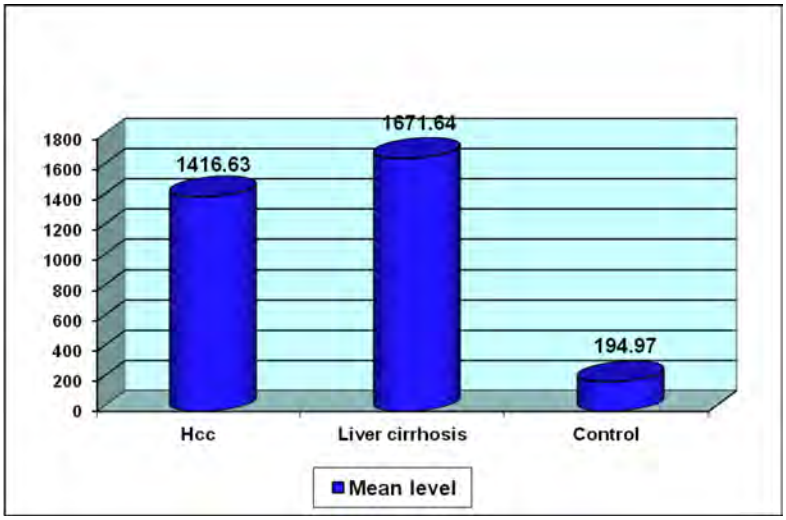


**Figure (1):** Angiopoietin-2 serum level in patients with HCC, Liver cirrhosis and Healthy control .

**Table (III):** Comparison of Angiopoietin-2 serum level in patients with HCC according to tumor characteristics

| No. of nodules | No. of patients | Range | X±SD | P-value |
|---|---|---|---|---|
| **Tumour Nodularity** | | | | |
| Uninodular | 35 | 625.95-1970.98 | 1485.2 ± 490.07 | 0.23 |
| Multi | 15 | 904.8-1902.2 | 1256.6 ± 511.9 | |
| **Tumour size** | | | | |
| < 5 cm | 24 | 473 -1971 | 1431.4±481.6 | 0.83 |
| ≥5 cm | 26 | 499.4-1998.03 | 1122.1.4±509.6 | |
| **Portal vein thrombosis** | | | | |
| Negative | 33 | 472.99- 1996.88 | 1389.7±519.7 | 0.49 |
| Positive | 17 | 729.73 - 1998.03 | 1468.9±478.8 | |
| **Porta hepatis lymphadenopathy** | | | | |
| Negative | 46 | 472.99- 1998.03 | 1445.07±490.2 | 0.18 |
| Positive | 4 | 729.7 -1996.88 | 1089.6±606.9 | |
| **AFP cut off** | | | | |
| < 200 | 18 | 472.99-1890.22 | 1279.12±476.6 | 0.059 |
| > 200 | 32 | 517.84-1998.03 | 1493.98±507.7 | |

**Table (IV):** Correlation analysis between Angiopoietin and other studied variables in HCC and cirrhosis group

| Angiopoietin | R | P-value | R* | P-value* | R** | P-value** |
|---|---|---|---|---|---|---|
| Age | - 0.07 | 0.62 | 0.05 | 0.78 | - 0.08 | 0.45 |
| AFP | 0.14 | 0.32 | - | - | 0.14 | 0.32 |
| Bilirubin | 0.22 | 0.13 | 0.12 | 0.51 | 0.23 | 0.04 |
| ALT | 0.03 | 0.84 | 0.11 | 0.57 | 0.08 | 0.47 |
| AST | 0.03 | 0.83 | 0.05 | 0.43 | 0.07 | 0.53 |
| ALP | - 0.09 | 0.52 | 0.09 | 0.62 | - 0.13 | 0.25 |
| GGT | - 0.08 | 0.56 | - 0.03 | 0.88 | - 0.16 | 0.16 |
| Albumin | - 0.33 | 0.02 | - 0.23 | 0.21 | - 0.39 | 0.001 |
| Prothrombin | - 0.16 | 0.27 | - 0.3 | 0.1 | - 0.26 | 0.02 |
| INR | 0.03 | 0.84 | -0.91 | 0.75 | 0.03 | 0.84 |
| Hb | - 0.11 | 0.44 | 0.13 | 0.34 | - 0.11 | 0.44 |
| TLC | 0.03 | 0.83 | 0.03 | 0.53 | 0.03 | 0.83 |
| Platelets | - 0.13 | 0.38 | - 0.47 | 0.05 | - 0.23 | 0.04 |
| Total tumor diameter | 0.008 | 0.96 | | | | |

R for HCC group R* for liver cirrhosis group R** for HCC & liver cirrhosis together

**Table (V):** Comparison of Angiopoietin-2 serum level in patients with HCC and Liver Cirrhosis according to Child-Pugh grade .

P: comparison between the 3 groups.
P* = comparison between Child A and Child B.
P** = comparison between Child B and Child C.
P*** = comparison between Child C and Child A.

| Angiopoietin-2 ug/L | | Group HCC+ LC (n=80) | P- Value | |
|---|---|---|---|---|
| Child class | A | 472.9- 1997<br>1221.9 ± 573.9 | | 0.01* |
| | B | 787.37 - 1998<br>1611.3 ± 394.56 | 0.011 | 0.96** |
| | C | 682.7 ± 2006.5<br>15881.1 ± 404.98 | | 0.024*** |

P: comparison between the 3 groups.
P* = comparison between Child A and Child B.
P** = comparison between Child B and Child C.
P*** = comparison between Child C and Child A.

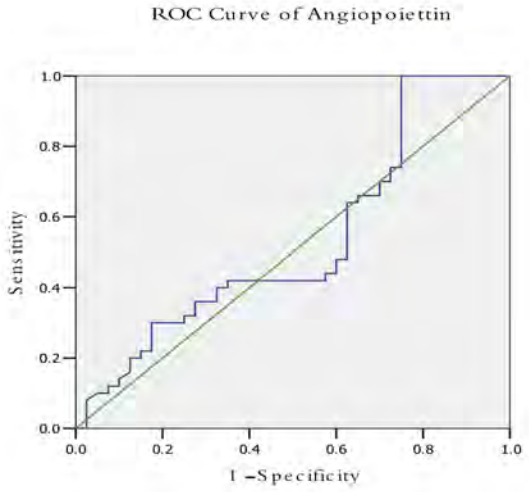


**Fig. (2):** Sensitivity, specificity and accuracy of Angiopoietin.

| Studied variables | Sensitivity | Specificity | AUC | Specificity | Accuracy | P- value | 95 % C.I. |
|---|---|---|---|---|---|---|---|
| Angiopoietin (Cut-off level 1105.7 µg /L) | 70 % | 30 % | 0.53 | 30 % | 53.4 % | > 0.05 | 0.41 – 0.66 |

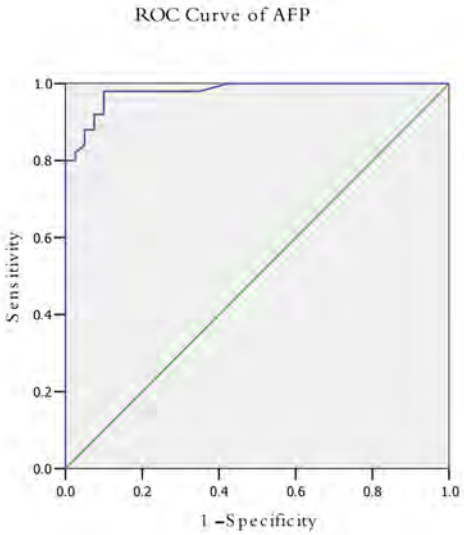


**Fig. (3):** Sensitivity, specificity and accuracy of AFP

| Studied variables | Sensitivity | Specificity | AUC | Accuracy | P- value | 95 % C.I. |
|---|---|---|---|---|---|---|
| AFP (Cut-off level 10 ) | 90 % | 92.5 % | 0.98 | 98.6 % | < 0.01** | 0.96 – 1 |

**Table (VI):** AFP and Angiopoietin in HCC group versus non-HCC groups (Cirrhosis and control groups)

| Both AFP and Angiopoietin as a screening tool | Clinical diagnosis | | Total |
|---|---|---|---|
| | HCC | Non HCC | |
| Positive | 32 | 3 | 35 |
| Negative | 18 | 37 | 55 |
| Total | 50 | 40 | 90 |

Sensitivity of both AFP and Angiopoietin = 64 %.
Specificity of both AFP and Angiopoietin = 92.5 %.
Positive predictive value of AFP and Angiopoietin = 91.4 %.
Negative predictive value of AFP and Angiopoietin = 67.3 %
Accuracy of AFP and Angiopoietin = 76.7 %.

## Discussion

Because of the alarming recent reports from Egypt pointing to a rising prevalence of HCC [14], early identification of liver cirrhosis as well as early HCC is needed to reduce mortality.

In the present work, we studied the role of serum Ang-2 in a group of 50 HCV-related HCC patients, 30 HCV-related cirrhosis patients in addition to 30 healthy control persons. The serum levels of Ang-2 in the studied series of HCC were significantly higher than those of healthy control conforming to the results of Scholz et al., [12] but the mean levels of Ang-2 detected in HCC were lower than those detected among patients with liver cirrhosis in contradiction to the results of the same group.

In the study by Scholz, [12] Ang-2 was expressed by the majority of tumors cells but in accordance with our results, the serum levels did not correlate with either tumor grade or stage. In our series, there was no correlation with either tumor diameter, multiplicity of nodules, or even the presence of adverse tumor characteristics such as portal vein thrombosis or porta--hepatis lymphadenopathy. All these parameters were not studied by Scholz et al. [12] Contrary to our results, Zhang et al., [2] found that Ang-2/Ang-1 ratio, Ang-2 and VEGF were all associated with clinicopathological characteristics such as tumor size, tumor capsule invasion, portal vein invasion and metastasis indicating that VEGF and angiopoietin/Tie2 system contribute greatly to the progress of HCC. These findings are perplexing because HCC diagnosis largely depends on its hypervacularity. Besides, many anti-angiogenic drugs such sorafinib and sutinib have shown success in the treatment of HCC. However, this could be explained by the findings of Zeng et al. [15] They reported that HCC in non-cirrhotic and cirrhotic livers expressed VEGF and its receptors to a similar extent as normal liver, and concluded that the angiogenesis in HCC is not dependent mainly on the vessel sprouting mediated by VEGF/Ang-2 but rather that the liver sinusoids represent a vascular bed that facilitates pathology related demands by vascu-

lar remodeling. Possibly, epidermal growth factor, family members including platelet derived growth factor influence tumor neovascularization or fibroblast growth factor plays a role in vascular remodeling.

Alpha fetoprotein has been the most accepted and largely studied tumor marker for HCC but around one third of HCC have low levels of AFP. In this series there was no difference in serum Ang-2 levels in patients with high AFP compared to those with low AFP in agreement with results of Scholz et al.[12] Serum angiopoietin levels among HCC cases did not show an overlap with healthy control. Adding serum angiopoietin to measurement of AFP did not yield an increase in the value of AFP in diagnosis of HCC.

In this study, Ang-2 serum levels were significantly increased in patients with cirrhosis compared to patients of HCC and healthy control groups, in agreement with Scholz et al.[12] Regenerative tissue in the cirrhotic liver participates less from portal perfusion than normal liver tissue. Arterialization of liver is the result, necessitating the formation of new arterial vessels. New vascular structures associated with inflammatory infiltrates in periportal areas develop in CHC. [16] The occurrence of hepatic angiogenesis may play a pathogenic role in the development of liver damage. Angiogenesis is a multifactorial, extremely complex process involving a huge number of molecules and cells regulated at different levels by various factors. It may be hypothesized that the identification of surrogate markers in the serum that provide information about the extent of angiogenesis may represent a useful tool for disease characterization and follow-up. [17]

We also found inverse relationship between serum angiopoietin and serum albumin whereas serum Ang-2 level correlated positively with serum bilirubin. Serum angiopoietin also correlated positively with ascites which is an important clinical manifestation of portal hyprtension and the most important pathophysiologic mechanism of which is neovascularization and development of collater-

als. We also found that serum angiopoietin 2 was significantly higher among Child B and C cases compared to Child A. One can extrapolate that serum angiopoietin 2 is related to advancing fibrosis. Therefore, in view of the lack of correlation between serum angiopoietin 2 and various tumor characteristic in HCC cases and the overlap between its serum levels among HCC patients and liver cirrhosis patients, it is conceivable that angiopoietin is stimulated and/or regulated by a process, unrelated to malignant growth, be it inflammation, fibrosis-associated hypoxia or nonspecific cell damage.

In conclusion, serum Angiopoietin-2 is a good marker of increasing severity of liver cirrhosis but does not represent an additive tool to diagnose HCC among patients with HCV-related liver cirrhosis. However, further studies are required in the future, with larger cohorts of patients, in order to confirm the potentially useful role of angiopoietin-2 as a marker of prognosis and progression of liver cirrhosis.

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# BENHA MEDICAL JOURNAL


## STUDY OF SERUM ANGIOPOIETIN-2 IN EGYPTIAN PATIENTS WITH LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA


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# PRIMARY BIPOLAR HIP ARTHROPLASTY FOR UNSTABLE INTERTROCHANTERIC FRACTURES IN THE ELDERLY

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## Abstract

*The purpose of this study is to evaluate the functional and clinical outcomes of cemented bipolar arthroplasty as a primary treatment for unstable intertrochanteric fracture in the elderly patient*

***Methods:*** *Twenty hips with unstable intertrochanteric fractures were followed for more than 2 years after cemented bipolar hemiarthroplasty. The mean age was 69 years and the mean follow-up period was 30.5 months. We evaluated the results by Harris hip score, walking ability, activity of daily living, complications, and radiologic findings. Results: At the last follow-up, the mean Harris hip score was 83.3 points. There was one case of hip pain. 15 (75%) out of 20 patients regained their pre-operative walking ability. Radiologically, there were no case of osteolysis.. All stems were stable without significant changes in alignment or progressive subsidence.*

***Conclusions:*** *We consider bipolar hemiarthroplasty with calcar reconstruction is a good option for unstable intertrochanteric fractures in elderly patients with severe osteoporosis in view of the stable fixation, early ambulation and the low rate of complications and reoperations.*

***Keywords:*** *Old age, Intertrochanteric fracture, Cemented bipolar hemiarthroplasty.*

## Introduction

Intertrochanteric fractures are common in the elderly populations, and usually occur as a result of pre-existing osteoporosis and minor trauma [1].

Internally fixed unstable trochanteric fractures might be difficult to retain in position during heal-

ing. Secondary displacement might lead to malunion and poor functional result [2].

These complications divert some authors 3,4,5 to use hip replacement as a primary treatment of such fractures. Our study was made with the purpose of presenting our results with bipolar hip arthroplasty as a primary treatment for unstable intertrochanteric fractures in elderly patients.

## Patients and Methods

In a prospective study, between sepetmber 2006 and October 2008, 20 patients having unstable intertrochanteric fractures (Muller type A2.2 and A2.3) 6 were treated with cemented bipolar hip replacement. There were 12 female and 8 males with an average age of 69 years (range 65- 87 years).

Exclusion criteria included patients unable to walk before the fracture, patients less than 65 years old, patients with pathological fractures, patients with a previous contralateral hip fracture, and patients with stable fractures and intact lesser trochanters.

**Surgical technique:**

All patients were operated under spinal or general anesthesia on average 5 days (mean 2-8 days) of trauma. Hip replacement was performed through a posterior approach. After splitting and retraction of the fibers of the gluteus maximus and the gluteus medius, the short external rotators muscles of the hip were divided close to their insertion and an inverted T shaped incision was made on the joint capsule. Dislocation of the hip joint was done followed by osteotomy of the neck femur.

The fragments of the greater trochanter were reduced and fixed temporarily by using bone forceps before the fragments of the greater trochanter was fixed with tension band stainless steel wire. Isolated displaced fragments of the lesser trochanter were reduced and fixed with stainless steel wire. The wire is placed through a drill hole in the lesser trochanter and the cable tightened, but not crimped. Final tightening is performed when the implant is cemented in place since the osteoporotic proximal bone may collapse (fig. 1).

Preparation of the femoral canal and insertion of the cemented bipolar hip prosthesis was done. Range of motion and stability were checked after reduction. The capsule was repaired and the short external rotators were reattached. The wound was closed over a closed suction drain.

**Postoperative follow up:**

Vacuum drain was removed after 48 hours. All patients were instructed to avoid excessive hip flexion and adduction. Plain X-ray was done to check the hip joint. Weight bearing and gait with aid of a walker was allowed two weeks postoperatively. Blood picture was assessed during the first 3 postoperative days and blood transfusions were given whenever required.

Patients were evaluated according to the Harris hip score 7 and followed up monthly for the first 6 months and then yearly. Postoperative hip pain was analyzed clinically. The level of pain in the groin and thigh after surgery was divided into 3 grades; mild, moderate, and severe. The changes in walking ability from before to after surgery were investigated. The activities of daily living 8 were graded into 4 categories; excellent (no pain or limitation of movement), good (slight pain or limitation of movement not interfering with walking and personal hygiene), fair (moderate pain or limitation of movement requiring assistance in walking), and poor (severe pain, limitation of movement or poor general condition that made walking impossible).

Radiologically, The changes in the alignment and subsidence of the femoral components were measured from after surgery to the last follow-up; ≥ 3° of valgus or varus and ≥ 5 mm longitudinal change were considered significant 8, 9. Loosening of the femoral stem was defined as the appearance of a radiolucent line progressive or > 1 mm in all zones, or the presence of continuous subsidence or migration of the femoral stem.

## Results

Twenty patients with unstable intertrochanteric fractures were followed an average follow up time 24 months (range, 13-40 months).

The mean Harris hip score at the last follow-up was 83.5 ± 4.2. excellent in 5 (25%), good in 12 (60%), fair in 3 (15%). Mild groin pain were noted in one patient (5%). 15 (75%) out of 20 patients regained their preoperative walking ability. one patient could not walk due to a poor general condition after surgery. 19 (95%) could carry out their daily living activities and personal hygiene after surgery; 6 (30%), 10 (50%), 3 (15%), and 1 (5%) patients had excellent, good, fair and poor results, respectively.

The mean hospital stay was 16 days. The average operative time was 140 minutes (range 110-170 minutes). Average intraoperative blood loss was 160 ml (range, 110-180 ml). An average of one unit of blood transfusion was required within the first week.

There were no infections and no dislocation, sciatic nerve paralysis, deep venous thrombosis.

Radiological examination showed no loosening of the prosthesis. All trochanteric fractures had united. With regard to the changes in the alignment of the implant, there was no changes in alignment. There was 3mm subsidence of the femoral stem in one patient.

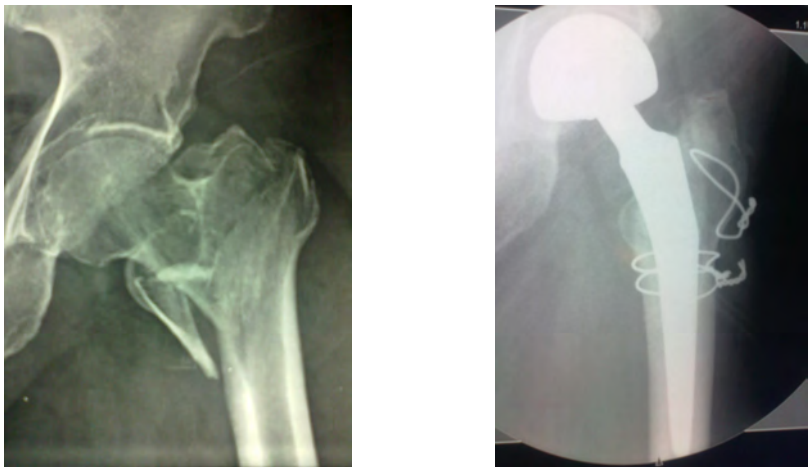

**Fig. 1 :** X-ray r hip joint anteroposterior view in (a) an 67 year old man with unstable intertrochanteric fracture (b) treated with bipolar hip arthroplasty.

## Discussion

Intertrochanteric hip fractures account for approximately half of the hip fractures in the elderly; out of this more than 50% fractures are unstable. Unstable pattern occur more commonly with increased age and with low bone mineral density[10].

Unstable intertrochanteric fractures in elderly patients are associated with high rates of morbidity and mortality. In the elderly, fracture instability, comminution and osteoporosis worsens the prognosis[11,12]. Internal fixation in these cases usually involves prolonged bed rest or limited ambulation, to prevent implant failure secondary to osteoporosis, leading to a high rate of general complications due to prolonged recovery time taken after surgery [13]. Reported overall failure rate with internal fixation in intertrochanteric fractures has been reported to be 3-10.5% [14,15,16]. Thus fracture stability, bone strength, and early rehabilitation determined the final results in cases of intertrochantric fractures.

To avoid the complications related to restricted weight bearing and prolonged bed rest as well as high failure rate with internal fixation of such pattern of fracture in osteoporotic bone, many authors[17,18] have advocated the use of prosthetic arthroplasty as primamary treatment of these fractures. Our results proved the same advantages: all cases began partial weight bearing within two week postoperatively. Haentjens et al.[18] in his retrospective study compared Primary bipolar arthroplasty with internal fixation in patients with unstable intertrochanteric fractures The results were comparable among groups with a significant reduction in the incidence of pneumonia and pressure sores in those undergoing prosthetic rep1acement. This was thought to be due mainly to rapid mobilization of the prosthetic group as compared with those fixed internally.

Some surgeons may worry about the probable increase of the blood loss induced by hemiarthroplasty. Parker 19 in his study found that patients with arthroplasty required more blood transfusions than group with internal

fixation due to per-operative blood loss. The retrospective study reported by Broos et al. [20] indicated that operative time, blood loss, and mortality rates were comparable between the two groups. Likewise, in the randomized trial described by Stappaerts et al.[21], operative time and blood loss were comparable between the two groups. According to our results, blood loss will not increase if hemostasis cautiously and thoroughly during the operation. The average blood transfusion in this series was 400 ml, which was not the real amount of blood loss. The real amount of blood loss was less than 400 ml. The purpose of blood transfusion is to improve the general status and enhance the immunity of the patients.

Grimsrud et al.[22] studied 39 patients of unstable intertrochanteric fractures treated with a cemented bipolar hip arthroplasty. They concluded that these fractures can be treated with a standard femoral stem and cerclage cabling of the trochanters. The technique allows safe and early weight bearing on the injured hip and had a relatively low rate of complications. In this series to treat this complex fracture, cemented bipolar hip arthroplasty was chosen instead of open reduction and internal fixation to obviate the danger of non union, delayed union, malunion and the cutting out of the implant through the femoral head and neck in these unstable fractures. Those in favor of internal fixation reported decreased operative time, blood loss, and mortality rates [23]. However, when converting failed fixation of a hip fracture to an arthroplasty, difficulties with dislocation and intraoperative fracture have been reported [24]. The internal fixation device which has failed, often has broken screws which must be removed, also the ununited head and neck fragments may be in a deformed position requiring mobilization before being excised. This requires additional dissection placing nearby neurovascular structures and muscles at risk and leading to increased blood loss [24].

The question as to whether prosthetic replacement or internal fixation should be performed in this population of elderly osteop-

orotic patients will probably remain controversial. One set of problems associated with arthroplasty is limb length discrepancy, dislocation. On the other side problems with internal fixation include implant failure, hardware cutting out. However limb length discrepancy problem has been overcome in this study by using the modularity of the bipolar prosthesis that allow for greater flexibility in "customizing" prosthetic sizing so that soft tissue tension and limb length equalization can be improved by ability to use variable neck lengths intra-operatively. Being this fracture is extracapsular type, Purse string type closure the entire capsule may enhance hip stability. Also, the large diameter of the head and self-centering cup that was used in the bipolar arthroplasty might explain the decreased tendency to dislocate [25].

Two options exist when treating an unstable intertrochanteric hip fracture with hemiarthroplasty. The deficient proximal medial femur can be augmented with a calcar replacing prosthesis, or the calcar can be reconstructed. Several investigators[26,27] have reported good to excellent functional results with use of a calcar replacing femoral component. However calcar replacing stems are not readily available in our hospital, so, our protocol was to reconstruct the calcar with cerclage stainless steel wire.

## Conclusions

We consider bipolar hemiarthroplasty with calcar reconstruction is a good option for unstable hip fractures in elderly patients with severe osteoporosis in view of the stable and rigid fixation, early ambulation and the low rate of complications and reoperations.

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## PRIMARY BIPOLAR HIP ARTHROPLASTY FOR UNSTABLE INTERTROCHANTERIC FRACTURES IN THE ELDERLY


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# STUDY OF FIRST TRIMESTER 3-D PLACENTAL VOLUME, 3-D PLACENTAL POWER DOPPLER FLOW INDICES AND SERUM PREGNANCY-ASSOCIATED PLASMA PROTEIN-A (PAPP-A) LEVELS AND THEIR CORRELATION WITH SECOND TRIMESTER UTERINE ARTERY DOPPLER FLOW VELOSIMETRY

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## Abstract

***Objectives:*** *to investigate whether the first trimester 3-D placental volume, 3-D placental flow indices and pregnancy-associated plasma protein-A correlate with each other and with second trimester uterine artery Doppler velocities.*

***Methods:*** *Two hundred and fourteen pregnant with a singleton pregnancy were enrolled in this study. At 11 to 14 weeks, transabdominal 3-D scan to measure the placental volume and placental power Doppler flow indices [vascularization index (VI), flow index (FI), and vascularization flow index (VFI)]. Placental quotient (PQ) was calculated and maternal serum pregnancy-associated plasma protein-A levels was measured at the same time. At 18–22 weeks Uterine artery Doppler flow velocity was evaluated for the presence of protodiastolic notch and pulsatility index was measured.*

***Results:*** *Placental volume and PQ had a significant positive correlation with maternal serum pregnancy-associated plasma protein-A and each three of the vascular indices (VI, FI, and VFI). Similarly, maternal serum pregnancy-associated plasma protein-A had a significant positive correlation with each three of the vascular indices (VI, FI, and VFI). While second trimester uterine artery pulsatility index was inversely correlated with placental volume, PQ, maternal serum pregnancy-associated plasma protein-A and each three of the vascular indices (VI,*

*FI, and VFI). The stepwise logistic regression analyses indicated that there was a significant influence of first trimester PQ, power Doppler VFI and maternal serum pregnancy-associated plasma protein-A on second trimester high uterine artery PI and persistence diastolic notch. First trimester small PQ, lower power Doppler VFI and lower plasma levels of maternal serum pregnancy-associated plasma protein-A indicate a higher probability of second trimester high uterine artery pulsatility index and persistence diastolic notch.*

***Conclusions:*** *First trimester 3-D placental volumetry, 3-D placental flow indices and maternal serum pregnancy-associated plasma could be important and efficient method for early and simple identification of impaired trophoblast invasion. Thus, they may be useful for the later clinical applications and in screening for high-risk pregnancies*

***Keywords:*** *3-D Placental volume; 3-D placental power Doppler flow indices; pregnancy-associated plasma protein-A; uterine artery Doppler flow velocity.*

## Introduction

Early identification of pregnancies at risk to develop severe complications such as pre-eclampsia (PE) and Intrauterine growth restriction (IUGR) is one of the main objectives of perinatal medicine [1]. Although the underlying mechanism of these complications is still poorly understood there are increasing evidences of a relationship between impaired placentation and the subsequent development of PE and IUGR [2]. The prevention of PE and IUGR remains a considerable challenge in obstetrics [3]. Although the symptoms of PE and IUGR generally manifest in the second to third trimester of pregnancy, their underlying pathology takes place in the first trimester [4]. One possible reason why preventive strategies have proven very disappointing at present is that the proposed interventions have commenced in the mid to late second trimester, when the underlying placental dysfunction may already be established. So, earlier assessment before the establishment of placental dysfunction may have the potential to improve predictive value for clinical practice [3].

Shallow invasive activity of the

extravillous trophoblast may result in small placental volume secondary either to a reduction in oxygen tension in the intervillous space or to the activation of inhibitors of trophoblast differentiation and proliferation[5]. Defective trophoblast invasion was shown to occur in the first trimester of pregnancy[6]; however, its consequences could be identified and diagnosed only in the second trimester by measuring the uterine artery Doppler perfusion. This usually delays the start of treatment and, therefore, the benefits of each therapy become questionable even if they are justified [7]. These limitations led to the idea that placental volumetry and vascularization might be useful as first and principal parameters for early and simple identification of women at risk [8]. Decreased placental volume and Placental flow indices at 11-14 weeks of gestation have been implicated in the subsequent development of PE and IUGR [9,10]. A possible method to detect impaired trophoblast invasion in the first trimester, might be three-dimensional (3D) ultrasonographic measurement of placental volume (PV) and vascularization [11]. High impedance of the uterine arterial blood flow and the persistence of an early diastolic notch in the flow velocity waveform give an indication of an insufficient physiological conversion of the spiral arteries. The best time to perform uterine artery Doppler scans is between 22-24 weeks, although this is relatively late for the start of an efficient prophylaxis [12]. pregnancy-associated plasma protein-A (PAPP-A) is an insulin-like growth factor binding protein (IGFBP) protease with specificity for IGFBP 2 and 4. Reduced levels of PAPP-A may result in increased amounts of insulin-like growth factor (IGF) being bound to its carrier proteins and hence not available at the cell receptor level to stimulate fetal growth and trophoblast invasion of the decidua [13]. Many studies have reported reduced maternal serum PAPP-A concentration at 11-14 weeks and increased risk for subsequent development of PE, IUGR and preterm delivery[8,14]. The study was carried out to investigate whether the first trimester 3D placental volume, 3-D placental flow indices and PAPP-A correlate with each other and with

second trimester uterine artery Doppler velocities.

## Patients and Methods

Two hundred and fourteen pregnant women with viable singleton pregnancies in the first trimester, between 11-14 weeks of gestation were recruited during the period from March 2008 to January 2011. All patients had no obstetric or gynecologic complications in their past medical history. Inclusion criteria were: singleton pregnancies, absence of pre-existing maternal diseases such as chronic hypertension, diabetes, collagen vascular diseases absence of fetal anomalies, and whose fetal crown-rump length (CRL) was 45-84 mm. The study was approved by the local ethical committee and informed consent was obtained from all women. An ultrasound scan was performed to measure CRL, nuchal translucency and PV by using a Voluson 730 Pro scanner (GE Medical Systems, Milwaukee, WI, USA) with a 3.5 MHz 4D transducer. For assessment of PV, three volumes were measured and recorded for offline analysis using Virtual Organ Computer-aided AnaLysis (VOCAL), and the mean PV was calculated by averaging the three volume values obtained. Placental 3-D power Doppler vascular indices [vascularization index (VI), flow index (FI), and vascularization FI (VFI)] were also calculated [15,16].

As PV increases with advancing CRL, in order to compare placental size across different CRL values, a PV/CRL quotient (PQ) was calculated as previously reported[16]. All measurements were recorded by one of two persons. At 22 weeks of gestation, a Doppler assessment for uterine arteries was done. Both right and left uterine arterial blood flows were examined using color Doppler imaging. The Doppler gate was placed at the proximal uterine arteries according to the method described by Bower et al. [17]. Doppler flow velocity waveforms were recorded in five subsequent cycles and the pulsatility index (PI) was measured. The average of the measurements from the left and right uterine arteries was used for the analysis and presence of notch in either of the uterine arteries was measured.

Maternal blood samples were taken for measurement of PAPP-A using a Kryptor analyzer (Brahms Diagnostica GmbH, Berlin, Germany) for biochemical assays, and the results were expressed as multiples of the median (MoM) values using Astraia software (Astraia Software GmbH, Munich, Germany).

**Statistical analysis :**

Statistical analysis was performed by using Statistics Package for Social Sciences (SPSS Inc., Chicago, IL). The data were reported as mean±SD. Correlation analysis was performed using Pearson correlation test. Stepwise linear regression analysis was used to find out if any correlation exists between PQ and other parameters. Also, stepwise logistic regression analyses were calculated to ascertain whether PV or PQ as well as placental power Doppler flow indices, PAPP-A, age, gravidity or BMI have any influence on uterine artery PI and on the occurrence of unilateral or bilateral notching in second trimester. A P-value < 0.05 was considered to be statistically significant.

## Results

Two hundred and fourteen pregnant women were recruited. Eight women were excluded from the analysis for the following reasons: 5 women were lost to follow up at 22 weeks for uterine artery Doppler and 3 had a second-trimester spontaneous miscarriage. Thus, 206 pregnancies were available for the final analysis. Demographic features, ultrasonographic measurements of fetal and placental parameters, uterine artery Doppler studies and PAPP-A measurements are listed in Table 1.

PV and PQ had significant positive correlation with PAPP-A and each three of the vascular indices (VI, FI, and VFI). While PV and PQ were inversely correlated with second trimester uterine artery PI (Table 2).

PAPP-A had significant positive correlation with CRL, PV, PQ and each three of the vascular indices (VI, FI, and VFI). While second trimester uterine artery PI was inversely correlated with CRL, PV, PQ, PAPP-A and each three of the

vascular indices (VI, FI, and VFI). (Table 3).

In a stepwise logistic regression model PQ and all other parameters (demographic parameters, uterine artery Doppler parameters, PAPPA, VI, FI, and VFI) were compared. PAPP-A and VFI values were affecting PQ ($P<0.001$, positive estimator). None of demographic variables show a significant influence on either PV or PQ in the stepwise regression analyses (Table 4).

The stepwise logistic regression which takes the dichotome PI ('high' and 'normal' resistance i.e. <95 centile versus >95 centile) as a regressor variable demonstrates that it depends on PQ, placental power Doppler indices and PAPP-A ($P<0.0001$, negative estimator) regardless of whether the PI is high on the left, on the right or on both sides. The stepwise logistic regression analyses indicate that there is a significant influence of PQ, power Doppler indices (VI, FI, VFI) and PAPP-A on the occurrence of bilateral notching. Gravidity, placental power Doppler indices (VI, FI, and VFI), PQ and PAPP-A have negative estimators. This suggests that the probability of bilateral notching is higher in nulliparous women and decreases with increasing gravidities. A small PQ, lower placental power Doppler indices and lower plasma levels of PAPP-A indicate a higher probability of bilateral notching. The occurrence of a notch, bilateral or unilateral, depends significantly on the PQ, PAPP-A and placental power Doppler indices, as well as on age and gravidity.

Significant inverse correlations between second trimester uterine artery PI and first trimester PV and PQ are shown in Fig. 1.

A significant positive correlation between PAPP-A and placental power Doppler indices (VI and VFI) is shown in Fig. 2.

**Table 1:** Characteristics or values related to the mother and to the placenta.

| Variable | n = 206 |
|---|---|
| Age (years) | 29.7±3.7 |
| BMI | 26.5±4.9 |
| Parity, n(%) | 1.9±0.93 |
| CRL (mm) | 67.4±9.7 |
| Placental volume | 66.8±16.5 |
| Vascularization index | 4.2±3.5 |
| Flow index | 41.3±6.9 |
| Vascularization flow index | 1.97±1.69 |
| Uterine artery pusatility index | 1.18±0.74 |
| PAPP-A (IU/mL) | 5.48±3.46 |
| PAPP-A (MoM) | 1.3±0.53 |
| placental quotient (PV/CRL) | 0.96±0.27 |

Values are given as mean±SD; PAPP-A: pregnancy-associated plasma protein-A; MoM: multiple of median; CRL: crown-rump length; VI: vascularization index; VFI: vascularization flow index; PI: pulsatility index

**Table 2:** Correlation of placental volume and placental quotient with other factors.

| Variable | Placental volume | | Placental quotient | |
|---|---|---|---|---|
| | r * | P** | r | P** |
| Placental volume | --- | --- | 0.93 | <0.001 |
| Placental quotient | 0.93 | <0.001 | --- | --- |
| VI | 0.66 | <0.001 | 0.64 | <0.001 |
| Flow index | 0.81 | <0.001 | 0.76 | <0.001 |
| VFI | 0.62 | <0.001 | 0.59 | 0.001 |
| PAPP-A (MoM) | 0.85 | <0.001 | 0.72 | <0.001 |
| Uterine artery PI | -0.72 | <0.001 | -0.71 | <0.001 |

*r = Pearson correlation; **Correlation is significant at the 0.01 level (2-tailed).
PAPP-A: pregnancy-associated plasma protein-A; MoM: multiple of median; CRL: crown-rump length; VI: vascularization index; VFI: vascularization flow index; PI: pulsatility index

**Table 3:** Correlation of PAPP-A (MoM) and second trimester second trimester uterine artery PI with other factors.

| Variable | PAPP-A (MoM) | | Uterine artery PI | |
|---|---|---|---|---|
| | r º | P | r | P |
| Placental volume | 0.85 | <0.001* | -0.72 | <0.001* |
| Placental quotient | 0.72 | <0.001* | -0.71 | <0.001* |
| CRL (mm) | 0.83 | <0.001* | -0.82 | <0.001* |
| VI | 0.76 | <0.001* | -0.42 | 0.031** |
| Flow index | 0.87 | <0.001* | -0.62 | 0.001* |
| VFI | 0.67 | <0.001* | -0.57 | 0.002* |
| PAPP-A (MoM) | ---- | ---- | -0.64 | <0.001* |
| Uterine artery PI | -0.64 | <0.001* | ---- | ---- |

r º = Pearson correlation; *Correlation is significant at the 0.01 level (2-tailed).
**Correlation is significant at the 0.05 level (2-tailed).
PAPP-A: pregnancy-associated plasma protein-A; MoM: multiple of median; CRL: crown-rump length; VI: vascularization index; VFI: vascularization flow index; PI: pulsatility index

**Table 4:** Stepwise linear regression model: demographic parameters, VI, FI, VFI, PAPP-A (MoM), uterine artery PI and notch values compared with PQ.

| Model | R square | Adjusted R square | F change | P |
|---|---|---|---|---|
| PAPP-A (MoM) | 0.723 | 0.720 | 346.3 | <0.001 |
| PAPP-A, PI, notch value | 0.771 | 0.768 | 222.6 | <0.001 |
| PAPP-A, PI, notch value, VFI | 0.788 | 0.783 | 162.5 | <0.001 |

PQ: Placental quotient; PAPP-A: pregnancy-associated plasma protein-A; MoM: multiple of median; VFI: vascularization flow index; PI: pulsatility index

**Table 5:** Stepwise linear regression model: demographic parameters, PQ, VI, FI, VFI and PAPP-A (MoM) compared with uterine artery PI.

| Model | R square | Adjusted R square | F change | P |
|---|---|---|---|---|
| PQ | 0.670 | 0.668 | 270.2 | <0.001 |
| PQ, PAPP-A | 0.697 | 0.692 | 151.8 | <0.001 |
| PQ, PAPP-A, VFI | 0.724 | 0.718 | 114.6 | <0.001 |

PQ: Placental quotient; PAPP-A: pregnancy-associated plasma protein-A; MoM: multiple of median; VFI: vascularization flow index; PI: pulsatility index

**Fig. 1:** correlation between second trimester uterine artery pulsatility index and first trimester both placental volume and placental quotient.

r = -0.72; P = <0.001 (for placental volume)

r = -0.71; P = <0.001 (for placental quotient)

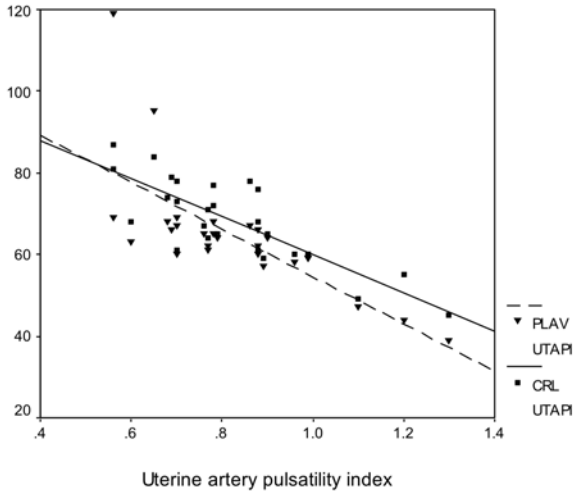
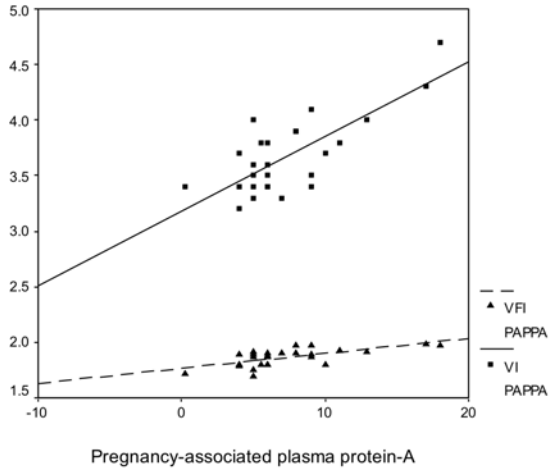



**Fig. 2:** correlation between first trimester pregnancy-associated plasma protein-A (MoM) and placental power Doppler indices (VI and VFI).

r = 0.76; P = <0.001 (for vascularization index)

r = 0.67; P = <0.001 (for vascularization flow index)



## Discussion

The potential advantage of first-trimester screening and selection of high-risk pregnancies is the provision of early intervention [18]. As the invasive activity of the extravillous trophoblastic cells is at a maximum during the first trimester of gestation, peaking at around 10-12 weeks and declining thereafter, treatment started in the first trimester may be more effective than that given in the second trimester [5]. Shallow invasive activity of the extravillous trophoblast may result in small placental volume secondary either to a reduction in oxygen tension in the intervillous space or to the activation of inhibitors of trophoblast differentiation and proliferation [6,8].

Results of the present study showed that of the present study showed that PAPP-A had a significant positive correlation with PV, PQ and placental power Doppler vascular indices (VI, FI, and VFI). These results agreed with those previously reported [8,10].

Decreased first trimester PAPP-A concentrations are associated with altered 3D placental Doppler indices[19], IUGR, and adverse pregnancy outcomes, such as preterm labor, and preeclampsia [13,20]. The value of PAPP-A in prediction of preeclampsia and IUGR is reviewed by Pilalis et al.[21]. A high PAPP-A level was associated with lower IGF binding proteins and higher free IGF's [13], which are important regulators promoting fetal and placental growth at the very beginning of pregnancy [22].

Current results showed that vascular indices of the placenta (VI, FI, and VFI) increase with increasing PV. Higher PAPP-A levels was associated with the higher VI, FI, and VFI values. So, PAPP-A might be related with the vascular and volumetric growth of the placenta. These findings are consistent with those of Rizzo et al. who investigated placental vascular indices among pregnancies with low serum PAPP-A. They observed that, altered vascular indices were associated with IUGR and abnormal umbilical artery Doppler findings[19]. Very low PAPP-A levels were also associated with altered placental vascularization indices

and reduced number of villous capillary vessels [23].

Impaired uteroplacental blood flow, as a result of this altered vascularization process was considered to have a major role in pathophysiology of IUGR[4]. This events usually start early in placental vascularization, later on affecting placental structure and finally the fetus [3,5].

Results of the present study showed that placentae of women with pathologic uterine perfusion in the second trimester of pregnancy are already remarkably small at the end of the first trimester. Uterine artery protodiastolic notch (either unilateral or bilateral) as well as increased PI is associated with a significant first trimester reduction in PAPP-A, placental size and 3-D power Doppler placental flow indices. Similar results have been previously reported (8,22). Placentation in the first trimester of pregnancy may therefore play an important role in determining the risk of subsequent late pregnancy complications such as PE and IUGR through inadequate trophoblastic invasion of the maternal spiral arteries [1] or the smaller trophoblastic mass that may cause a decrease in production of vasoactive substances or antioxidative agents leading to endothelial dysfunction [24].

Previous studies reported that persistence of uterine artery protodiastolic notch in second trimester is valuable in detecting pregnancies at high-risk, although the second trimester is too late for patients to benefit from treatment[5,7]. Placental power Doppler studies measuring VI, FI, and VFI may reflect abnormal vascularization pattern and thus may predict high-risk pregnancies (such as IUGR) at an early stage, especially when interventions might still be effective.

First trimester PV and uterine artery PI measurements were found to be valuable parameters for predicting preeclampsia [1], and adverse pregnancy outcomes, such as fetal growth restriction, pregnancy induced hypertension and abruption (9). Hafner et al. compared first trimester PQ and second trimester uterine artery

Doppler PI values and found similar predictive values of each parameter for prediction of preeclampsia [7]. However, Low et al. observed that first trimester PV and second trimester PV and uterine artery Doppler were independent markers and offer similar predictive values of IUGR [18].

## Conclusions

First trimester evaluation of the placenta by 3D volumetric measurements and 3D power Doppler might improve investigation of early placental vascularization and its association with fetal growth as they could reflect the second trimester uterine perfusion parameters. These results could be of great importance for the predictive and diagnostic evaluation of fetal growth restriction presenting with PV and vascular tree alterations as early as first trimester.

**Conflict of interest**

The author has no conflicts of interest.

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REPRINT


# BENHA MEDICAL JOURNAL


## STUDY OF FIRST TRIMESTER 3-D PLACENTAL VOLUME, 3-D PLACENTAL POWER DOPPLER FLOW INDICES AND SERUM PREGNANCY-ASSOCIATED PLASMA PROTEIN-A (PAPP-A) LEVELS AND THEIR CORRELATION WITH SECOND TRIMESTER UTERINE ARTERY DOPPLER FLOW VELOSIMETRY


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# SALIVARY TESTOSTERONE : IS IT A MARKER FOR POLYCYSTIC OVARIAN SYNDROME?

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## Abstract

***Objective:*** *to evaluate the role of salivary testosterone (ST) a reliable hormonal marker for diagnosis of PCO and comparing it with serum free testosterone (SFT).*

***Material and Methods :*** *this prospective study was conducted in the Departments of Obstetrics & Gynecology and Clinical Pathology, Mansoura Faculty of Medicine, Egypt, from April 2009 to October 2010. Thirty five women with clinically diagnosed PCOs as study group and 25 normal women as control group. All cases were assayed for salivary testosterone by (ELISA), serum free testosterone by radioimmunoassay (RIA) and FSH, LH. Transvaginal sonography for all cases to assess ovarian morphology and volume*

***Results:*** *significant positive correlation between salivary testosterone assayed by ELISA and serum free testosterone assayed by RIA. (r value : 0.38, P value: <0.01) by using receiver operating curve ST was found more sensitive than SFT (85% versus 65%) in diagnosing PCOS patients*

***Conclusion:*** *Salivary testosterone (ST) provides a sensitive, simple, reliable, non invasive diagnostic tool for PCOS.*

***Key words :*** *Salivary testosterone (ST) - serum free testosterone (STF) - Polycystic Ovarian Syndrome (PCOs).*

## Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women in reproductive age, affecting 5-10% of women in this age group. The underlying defect in PCOS remains unknown, but there is growing consensus that key features include insulin resistance, androgen

excess, and abnormal gonadotrophin dynamics[1,2].

Current studies suggest that the ovary is the principle site of excess androgen, but some women with PCOS may have an adrenal contribution of the increased androgen production[3].

Testosterone-bound sex hormone binding globulin (SHBG) is considered a biologically inactive measurement, so the measurement of free testosterone is a most important method of assessing hyperandrogenemia[1,2].

Testosterone is synthesized in females in small quantities by the theca interna of the ovaries where it is converted to estradiol. Testosterone in women is carried in blood 2% as free form, 66-78% are bound to sex-hormone binding globulin (SHBG) and 20-30% is bound to albumin[3].

It was found that assessment of serum free testosterone by widely spread radioimmunoassay (RIAs) revealed non accurate results which aroused the search for another more accurate method of assessment of free testosterone. The use of saliva has provided a substantial addition to the diagnostic field as an investigative tool for disease processes and disorders[5,6].

Testosterone is secreted in saliva in the free form without the SHBG and albumin[6,7]. The aim of this work was to evaluate salivary testosterone (ST) level as a simple reliable and sensitive diagnostic tool in diagnosing cases of PCOS and comparing it with serum free testosterone (FT).

## Material & Methods

This prospective study was conducted in the Departments of Obstetrics & Gynecology and Clinical Pathology, Mansoura Faculty of Medicine, Egypt, from April 2009 to October 2010

Thirty Five women with clinically diagnosed PCOS as study group and twenty five normal women with normal reproductive outcome as control group were selected for the study, all aged between 20 and 35 years.

Inclusion criteria were accord-

ing to Rotterdam criteria[9] for PCOS: 1- anovulation, oligoovulation or both 2- clinical or biochmmeical signs of hyperandrogenism or both 3 - polycystic ovaries on ultrasound (US) examination.

We ex-cluded woman having diabetes, hypertension, hyperthyroid-ism, hyperprolactinemia or receiving hormonal medication within 3 months before or having oral diseases or oral inflammations were excluded from the study.

**All participants were subjected to:** Full history taking, laboratory tests including: serum free testosterone (serum FT), salivary testosterone (ST) and serum LH and FSH to confirm the diagno¬sis of PCOS.

Vaginal ultrasonography was also done to determine the mean ovarian volume, patients underwent transvaginal ultrasonography in the early follicular phase (cycle days (2-3) if they were oligomenorrheic or at random if amenorrheic. Each patient underwent examination of the morphology of the ova-ries, ovarian volume, follicle distribution, number and diameter were recorded. Ovarian volume, follicle distribution, number and diameter were recorded. Ovarian volume determination was made using the formula for the volume of an ellipsoid : 0.523 x length x width x thickness. All cases given written informed consent.

**Hormonal assay :**

Seven milliliters of venous blood were withdrawn from each subject and collected in a centrifuge tube on the 2nd or 3rd day of the cycle unless the subject was amenorrheic. The samples were left for 10 min to clot and then centrifuged. The serum was separated and stored at -20C till the time of the assay. Haemolysed and lipemic samples were avoided.

- Determination of serum free testosterone was done by direct competitive radioimmunoassay (RIA) analog tracer. This method utilizes an [I 125]-labelled testosterone analog which has low affinity for SHBG and albumin, hence, no extraction was needed . The kit used was supplied by Diagnostic Systems Laboratories Inc. (445

Medical Center Blvd., Texas 77598 - 4217, USA)[10]. Determination of serum LH and FSH: by an automated chemiluminescent immunometric assay using Immulite 2000 FSH & LH Chemiluminescent kit (supplied by DPC, Biermann GmbH-61231 Bad Nauheim, Germany).

It is a solid-phase, sandwich chemiluminescent assay. The FSH/LH ratio was calculated[11,12].

**Determination of salivary testosterone:** the patients were asked to avoid eating or drinking or brushing their teeth for 30 min before sampling. In addition, they were asked to rinse their mouth thoroughly with cold water 5 min prior to sampling. No samples were collected from patients with oral diseases, inflammations or lesions to avoid blood contamination which gives false results. Three milliliters of saliva were collected in glass tubes by direct spitting into the tubes following salivary stimulation by chewing a piece of parafilm[7]. The samples were stored at -20C till the time of the assay. Salivary testosterone (ST) was assayed by competitive enzyme linked immuno sorbent assay (ELISA)[13]. The kit was supplied by DRG Diagnostics, GmbH, Frauenbergstr. 18, D-35039 Marburg, Germany.

## Results

Table I shows clinical and sonographic parameters among study and control group as regard age, body mass index BMI and ovarian volume .

Table II shows hormonal assay of salivary testosterone ng/ml and serum free testosterone ng/ml, among both groups .

Table III: shows that positive correlation between salivary testosterone assayed by ELISA and serum free assayed by RIA .

Figure(1) : shows ROC curve for sensitivity and specificity for diagnosed of PCO by using salivary T and serum FT, salivary T was more sensitive than serum FT (85% VS 65%) while specificity of serum free testosterone was (95% VS 88% ) for salivary T.

**Table I :** Clinical and sonographic characteristic among study and control group .

| Parameters | Study group | Control group | P value |
|---|---|---|---|
| **Age (years)** | 28.5±3.2 | 27.2±4.1 | 0.14 |
| **BMI kg/m$^2$** | 34.5±4.6 | 23.9±2.8 | <0.05 |
| **Ovarian Volume (mm$^3$)** | 16.48 ±4.32 | 8.01±2.23 | <0.05 |

BMI = body mass index

**Table II:** Hormonal assay among study and control group.

| Parameters | Study group | Control group | P value |
|---|---|---|---|
| **Salivary T ng/ml** | 0.042 ± 0.016 | 0.015 ± 0.006 | <0.05 |
| **Serum FT ng/ml** | 0.97 ± 0.31 | 0.6 ± 0.12 | <0.05 |

**Table III:** Correction coefficient between different parameters among study and control group

| | Salivary ng/ml T | Serum FT ng/ml | Ovarian volume | LH/FSH |
|---|---|---|---|---|
| **r value** | 0.0003 | 0.36 | 0.43 | 0.39 |
| **P value** | <0.01 | <0.01 | <0.01 | <0.01 |

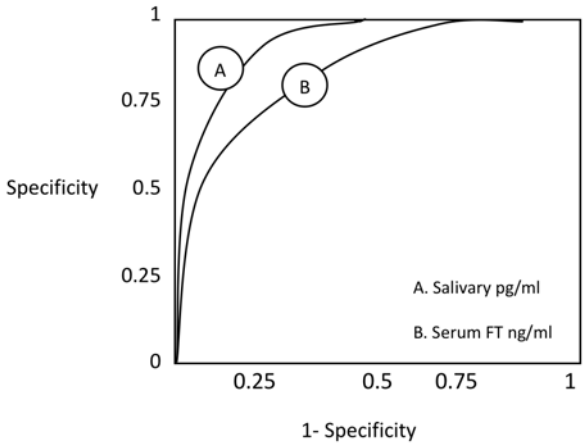


**Figure (1) :** ROC curve receiver (operating curve ) of salivary and serum free testosterone.

## Discussion

Polycystic ovarian syndrome (PCOS) is a widely spread disease among females during their reproductive period. One of the criteria on which the diagnosis of PCOS is built on, assessment of testosterone hormone which is often elevated in such cases[9].

Although PCOS is primarily a clinical diagnosis (given a history of chronic anovulation and androgen excess) with the exclusion of the very uncommon condition of non-classical congenital adrenal hyperplasia, however, elevated free testosterone level represents the most sensitive biochemical marker supporting the diagnosis. A raised LH concentration, although a useful marker of the syndrome, is now less favored as a diagnostic tool. A ratio of LH/FSH equal or greater than 2:1 is certainly consistent with PCOS, as a diagnostic test, however, the LH/FSH ratio is often in the normal range and therefore is an insensitive test[3,4].

The present study was made to evaluate the reliability of salivary testosterone level in diagnosing cases of PCOS, and comparing its reliability with serum free testosterone. The mean salivary testosterone in the normal control group was 0.015 ± 0.006 ng/ml vs. 0.042 ± 0.016 ng/ml in PCOS cases with a high statistically significant ($p<0.005$) in the PCOS cases.

These results agree with the study conducted by Dipankar et al.[15], which shows that salivary testosterone shows higher significance in PCOS cases ($p <0.0001$) than does the serum total testosterone ($p< 0.01$). The same results were obtained by Sharquie et al.[16], Previous studies showed that salivary testosterone gives a useful indication of levels of biologically available androgen in hyperandrogenic women. Salivary testosterone was of greater diagnostic use in hirsutism than any of the currently used serum androgen assays at that time [15,16,17].

The present study showed a significant positive correlation between salivary testosterone, measured by ELISA, and serum free testosterone measured by

RIA (r = 0.38, p =0.01) suggesting that salivary testosterone reflects changes in the levels of available biologically active hormone in the circulation. These findings were similar to those cited in other studies [17,18].

The reliability of salivary testosterone was proved using the receiver operator curve (ROC) which showed that salivary testosterone was found to be more sensitive than serum free testosterone (85% vs. 65%) in diagnosing PCOS based on clinical criteria, while the specificity of serum free testosterone was 95% vs. 88% for salivary testosterone. The salivary testosterone was not only related to the clinical criteria of PCOS but also to its biochemical markers, as strong correlation was found between salivary testosterone and LH/ FSH ratio (r= 0.39, p =0.01), the main biochemical standard in diagnosing PCOS. Serum free testosterone was also found to correlate with LH/FSH ratio (r = 0.36, p =0.01) in PCOS cases which agrees with other studies [17,19].

However, variability in salivary testosterone results between laboratories using different RIAs has been reported [20]. In their study, saliva was assayed for testosterone by isotopic immunoassay methods. Serum was assayed by commercially available RIA kits for free testosterone. Salivary testosterone was assayed in our study using non-isotopic immunoassay (ELISA) which helped to give more reliable results. It seems that determination of salivary testosterone is a reliable method to detect changes in the concentration of available biologically active testosterone in the serum[17].

In conclusion, salivary testosterone (ST) provides a sensitive, simple, reliable, non invasive diagnostic tool for PCOS.

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## SALIVARY TESTOSTERONE : IS IT A MARKER FOR POLYCYSTIC OVARIAN SYNDROME?


**Raafat Abdel Fatah MD and Ahmed Elewa MD**



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# COMPARISON BETWEEN TRANSVAGINAL DOPPLER ULTRASONOGRAPHY AND HYSTEROSCOPY IN PREDICTION OF ENDOMETRIAL HYPERPLASIA

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## Abstract

***Objective:*** *to assess the role of transvaginal Doppler ultrasound (U/S) in prediction of endometrial hyperplasia during perimenopausal period and comparing it with the hysteroscopy and endometrial biopsy.*

***Setting:*** *obstetrics and gynecology department, Menofiya university.*

***Subjects:*** *two hundred patients with perimenopausal bleeding.*

***Interventions:*** *color Doppler ultrasound (U/S) on uterine arteries, hysteroscopy, and D & C biopsy.*

***Main outcome measures:*** *resistance and pulsatility index on the uterine arteries were measured as well as ability of the hysteroscopy to predict endometrial hyperplasia.*

***Results:*** *from the 200 cases with perimenopausal bleeding, 30 cases were diagnosed as endometrial hyperplasia by D & C biopsy (15%). Hysteroscopy was successful in predicting endometrial hyperplasia in 22 cases out of the 30 cases diagnosed by the D & C biopsy (73.3%) with false negative result in 8 cases (26.7%) and false positive result in 2 cases. As regards to Doppler U/S, resistance index predicted hyperplasia in 25 cases (83.3%) with false negative result in 5 cases (16.7%) and false positive result in 3 cases. Pulsatility index diagnosed hyperplasia in 23 cases (76.7%) with false negative result in 7 cases (23.3%) and false positive result in 4 cases. There is no significant difference between Doppler U/S and hysteroscopy in predicting endometrial hyperplasia.*

***Conclusions:*** *this study recommends Doppler U/S as a screening tool in the situation of suspicion of endometrial hyperplasia as it is non-invasive method but the routine D & C biopsy is a mandatory procedure for reaching final proper diagnosis.*

***Key words:*** *Doppler, Hysteroscopy, Endometrial hyperplasia.*

## Introduction

Endometrial hyperplasia is an increased ratio of endometrial glands to stroma greater than one to one. In simple hyperplasia, the ratio is only slightly increased and the glands may be cystically dilated. In complex hyperplasia, the glands are irregular, the ratio of glands to stroma may be three to one or more and there may be back- to- back crowding of glands. These types of hyperplasia are further subdivided into those with typical or atypical cytology [1].

Many methods are used for prediction and diagnosis of endometrial hyperplasia such as hysteroscope, transvaginal ultrasonography (TVUS), transvaginal Doppler ultrasonography on uterine arteries and endometrial biopsy [2].

As TVUS alone is not reliable enough to obviate endometrial biopsy in diagnosis of endometrial pathology, color Doppler measurement of uterine arteries have been suggested to improve the sensitivity of the TVUS by detection of low impedence and high diastolic blood flow in collateral vessels caused by tumour angiogenesis [3].

Hysteroscopy has become the tool of choice for the evaluation of the endometrial cavity, including assessment of abnormal uterine bleeding [4].

This study was performed to compare the ability of the hysteroscopy and the Doppler U/S on uterine arteries in evaluation of the endometrial cavity and prediction of endometrial hyperplasia.

## Material and Methods

This study included two hundred patients with perimenopausal bleeding attending outpatient clinic in Obstetrics and Gynecology department, Menoufiya University Hospital. The study was approved by the ethical committee of Menoufiya University. A signed written consent was obtained from each patient after explaining the whole study for each. Our inclusion criteria were any patient with perimenopausal bleeding without any medical disorder and without any macroscopic pathology like myoma proved by U/S. Patients on hormone therapy were

excluded from the study.

Patients included in this study were subjected to detailed full medical history, general, abdominal, and pelvic examination.

Transvaginal color Doppler U/S on uterine arteries for determination of (resistance index `RI` and pulsatility index `PI`) was done for all patients before performing hysteroscopy and D & C biopsy. It was carried out in Radiology department using 6.5- mega Hertez transvaginal probe (Siemens Co., Model no.: GM- 6601A2E00) to look for the presence or absence of colour coded zones that represent vascularized areas when colour signals are obtained. Blood flow velocity waveform was recorded by placing the sample volume over the colored vessels and activating the pulsed Doppler.

Both uterine arteries are located lateral to the cervix by colour Doppler imaging and the angle of insonation was adjusted to obtain maximum colour intensity. The pulsed Doppler gate was placed over the artery and the resulting waveform obtained from the pulsed Doppler was analysed by these two indices: the first is the resistance index (RI) and the other is the pulsatility index (PI). Both of them were automatically calculated.

Hysteroscopy and dilatation and curettage (D & C) biopsy were done for all patients under general anaesthesia. Hysteroscopy was performed without cervical dilatation, using the Hamou- microhysteroscope II (Karl Storz, GMBH, Tuttlingen, Germany). Hysteroscopic prediction of endometrial hyperplasia was based on the presence of one or more of the following criteria: (focal or diffuse increase in endometrial thickness, irregular aspect of the endometrial surface, button like proliferations or large protruding cyst in uterine cavity, dilated glandular openings of yellowish colour, or presence of large superficial vessels in panoramic view[5].

For prediction of endometrial hyperplasia by the Doppler U/S, the cut off value of the RI used was 0.75 or less and that of the PI was 1.83 or less. These values are chosen according to the study of

Elusseiny [6] about uterine assessment by Doppler in cases of abnormal uterine bleeding.

The data collected were tabulated and analyzed by SPSS statistical package version II on IBM compatible computer. Quantitative data were expressed as mean ± standard deviation (SD). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Qualitative data were expressed as number and percentage and analyzed by applying chi-square test. Pearson correlation (r) was used to measure association between quantitative variables. The test is considered significant when P value is < 0.05.

## Results

In this study two hundred perimenopausal patients were included with age ranged from 40-51 years, with mean age of 45.2±3 years. The parity ranged from 1-10 with mean of 5.4±2.1. The weight ranged from 78-112 kilograms (Kg), with mean of 88.9± 8.6 Kg.

The patients presented either with menorrhagia (n= 60 [30%]), metrorrhagia (n= 65 [32.5%]), menometrorrhagia (n= 75 [37.5%]).

In the 30 cases diagnosed by D & C biopsy, endometrial thickness was more than 17 mm in 14 cases (46.7%) with mean thickness of 19.4 ± 1.4 mm followed by 13 - 17 mm in 9 cases (30%) with mean thickness of 15.9 ± 1.3 mm. Finally, endometrial thickness was 8 - 12 mm in 7 cases (23.3%) with mean thickness of 10.3 ± 2.1 mm (table 1). As regards to the type of endometrial hyperplasia, the simple type was the most common (100%) among the group of 8 - 12 mm, the same was in the group of 13 - 17 mm but with the percentage of (88.9%). Lastly. In the group of endometrial thickness of > 17mm, both simple and complex hyperplasia were present (50% and 42.9% respectively) (table 1).

As regards to the resistance index (RI) measured by Doppler velocimetry of the uterine arteries, 25 cases from the thirty cases diagnosed by D & C biopsy (83.3%) were suspected to have endometrial hyperplasia with false negative result in 5 cases (16.7%) (table 2)

and false positive result in 3 cases. The sensitivity, specificity, (PPV), and (NPV) of the RI were 71%, 89%, 89%, and 97% respectively (figure 1).

In addition, in the group of endometrial thickness of 8 - 12 mm, there was non significant decrease in the RI, in the group of endometrial thickness of 13 - 17 mm, there was significant decrease in RI (high incidence of hyperplasia), finally, in the group of endometrial thickness of > 17 mm, there was highly significant decrease in the R.I (very high incidence of hyperplasia) (table 2).

In addition, pulsatility index predicted hyperplasia in 23 cases from the thirty cases diagnosed by D & C biopsy (76.7%) with false negative result in 7 cases (23.3%) (table 3) and false positive result in 4 cases. The sensitivity, specificity, PPV, and NPV of the PI were 62%, 98%, 85%, and 96% respectively (figure 1). In addition, in the group of endometrial thickness of 8 - 12 mm, there was non significant decrease in the PI, in the group of endometrial thickness of 13 - 17 mm, there was significant decrease in PI (high incidence of hyperplasia), finally, in the group of endometrial thickness of > 17 mm, there was highly significant decrease in the PI (very high incidence of hyperplasia) (table 3).

On the other side, from the thirty cases diagnosed by D & C biopsy, hysteroscopy was able to predict endometrial hyperplasia in 22 cases (73.3%) with false negative result in 8 cases (26.6%) (table 4) and false positive result in 2 cases. The sensitivity, specificity, PPV, and NPV of the hysteroscopy in predicting endometrial hyperplasia were 58%, 99%, 92%, and 96% respectively (figure 1).

In addition, in the group of endometrial thickness of 8 - 12 mm, there was significant difference in the hysteroscopic results. In the group of endometrial thickness of 13 - 17 mm, there was significant difference in the hysteroscopic assessment (high incidence of hyperplasia). Finally, in the group of endometrial thickness of > 17 mm, there was highly significant difference in the hysteroscopic assessment (very high incidence of hyperplasia) (table 4).

Obviously, in the group of endometrial thickness of > 17 mm, there was highly significant predictable changes seen by both Doppler studies and hysteroscopy.

When comparing hysteroscopy and transvaginal Doppler velocimetry measurements, there was no significant difference between both in predicting endometrial hyperplasia as shown in figure 1.

**Table 1:** Relation between endometrial thickness and types of endometrial hyperplasia.

| Endometrial thickness (mm) | Types of endometrial hyperplasia | | | | | | Total | P-value |
|---|---|---|---|---|---|---|---|---|
| | Simple | | Complex | | Atypical | | | |
| | No. | % | No. | % | No. | % | | |
| 8 - 12 | 7 | 100 | 0 | 0 | 0 | 0 | 7 | High Sign. |
| 13 – 17 | 8 | 88.9 | 0 | 0 | 1 | 11.1 | 9 | High Sign. |
| > 17 | 7 | 50 | 6 | 42.9 | 1 | 7.1 | 14 | NS |
| Total | 22 | | 6 | | 2 | | 30 | |

**Table 2:** Relation between endometrial thickness and uterine artery resistance index (RI).

| Endometrial thickness (mm) | Resistance index | | | | Total | P-value |
|---|---|---|---|---|---|---|
| | ≤ 0.75 | | > 0.75 | | | |
| | No. | % | No. | % | | |
| 8 - 12 | 4 | 57.1 | 3 | 42.9 | 7 | NS |
| 13 – 17 | 7 | 77.8 | 2 | 22.2 | 9 | Sign. |
| > 17 | 14 | 100 | 0 | 0 | 14 | High Sign. |
| Total | 25 | | 5 | | 30 | |

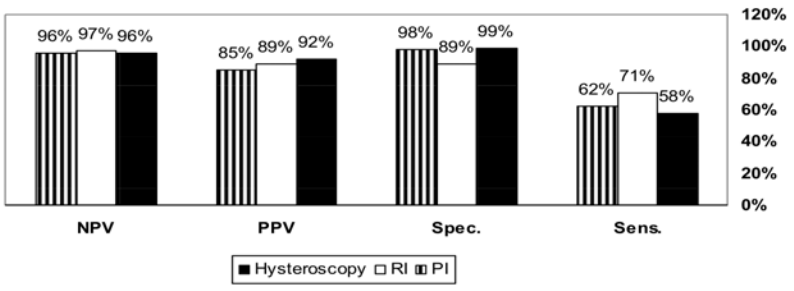
**Table 3:** Relation between endometrial thickness and uterine artery pulsatility index (PI).

| Endometrial thickness (mm) | Pulsatility index | | | | Total | P-value |
|---|---|---|---|---|---|---|
| | ≤ 1.83 | | > 1.83 | | | |
| | No. | % | No. | % | | |
| 8 – 12 | 3 | 42.9 | 4 | 57.1 | 7 | NS |
| 13 – 17 | 8 | 88.9 | 1 | 11.1 | 9 | Sign. |
| > 17 | 12 | 85.7 | 2 | 14.3 | 14 | High Sign. |
| Total | 23 | | 7 | | 30 | |

**Table 4:** Relation between endometrial thickness and hysteroscopic prediction of endometrial hyperplasia.

| Endometrial thickness (mm) | Hysteroscopic assessment | | | | Total | P-value |
|---|---|---|---|---|---|---|
| | +ve | | -ve | | | |
| | No. | % | No. | % | | |
| 8 - 12 | 1 | 14.3 | 6 | 85.7 | 7 | Sign. |
| 13 – 17 | 7 | 77.8 | 2 | 22.2 | 9 | Sign. |
| > 17 | 14 | 100 | 0 | 0 | 14 | High sign. |
| Total | 22 | | 8 | | 30 | |

**Figure 1:** Sensitivity, Specificity, PPV and NPV of the RI, PI, and hysteroscopy in prediction of endometrial hyperplasia showing statistically insignificant difference between them.



## Discussion

Endometrial hyperplasia covers a group of abnormalities encompassing premalignant lesions of the endometrium. It is classified according to cellular and structural appearance, and is recognized as an estrogen dependent condition [7].

Many methods were tried for evaluation of perimenopausal bleeding to reach an accurate method that carries high sensitivity and specificity in diagnosis. These methods are either non- invasive or invasive. The non- invasive methods as imaging techniques are very helpful in diagnosis, such as pelvic U/S, transvaginal color Doppler U/S and MRI [8].

Invasive techniques are also very helpful in diagnosis such as hysteroscope and endometrial sampling [9].

Color Doppler scanners are able to superimpose a color map over the grey scale image, the ability to assess blood supply and tumour vascularity makes color Doppler U/S a potentially useful tool to differentiate benign from malignant lesions [6].

In this study, uterine cavity assessment was done and any uterine lesion such as myoma, polyp, or adenomyosis has been excluded.

Hysteroscopic assessment was performed in the operating theatre under general anesthesia, then D & C biopsy was done in the same setting after diagnostic hysteroscopy. Endometrial biopsies were examined histopathologically. Transvaginal color Doppler U/S was done on uterine artery before the operative intervention (hysteroscopy and D & C biopsy).

In our study, uterine artery RI cut off value of 0.75 was selected according to the coordination of Receiver Operating Characteristics (ROC) curve of the RI in patients with endometrial hyperplasia, and this cut off point had the sensitivity, specificity, PPV, and NPV of 75%, 88%, 60%, and 96% in predicting endometrial hyperplasia respectively [6].

The Doppler results of this

study are in agreement with Arslan et al [3], and Emoto et al[10], who concluded that uterine artery RI has 100% sensitivity and 62% specificity in detecting endometrial pathology.

Also, Drajojevic et al[9], have stated that, transvaginal colour Doppler sonography as sensitive, specific and non-invasive method has significant place in the diagnostic procedure for evaluation of abnormal uterine bleeding in perimenopausal and postmenopausal women. Colour Doppler sonography can help in differentiating benign from malignant endometrial changes, and in deciding on the most efficient therapeutical regime.

On the other side, Vuento et al[11] and Sladkevicius[12] reported that transvaginal U/S is a better method for discriminating between normal and pathological endometrium than Doppler velocimetry of the uterine arteries.

In the present study, as regards to the PI, the cut off value of 1.83 has been chosen and this value has a sensitivity, specificity, PPV, and NPV of 75%, 85%, 43%, and 96% respectively in the prediction of endometrial hyperplasia [6] and[13].

In contradiction with our results, Wilailak et al[14] reported that PI and RI values for the uterine arteries does not help in discrimination between normal and pathologic endometrium and that is probably because uterine arteries are too large to show any hemodynamic disturbance, whereas, intra-lesion vessels may show some difference.

The divergence in the results between the present study and the other reports might be attributed to many variables that can affect Doppler measurements such as variation in the angle of insonation and type of the Doppler beam used, the frequency used, machine resolution, the sample size, quality of the produced image, patient cooperation during examination, and the skills of the examiner.

Regarding to hysteroscopic results, it has been succeeded in predicting endometrial hyperpla-

sia in 22 cases (73.3%) and failed to predict it in 8 cases (26.7%) with sensitivity, specificity, PPV, and NPV of 58%, 99%, 92%, and 96% respectively and this is in agreement with Bassil et al[15], Garuti et al[16] and Giancarlo et al [17] who demonstrated that, the overall sensitivity, specificity, NPV, and PPV of the hysteroscopy were 63.7%, 91.7%, 91.3%, and 64.7%, respectively.

On the other hand, Loverro et al[5] studied the predictive accuracy of the hysteroscope in cases of endometrial hyperplasia, the sensitivity, specificity, PPV, and NPV were 98%, 95%, 63%, and 99% respectively with high rate of (42%) of false positive results. This was mainly due to presence of abnormal thickened endometrium as a main criterion for hysteroscopic diagnosis of endometrial hyperplasia and concluded that high diagnostic accuracy associated with minimal pain renders hysteroscope the ideal procedure for both diagnosis and follow up of conservative management of endometrial hyperplasia.

In accordance with our results, Birinyi et al[18] studied the predictive value of hysteroscope in diagnosis of intrauterine abnormalities, they reported that sensitivity, specificity, PPV, and NPV were 52%, 92%, 35%, and 95% respectively.

Also, Butureanu et al[19] in their study about hysteroscopy and endometrial hyperplasia, emphasized that the hysteroscopy was successful in predicting hyperplasia in 76% of cases.

Obviously, in this study, in the group of thickened endometrium > 17 mm, there were very high incidence of endometrial hyperplasia as proved by both Doppler and hysteroscopy and this enforces the concept of performing Doppler U/S as a screening non invasive method in patients with thick endometrium as it has high predictive value in this condition.

In the present study no statistically significant difference between sensitivity, specificity, PPV, and NPV of both hysteroscopy and transvaginal Doppler U/S on the uterine artery was noted. However, they are slightly higher in

Doppler U/S. Finally, Doppler U/S offers additional advantages of being non- invasive, rapid, easily applicable, painless with avoidance of the numerous complications of the hysteroscopy.

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# BENHA MEDICAL JOURNAL


## COMPARISON BETWEEN TRANSVAGINAL DOPPLER ULTRASONOGRAPHY AND HYSTEROSCOPY IN PREDICTION OF ENDOMETRIAL HYPERPLASIA


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# PLASTICITY OF HUMAN MESENCHYMAL STEM CELLS : ABILITY TO FORM BLOOD AND TO DIFFERENTIATE INTO HEPATIC CELLS

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## Abstract

***Objective:*** *To evaluate the ability of mesenchymal cells derived from nonhematopoietic organs to form blood and to differentiate into hepatic cells in vitro and in vivo.*

***Materials and Methods:*** *Because of its mesodermic derivation, human fetal kidney was used as a source of mesenchymal cells. Two populations of kidney cells were studied at a nonclonal level: a crude preparation, and an adherent fraction that was derived from the first by propagation in vitro (MNMC). Both populations were transplanted into sheep fetuses and analyzed at intervals for the presence of human cells in different organs by flow cytometry, PCR, immunohistochemistry, and in situ hybridization. Secondary transplantation studies were performed using human hematopoietic cells obtained from the bone marrow (BM) of primary recipients.*

***Results:*** *MNMC were Thy-1$^+$, CD51$^+$, CD44$^+$, CD45$^-$, and vimentin$^+$, a phenotype consistent with that of metanephric mesenchyme. The crude population displayed the same phenotype but was contaminated with 0.4% CD34$^+$ CD45$^+$ cells. Cells with hepatocyte-like morphology and phenotype were obtained from the MNMC after culture in specific inducing media. After transplantation, both populations of cells produced multilineage hematopoietic engraftment and gave rise to CD34$^+$ cells. Successful hematopoietic engraftment in secondary recipients demonstrated the generation of long-term engrafting hematopoietic*

*stem cells from MNMC. PCR analysis confirmed human hematopoietic engraftment and revealed that human cells were also present within other organs. Liver sections of transplanted animals contained human albumin-producing hepatocyte-like cells.*

***Conclusion:*** *A human metanephric mesenchymal cell population simultaneously gave rise to human blood and liver-like cells, suggesting that mesenchymal cells may represent a broad population of putative stem cells in multiple adult organs.*

## Introduction

Numerous studies have provided evidence that stem cells persist through adult life in several tissues, participating in normal replacement and repair within their respective tissues[1-4]. By examining stem cells from readily available adult sources, investigators have begun to gain new insights into the processes of cellular differentiation and organogenesis, and have started to provide evidence suggesting that some of these adult stem cells possess the ability to develop into cells of multiple tissues, in some cases including those of seemingly unrelated organs[5-16].

These findings have thus challenged the commonly held belief on development, that cellular differentiation and lineage commitment are irreversible processes and that the inter conversion of cells from one germinal derivation to those of another is not possible. This apparent plasticity of adult stem cells has opened vast potential for use of adult stem cells in cellular replacement therapy for a wide range of congenital and degenerative diseases.

Among the different populations of adult stem cells that have thus far been investigated, bone marrow mesenchymal stem cells (MSC) have been shown to harbor the ability to differentiate into a number of other different cells like blood, chondrocytes, adipocytes, myocytes, and endothelium, as well as cells of alternate germinal derivation such as neural cells, skin, and liver, suggesting that MSC likely represent one of the most promising stem cell sources for tissue replacement therapy [17-26].

Unlike many other stem cells that are thought to exist only in specific niches within the body, MSC presence is not restricted to the bone marrow; rather, they are broadly distributed in a variety of different tissues. For instance, among others, organs such as kidney, lung, and liver arise through the interactions and inductive signals between mesenchymal and embryonic epithelial tissues [27].

In the specific case of the kidney, a derivative of the intermediate mesoderm, the metanephric mesenchyme is considered to represent a homogeneous population of cells that are pluripotent in that they possess the ability to differentiate towards both epithelial and stromal cell lineages [28-32]. It is also known that during a particular phase of fetal development, the aorta-gonad-mesonephros (AGM) region serves as a source of multiple stem cells, including somatic cells in the male gonad and vascular endothelial cells, and gives rise to an initial population of hematopoietic stem cells that migrates to the fetal liver and the adult bone marrow [33,34].

Furthermore, the AGM has also been characterized as a region in which definitive stem cells are maintained in a pluripotent state. Thus, the role of the mesonephros in the early stages of hematopoiesis and the functional and structural similarities between the mesonephric and metanephric kidneys led us to perform the present studies to evaluate at a nonclonal level the ability of mesenchymal cells derived from metanephric mesenchyme to form blood and other tissues in vitro and in vivo.

Using the human sheep xenograft model of human hematopoiesis, we demonstrate that a human fetal metanephric mesenchymal cell population is capable of simultaneously giving rise to human blood and liver, suggesting that MSC may represent a broadly distributed population of residual putative stem cells that exists within multiple adult organs.

## Material and Methods

### Isolation and culture of metanephric mesenchymal cells:

Human fetal kidneys were obtained from Advanced Bioscience

Resources (ABR, Palo Alto, CA, USA) after approval from the Human Subjects Committee of the University of Nevada, Reno. The kidneys (n = 8) collected from eight 14- to 22-week-old fetuses were minced into thin slices, washed extensively with phosphate-buffered saline (PBS; Gibco BRL, Gaithersburg, MD, USA), and finally individually dissociated into single cell suspensions by incubation for 5 minutes with 2% collagenase/0.02% trypsin (Sigma Chemical, St. Louis, MO, USA), transferred to Dulbecco's modified Eagle's medium (DMEM; Gibco) with 10% fetal bovine serum (FBS) (HyClone Laboratory Inc., Logan, UT, USA), and then mechanically dissociated in glass homogenizers. After several washes in DMEM and filtration through a 70 μm cell strainer, the resultant cells were plated in gelatin-coated flasks at a concentration of 5-10 x $10^4$/mL in DMEM 12% FBS and the adherent fraction propagated in vitro for at least 4 passages.

**Phenotypic analysis :**

In order to assess the phenotype expressed by metanephric cells and to exclude the possibility of contamination by circulating hematopoietic stem cells, both freshly isolated and cultured metanephric mesenchymal cells (MNMC) were analyzed by flow cytometry using monoclonal antibodies against CD3, CD7, CD14, CD10, CD19, CD29, CD31, CD33, CD34, CD44, CD45, CD49d, CD49e, CD50, CD54, CD58, CD62E, CD62L, CD90, CD102, CD106, CD117 (Becton-Dickinson Immunocytometry Systems [BDIS], San Jose, CA, USA), and Gly-A (Immunotech, Miami, FL, USA).

**Exclusion of contaminating hematopoietic cells in MNMC layers by RT-PCR**

In order to confirm that our MNMC layers did not contain levels of $CD45^+$ cells that were undetectable by flow cytometry, we isolated RNA from the MNMC layers using NAqueous kit from Ambion (Ambion, Austin, TX, USA) according to the manufacturer's recommendations. RNA was reverse transcribed to cDNA using a commercially available kit according to manufacturer's instructions (Gibco BRL). We then performed 40 cycles of PCR amplification with

the following CD45-specific primers: forward primer 5-GGA ATT CCA AAG CCC AAC ACC TTC C-3, and reverse primer 5-GCG GAT CCA CTT GTG TAC AAT CAT GTA A-3.

**Induction of MNMC differentiation in vitro :**

In order to induce differentiation of MNMC into hepatocyte-like cells, MNMC were replated on collagen-coated plates at the concentration of $10^4$ cells/mL and cultured for 2 to 3 weeks in DMEM with transferrin (10 mg/mL), hcortisone - 21 - hemisuccinate (0.5 mg/mL), bovine albumin-fatty acid free (50mg/mL), 10 mM ascorbic acid, 0.5μg/mL hEGF, and insulin (final concentration107/M) (Clonetics, San Diego, CA, USA) with media changes every 2 to 4 days. Immunoperoxidase staining with an antibody against either albumin (Sigma) or an isotype control was performed on MNMC before and after induction of differentiation.

**Creation of human sheep chimeras :**

These studies were performed in 18 fetal sheep (13 primary recipients & 5 secondary recipients) following the transplantation procedure that has been described in detail previously[35,36]. In short, both freshly isolated metanephric mesenchymal cells and MNMC were injected intraperitoneally in a 0.5-mL volume into 55- to 60-day-old fetal sheep (term: 145 days) at a dose of $10^6$ cells/fetus for the MNMC and $1^6$-$10^6$ cells/fetus for the freshly isolated population. The transplanted sheep were then analyzed for donor (human) cell engraftment at intervals between 2 and 9 months post transplant.

At 2 months post transplant, cells isolated from the bone marrow (BM) of primary sheep recipients transplanted with the cultured MNMC fraction were used to perform secondary transplants. Secondary recipients were analyzed for human hematopoietic activity at 2 months post transplant.

**Assessment of human donor cell engraftment**

The presence of donor cells in hematopoietic tissues of the recipients (blood, marrow, liver, spleen, and thymus), was determined at intervals post transplantation us-

ing flow cytometric analysis and PCR using human HLA-specific primers. Flow cytometric analysis of the cell populations was performed on a FACScan (BDIS). Monoclonal antibodies to various cluster designations (CDs) directly conjugated fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were used according to the manufacturer's recommendation. These included: CD3, CD7, CD20, CD34, CD45, CD59, CD83, CD105 (BDIS) and glycophorin A (Coulter, Immunotech).

**Detection of human cells by PCR :**

To evaluate various organs for the presence/engraftment of human cells, single cell suspensions were prepared by passing each of the tissues through a glass homogenizer. Total genomic DNA was then extracted from $10^7$ cells from each tissue using a commercially available kit according to the manufacturer's recommendations (Master Pure genomic DNA purification kit, Epicentre Technologies, Madison, WI, USA). A segment from the human HLA-DQ gene was then amplified by polymerase chain reaction (PCR) using the primers A (5'-GTG CTG CAG GTG TAA ACT TGT ACC AG-3') and B (5'-CAC GGA TCC GGT AGC GGT AGA AGT TG-3') by performing 2 minutes of denaturation at 98°C and 5 minutes of annealing at 58°C followed by 40 cycles with the following parameters: 95°C for 45 seconds, 58°C for 1 minute, 69°C for 1 minute. After a 7-minute extension at 69°C, 1/10 of each of the reaction products was run on a 1% agarose gel and subsequently transferred to Gene Screen Plus membrane (NEN, Boston, MA, USA) under denaturing conditions. An oligonucleotide probe (5'-TGG ACC TGG AGA GGA AGG AGA CT-3') was then end-labeled with 32 P-ATP and hybridized to the resultant blot for 2 hours at 42°C in Rapid-Hyb hybridization solution (Amersham, Piscataway, NJ, USA). After 4 washes at 42°C under conditions of increasing stringency, the blots were exposed to Bio-Max MS film (Kodak, Rochester, NY, USA) for 2 to 12 hours at 70°C with 1Bio-Max intensifying screen.

**Detection of human cells by immunohistochemistry :**

Experimental and age-matched

control sheep liver sections were obtained and fixed in formalin. Paraffin sections 3 to 4µm in thickness were prepared on poly-L-Lysine coated slides from formalin-fixed paraffin-embedded tissues. Control and experimental sheep liver slides were incubated at 60°C for 12 to 16 hours and then deparaffinated by two 10-minute incubations in xylene followed by rehydration through a graded ethanol series to deionized water. To enhance antigen retrieval, slides were incubated at 90°C for 10 minutes in TUF (tissue unmasking fluid, Signet Laboratories, Inc., Dedham, MA, USA). Human cells were then detected with antibodies to human albumin (Sigma) and human hepatocyte-like cells (Dako, Carpinteria, CA, USA) using previously published methodology [20].

**ELISA for identification of human albumin in serum of chimeric sheep :**

In order to determine if the human hepatocyte-like cells within the sheep liver were functionally active, we looked for the presence of human albumin in the serum of the transplanted sheep. We used a two site immunoenzymatic assay using an amplified biotin/streptavidin detection system that allows detection of human albumin in serum up to around 200 pg/ml (Cygnus Technologies, Plainville, MA, USA). Serum from transplanted animals were collected immediately and analyzed according to the manufacturer's instructions or frozen until enough samples were collected to perform the assay.

## Results

**Characterization of metanephric mesenchymal cells :**

Two populations derived from fetal kidney were analyzed in these studies. The first one was obtained by a collagenase/trypsin digestion step followed by mechanical dissociation as described in the materials and methods section and consisted of a crude preparation of cells. Phenotypically, these cells were Thy-1$^+$, CD106$^+$ CD102$^+$, CD51$^+$, and CD44$^+$. As shown in table 1, these cells also expressed a number of other markers at varying levels. Of concern was the presence of 6 to 8% of CD34$^+$ cells that we thought might have resulted from

contamination by fetal blood, despite the extensive washes of the fetal kidneys with saline before processing. However, further analysis revealed that the majority of the $CD34^+$ cells in our kidney preparation were negative for the CD45 antigen and were positive for $CD106^+$ and $CD102T^+hy\text{-}1^+$ (6-8%), with only 0.4 to 0.5% of the $CD34^+$ population being double-positive for $CD45^+$. Becaue we wanted to obtain a population that was devoid of hematopoietic markers, and since these cells expressed Thy-1,CD44, and CD29, a phenotype similar to MSC obtained from adult BM, we decided to culture these cells in an identical way to that of BM MSC. An adherent population of cells with a spindle-like appearance was obtained (Fig. 1), and after propagation for at least 4 passages, we again analyzed the cells by flow cytometry. The population of cells obtained had a similar phenotype to the starting crude population in that the cells were positive for Thy-1, CD44, and CD51. However, these cultured adherent cells had become dim for CD102 and CD106. In addition, no CD45 or CD34 were expressed in this adherent fraction, and no other known hematopoietic markers were found (Table 2). Furthermore, RT-PCR using CD45-specific primers confirmed that our adherent layers did not contain levels of $CD45^+$ cells that were undetectable by flow cytometry (data not shown). Plating of these cells in methylcellulose under conditions identical to those used for the crude cell population demonstrated their inability to form hematopoietic colonies.

**MNMC are able to differentiate into hepatocyte-like cells in vitro :**

Since MNMC expressed high levels of c-met, we investigated whether we could induce MNMC towards a hepatocytic phenotype by plating them in media that induces/supports the growth of hepatocyte-like cells. Areas of cells with a flat cuboid morphology differing from the morphology of the starting population were obtained. These cells stained positive with an antibody specific for albumin, showing that these cells had acquired properties of hepatocyte-like cells (Fig. 2). Furthermore, ar-

eas of small cells morphologically similar to the bigger cells and staining strongly for human albumin were also seen, suggesting that in our culture system we also obtained colonies of small hepatocyte-like cells.

**Evaluation of the differentiative potential of the metanephric mesenchymal cells in vivo :**

To evaluate the in vivo differentiative potential of the metanephric mesenchymal cells, thirteen 55- to 60-day-old primary fetal sheep were transplanted with either the crud fraction of the metanephric cells (1-6 x$10^6$ cells/ fetus) or MNMC ($10^6$ cells / fetus). Recipients were evaluated for the presence / engraftment of human cells beginning at 2 months post-transplant and at intervals thereafter until 9 months post-transplant. At 2 months post-transplant, human hematopoietic cells were readily detectable in the hematopoietic system of the transplanted sheep. Levels of human CD45 cells in the primary sheep recipients transplanted with MNMC and crude metanephric cells ranged from 1.3 to 3.5% and 1.7 to 2%, respectively. At 3 months post-transplant, the levels of human cells as assessed by CD45 expression was essentially maintained from the previous time point, with levels of 1.5 to 2.2% in sheep transplanted with MNMC and 0.75 to 1.9% in animals that received the crude fraction of cells. At 9 months post-transplant, the sheep maintained their chimeric status with human cell levels between 0.2 and 1.3% in the MNMC transplanted sheep, while the sheep that received the crude fraction had levels of human cells between 1.8 and 2%. The presence of human hematopoietic cells in these primary recipients was multilineage, with the presence of CD20 [B cells], (0.3-3%); CD7 [T cells] (0.1-3.7%); CD34 [stem/progenitor cells], (0.8-1.3%), CD83 [dendritic cells], (0-0.1%), and human CD59 (0.1-13%). PCR analysis with human-HLA-DQ - pecific primers confirmed the engraftment of human cells in BM and peripheral blood (PB) and revealed the presence of human cells in brain, liver, heart, skin, lung, and muscle in different transplanted animals (Fig. 3).

**Long-term engrafting hematopoietic stem cells are present in BM of MNMC primary recipients**

The ability to engraft secondary recipients upon serial transplantation is a functional characteristic of hematopoietic stem cells and has been an essential tool to distinguish between short- and long-term engrafting cells [41,42]. Thus, in order to assess whether the hematopoietic cells that were generated in vivo following transplantation of MNMC had long-term engrafting capability, cells isolated from the BM of primary transplanted animals at 2 months post-transplant were transplanted into secondary recipients at cell doses of 2-$10^7$ cells/fetus (n-5). At 2 months posttransplant, BM and PB from secondary recipients were analyzed for the presence of human cells. As shown in Figure 4, human hematopoietic cells were detected by flow cytometry in PB and BM of all five animals at significant levels. The engraftment in secondary recipients was also multilineage (Table 3), and of particular note was the presence of human $CD34^+$ cells in the BM of these chimeric secondary animals.

**Human metanephric mesenchymal cells generate hepatocyte-like cells upon transplantation**

Since our in vitro studies had shown that under appropriate conditions the MNMC were able to transdifferentiate to produce hepatocyte-like cells in culture and synthesize albumin and PCR analysis had shown the presence of human cells in the liver, we obtained liver sections from the primary sheep recipients that had been transplanted with the crude mesenchymal cell population and the adherent MNMC population. Sections were first analyzed with an antibody to human hepatocytes (HEPAR-1). As can be seen in Figure 5, this antibody exhibited no cross-reactivity with hepatocyte-like cells within the liver section of an untransplanted control sheep (Fig. 5A), while human hepatocytelike cells could readily be detected within the sections from sheep transplanted in utero with the human mesenchymal cells, as can be seen in Figure 5B and C, which are representative sections from two of the sheep that received MNMC. In order to demonstrate that the hepatocyte-like

cells generated were functional, sections from the liver of these same animals were analyzed by immunohistochemistry with an antibody specific for human albumin. Figure 5D shows a liver section of a nontransplanted, age-matched sheep stained with the anti-human albumin antibody, while Figure 5E is a representative section from the liver of one of the sheep transplanted with MNMC. Human hepatocytelike cells present within the liver sections stained positively with this albumin antibody, demonstrating that the cells generated from the metanephric mesenchymal cells are functional. Overall, of the four sheep transplanted with the adherent MNMC, only three of the livers could be analyzed due to degradation of the morphology of one of the tissues. The liver from all three remaining sheep in this group exhibited human hepatocyte-like cells, with levels varying from sheep to sheep. One of the livers evaluated showed few isolated hepatocyte-like cells (1-2) located around the periportal area (Fig. 5B); the other two had clusters of human hepatocyte-like cells mostly around the periportal area, as shown in Figure 5F and G. Overall, levels of human hepatocyte-like cells ranged from 0.01 to 0.5% over the entirety of the liver sections. Despite the fact that all of the sections from the liver of these sheep stained positively with an anti-human albumin antibody, we were unable to find human albumin in the serum of these same sheep. From the nine sheep transplanted with the crude cell population, the livers from six of these sheep were analyzed and four were found to contain human hepatocyte-like cells. Again, the levels of hepatocyte-like cells varied from sheep to sheep, but in general, the levels were low, similar to those seen in the sheep transplanted with MNMC. Interestingly, in both groups, the levels of human hepatocyte-like cells appeared to correlate well with the levels of human hematopoietic cell engraftment, with animals containing higher levels of human hematopoietic cells exhibiting greater numbers of human hepatocyte-like cells within their liver. In all of the sections staining positive for human hepatocyte-like cells, the human origin of the hepatocyte-

like cells was confirmed by in situ hybridization with a human-specific Alu probe (data not shown) using methodology that has previouslybeen described in detail [20].

**Table 1.** Phenotype of freshly isolated metanephric mesenchymal cells by flow cytometry

| *Marker* | *Peresent positivity* |
|---|---|
| CD29 (Integrin1) | 60–75% |
| CD31 (PECAM-1) | 5–11% |
| CD34 | 8–10% |
| $CD34^{+}CD90^{+}CD106^{+}CD102^{+}$ | 6–8% |
| $CD34^{+}CD45^{+}$ | 0.4–0.5% |
| CD49d (Integrin a4) | 10–12% |
| CD49e (Integrin a5) | 6–8% |
| CD50 (ICAM-3) | 2–10% |
| CD54 (ICAM-1) | 5–7% |
| CD58 (LFA-3) | 0.5–1% |
| CD62E (E-selection) | 25–29% |
| CD62L (L-Selectin) | 14–16% |
| CD117 | 0.08–0.5% |
| FLT-3 | 0 |
| Tek | 0 |
| Tie | 0 |

**Table 2.** Phenotype of adherent metanephric mesenchymal stem cells (MNMC) by flow cytometry

| Marker | Peresent positivity |
|---|---|
| CD34 | 0% |
| CD44 | 96 - 97% |
| CD45 | 0% |
| CD51 | 96 - 98% |
| CD90 | 50 - 60 % |
| CD105 | 1 - 2% |
| CD117 | 0% |
| FLt-3 | 0% |
| Tek | 0% |
| Tie | 0% |

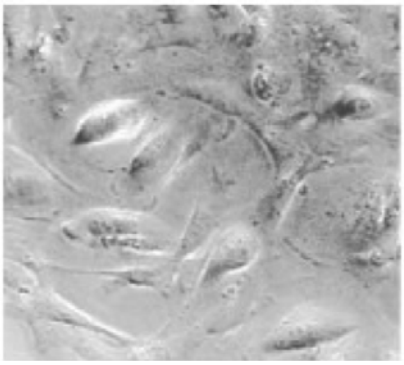
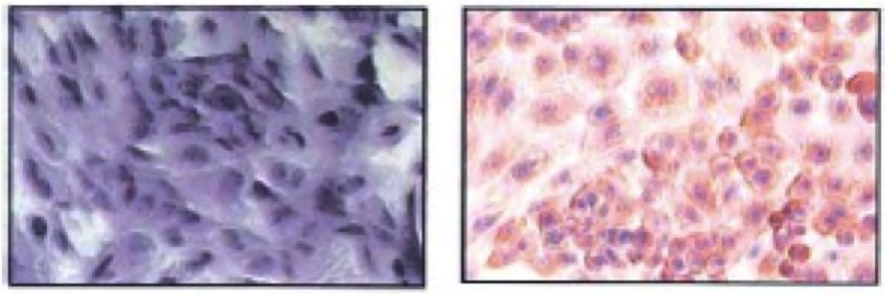


**Figure 1 :** Morphology of in vitro propagated MNMC. After 4 passages in vitro MNMC, grown in chamber slides & were examined by phase contrast microscopy.



**a** **b**

**Figure 2 :** MNMC are able to adopt a hepatocyte- like morphology and phenotype after induction in vitro. MNMC were cultured in a hepatocyte-differentiative media and stained with either isotype control (A) or antibody to human albumin (B).

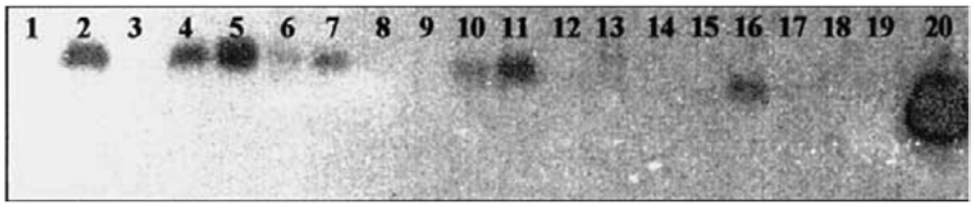



**Figure 3 :** Human HLA-DQ-specific PCR analysis of nonhematopoietic tissues from animals transplanted with MNMC. At 2 months posttransplant, single cell suspensions were prepared from liver, heart, skin, lung, muscle, PB, and BM of primary recipients and analyzed by human HLA-DQ-specific PCR. Samples are as follows: 1, Reagent control; 2, PB; 3, PB control; 4, BM; 5, Skin; 6, Lung; 7, Muscle; 8, BM control; 9, Skin control; 10, Liver; 11, Heart; 12, Lung control; 13, Muscle control; 14, Liver control; 15, Heart control; 16, Brain; 17, Brain control; 18, Spinal cord; 19, Spinal cord control; 20,Control.

**Table 3 :** Multilineage human hematopoietic cell activity in secondary recipients at 2 months posttransplant

| | *BM* | *PB* |
|---|---|---|
| *CD7+* | 0.2–0.5% | 1.0–3.1% |
| *CD20+* | 0.1–0.4% | 0.1–0.8% |
| *CD34+* | 0.2% | 0% |
| *CD83+* | 0.2–0.5% | 0.3–0.4% |
| Gly-A+ | 0.6–1.2% | 0.2–0.8% |

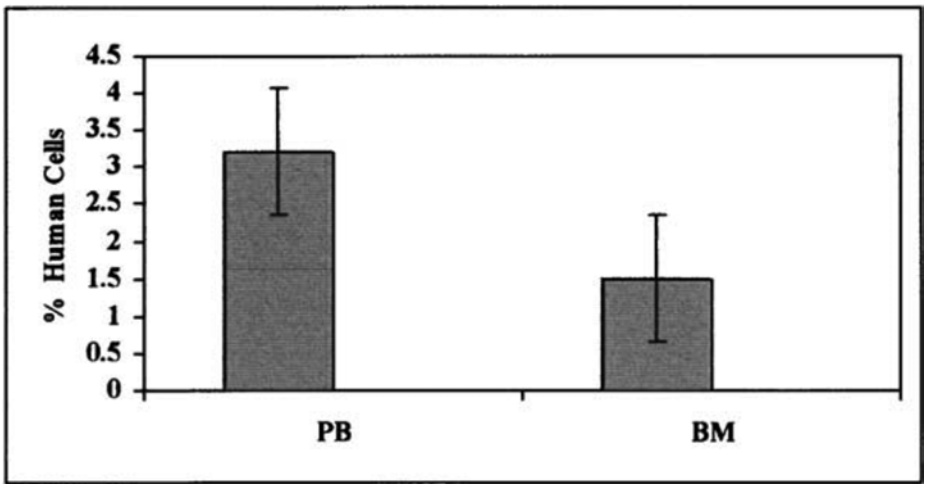



**Figure 4 :** MNMC produce serially transplantable HSC. Human cells were isolated from bone marrow of primary MNMC recipients and used to transplant secondary fetal sheep recipients. At 2 months posttransplant, the PB and BM of the secondary recipients were analyzed for human hematopoietic cells engraftment by flow cytometry with a human-specific antibody against CD4.

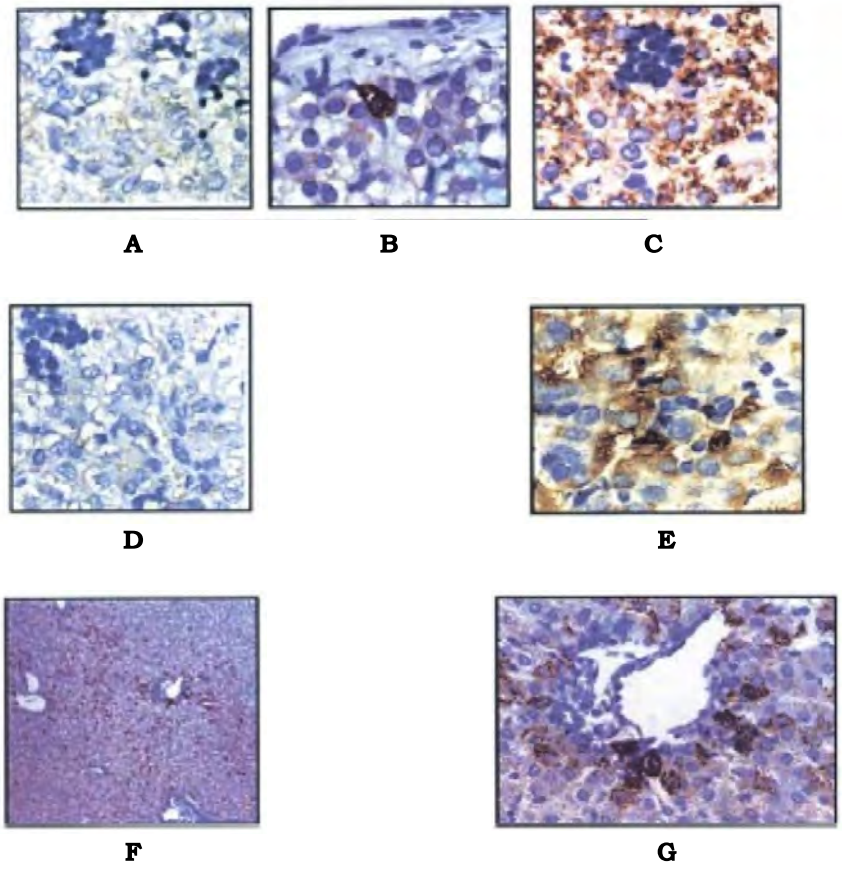



**Figure 5 :** Identification of functional human hepatocyte–like cells within the liver of sheep transplanted with metanephric mesenchymal cells. We performed immunohistochemistry on the liver sections from sheep transplanted in utero with metanephric mesenchymal cells to determine whether the PCR positivity of the liver was due to the formation of human hepatocyte-like cells. (A): Untransplanted control sheep liver sections stained with antibody specific for human hepatocyte -like cells (HEPAR-1).(B) and (C): Representative sections from the liver of two different sheep transplanted with MNMC stained with HEPAR-1. (D): Untransplanted control sheep liver sections, stained with antibody specific for human albumin. (E): Representative sections from the liver of a sheep transplanted with MNMC stained with antibody specific for human albumin. (F) and (G): Low and high magnification of a section of liver of a transplanted sheep stained with HEPAR-1. The human hepatocyte-like cells were found in clusters and mostly around the periportal area.

## Discussion

Recently, a great deal of evidence has demonstrated the ability of mesenchymal cells from adult human bone marrow to give rise to numerous other cell types both in vitro and in vivo, prompting investigators to begin looking for ways to exploit this apparent plasticity in efforts to develop novel therapeutic approaches for the treatment of both inherited and degenerative diseases of multiple organ systems. The majority of the research to date has focused on mesenchymal cells present within the bone marrow based on the ease with which cells from this organ can be obtained and the lack of immune compatibility issue when used for oneself[17-26]. In the present study, we reasoned that the fetal kidney might represent a rich source of pluripotent mesenchymal stem cells, based on both the mesenchymal origin of the kidney and the role played by the developing mesonephros in primitive hematopoiesis[27,34]. We examined two different populations of metanephric mesenchymal cells to test this hypothesis. The first of these was a crude cell population obtained by enzymatic digestion and subsequent homogenization of fetal human kidney. Despite the obvious heterogeneity of these cells, phenotypic analysis demonstrated that the majority of the cells expressed Thy, CD44, and CD29, a phenotype that is similar to that of mesenchymal stem cells obtained from adult human BM. Interestingly, 6 to 8% of these cells expressed the hematopoietic antigen CD34, raising the possibility that fetal blood may have contaminated our preparation; however, costaining with CD45 revealed that the majority of these CD34 cells (99.5%) were $CD45^-$. Given the fact that there have been reports suggesting that some hematopoietic $CD34^+$ cells within the fetal BM do not express CD45, we proceeded to a more thorough analysis of this population to fully exclude the possibility of large numbers of contaminating potential HSC. This analysis showed that these cells were $CD34^+$, $CD45^-$, $CD106^+$, suggesting that they possessed an endothelial- like phenotype. Because it was imperative to the unambiguous interpretation of the results of these studies to start with a population of cells that was completely

devoid of all detectable hematopoietic contaminants, and given that the phenotype of these cells was reminiscent of adherent MSC isolated from BM, we propagated the adherent fraction of this crude preparation for a minimum of 4 passages under conditions normally used for the derivation/ propagation of bone marrow-derived mesenchymal stem cells to obtain a relatively homogeneous population of adherent cells that possessed a mesenchymal morphology. Phenotypic analysis demonstrated that these cells were quite similar to the original starting population, but lacked expression of CD34, CD45, and other known HSC markers demonstrating that any hematopoietic contaminants had been removed by this culture method as well as all cells possessing an endothelial-like phenotype ($CD34^+$, $CD106^+$). In addition to markers indicative of early kidney mesenchyme, these cells also expressed c-met, the receptor for hepatocyte growth factor. Although c-met had previously been shown to be expressed within the developing kidney [40], this finding prompted us to examine whether these MNMC might possess the ability to generate hepatocyte-like cells under the appropriate conditions. To examine this possibility, we grew MNMC under conditions designed to drive hepatocytes into differentiation and confirmed that these MNMC possessed the ability to produce albumin-synthesizing cells that morphologically and phenotypically resemble populations of both mature and small hepatocyte-like cells[43]. To examine how broad a differentiative potential these cells possessed, we made use of our unique human-sheep xenograft model system in which populations of human stem cells are transplanted into early gestational sheep fetuses. We reasoned that the fetus would be the ideal setting in which to evaluate the full differentiative potential of this novel cell population, since the fetus has a need for multiple cell types created by its exponential growth rate, and each of its developing tissues possesses a wide array of inductive signals that should facilitate To examine this possibility, we grew MNMC under conditions designed to drive transdifferentiation if the transplanted cells harbor this potential. Upon

transplantation into preimmune fetal sheep, the crude metanephric mesenchymal cell population produced durable multilineage hematopoietic engraftment, including the generation of $CD34^+$ hematopoietic cells. The adherent population of metanephric mesenchymal stem cells (MNMC) also produced multilineage hematopoietic engraftment that persisted throughout the entire 9-month course of these studies. Importantly, the human hematopoietic cells generated in these primary recipients produced durable engraftment upon serial transplantation into secondary fetal sheep recipients, providing conclusive evidence that MNMC were capable of generating long-term engrafting HSC. Interestingly, the recipients of these adherent cells exhibited higher levels of hematopoietic cell engraftment at earlier time points than the crude population did, providing further evidence that any contaminating hematopoietic cells that were present within our initial crude cell population were not the cells responsible for hematopoietic potential we observed in our transplantation experiments.

Immunohistochemistry performed on liver sections obtained from both sheep receiving the crude cell preparation and MNMC demonstrated the presence of hepatocyte-like cells that were of human origin and were functional, producing human albumin; however, we were unable to detect circulating human albumin in the serum of these same sheep. We attribute this to the fact that the levels of hepatocyte- like cells generated were low and thus the amount of albumin produced eluded our method of detection.

In conclusion, we have shown in these studies that following in utero transplantation, populations of metanephric mesenchymal cells possess the ability to provide both durable, serially transplantable hematopoietic engraftment and hepatocyte- like cells within the same recipients, demonstrating that mesenchymal stem cells from the human fetal kidney possess a degree of plasticity that is similar to that which has previously been described for adult human bone marrow MSC. Our results suggest that MSC may represent a widely distributed pluripotent stem cell

pool that is present in multiple organs of the body, raising the possibility that by comparing MSC from several different tissues to determine their commonalities, it may become possible to arrive at the phenotype of a truly pluripotent MSC. It is important to note that the MNMC were not clonal in nature, raising the possibility that the observed transdifferentiation into multiple cell types within the same recipient was not in fact due to plasticity of a single cell, but rather due to the presence of several different tissue-specific stem cells within the MNMC.

Nevertheless, the presence in the kidney of stem cells with the ability to be extensively propagated in vitro and still produce durable hematopoietic engraftment and generate hepatocyte-like cells upon transplantation is clearly of scientific significance and may ultimately open the door to the findings of a common MSC phenotype.

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# BENHA MEDICAL JOURNAL


## PLASTICITY OF HUMAN MESENCHYMAL STEM CELLS : ABILITY TO FORM BLOOD AND TO DIFFERENTIATE INTO HEPATIC CELLS


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# STOPPA REPAIR VERSUS LICHTENSTEIN TECHNIQUE IN TREATMENT OF BILATERAL INGUINAL HERNIAS (PROSPECTIVE RANDOMIZED STUDY)

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## Abstract

***Background:*** *Bilateral inguinal hernias occur in about 6-8% of groin hernias and simultaneous repair seems more beneficial and economical, requiring only one admission, anesthetic and period of convalescence. The aim of this study is to evaluate and compare between Stoppa's repair versus Lichtenstein's simultaneous mesh repair, in the treatment of bilateral inguinal hernias as regard duration of operation, hospital stay, complications, postoperative pain, recurrence rate and quality of life.*

***Patients and Methods :*** *This prospective randomized study was conducted in general surgery department at Mansoura university hospital from March 2008 to December 2010, sixty consecutive patients who were treated for bilateral inguinal hernia of any type were eligible for the study. The patients were then randomized into two groups: group I underwent Stoppa technique; group II underwent bilateral Lichtenstein hernia repairs; duration of operation, hospital stay, complications, postoperative pain, recurrence rate and quality of life were recorded in each patient.*

***Results:*** *No significant differences were noted in patient characters or early postoperative complication. Operative time in group I were done in a significantly shorter time than group II, 52 ± 20.7 minutes and 75 ± 16 minutes, respectively (p < 0.00l), The length of incision in group I*

*was (10.6 ± 2.7 cm) which is significantly shorter than the sum of bilateral incisions in group II (15.5 ± 3.6 cm) (p < 0.001). While Mean Hospital study was (2.6 ± 1.8 days) group I and in group II was (4.9 ± 1.3 days) (p < 0.001) According to the visual analogue Pain Score Scale, patients in group I reported less pain, and used fewer analgesics than group II (P=0.02). Four cases (13.3%) in-group II showed recurrence (three unilateral and one bilateral) there was no recurrence in-group I., there was improvement in the quality of life in both groups but group I was superior than group II.*

***Conclusion:*** *Stoppa procedure is a reliable technique to repair bilateral inguinal hernia with less postoperative pain, faster return to normal daily activity, low recurrence rate and high patient satisfaction and should be offered to patients who are at risk for recurrence.*

## Introduction

Inguinal hernia surgery has been one of the most extensively debated and continues to evolve in search for the ideal technique [1].

Bilateral inguinal hernias occur in about 6-8% of groin hernias and simultaneous repair seems more beneficial and economical, requiring only one admission, anesthetic and period of convalescence [2].

The reconstruction of the posterior barrier of the groin represents one of the major objectives in groin hernia repair. Various techniques have been promoted for better patient comfort and lower recurrence rates.

There are 2 primary methods used to achieve this objective: "tissue-repair technique" and "tension-free repair". Recently, tension-free repair has become the gold standard procedure for repairing inguinal hernias. Many techniques have been described by different authors[3,4]. Tension-free repair involves the use of synthetic prosthetic materials for rebuilding the posterior inguinal wall.

The alloplastic materials can be installed via conventional inguinal access or. preperitoneal access[5].

The mesh contributes to a physiological healing process that creates a special bilateral anatomical reinforcement in the inguinal region, which effectively prevents inguinal hernia recurrence [6,7].

The repair of recurrent inguinal hernia is usually a difficult operation, requiring surgical experience and an accurate knowledge of the inguinal anatomy. This type of repair does not always have successful results, and high recurrence and complications rates have been reported[8,9,10]. The anterior mesh approach is the most commonly performed technique, but it has the disadvantage of reoperating through scar tissue with the risk of testicular damage and a large number of local haematoma. However, a preperitoneal (posterior) approach, open or laparoscopic, reduces these problems[11] the main advantages of the preperitoneal approach are mesh placement in the preperitoneal space where the hernia is produced and avoiding the disadvantage of reoperating through scar tissue.

Now the dominate techniques to the repair of primary, bilateral, recurrent, inguinal, and femoral hernia is preperitoneal approaches [12].

The French surgeon Rene´ E. Stoppa proposes his method for primary treatment of all complex and difficult hernias (giant, sliding, multiple, femoral)[13]. He recommended that a large polyester mesh should be interposed in the preperitoneal connective tissue between the peritoneum and transversals fascia. Implantation of a bilateral retroperitoneal prosthesis can be undertaken from the preperitoneal access and covers nearly the whole peritoneum of the anterior wall of the lower abdomen as an artificial endoabdominal fascia (5). Stoppa's technique called Giant Prosthetic Reinforcement of the Visceral Sac (GPRVS), which requires wide dissection of the subfascial preperitoneal space.

The aim of our study is to evaluate and compare between Stoppa's giant prosthetic reinforcement of the visceral sac (GPRVS) and Lichtenstein's simultaneous mesh repair, in patients with bilateral inguinal hernias. As regard

duration of operation, hospital stay, complications, postoperative pain, recurrence rate and quality of life.

## Patients and Methods

- This prospective randomized study was conducted in general surgery department at Mansoura university hospital during the period from March 2008 to December 2010,sixty consecutive patient who were treated for bilateral inguinal hernia of any type were eligible for the study (41 patients had primary hernia and 19 patients had recurrent hernia).

- Exclusion criteria Patients with strangulated hernias and ASA III &IV patients.

- Informed consent was obtained from all patients to be included in the study, after explanation of the nature of the disease and possible treatment (either Stoppa or bilateral Lichtenstein hernia repairs) the study was approved by the local ethics committee.

- All patients were subjected to thourghly 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 drown and opened by a nurse in the operating room.

- The patients were then randomized into two groups: group I underwent Stoppa technique; group II underwent bilateral Lichtenstein hernia repairs.

- All patient, are operated under spinal anesthesia (safe to the patients and avoid complication of general anesthesia) nine patients operated under general anesthesia after failed spinal anesthesia, or refused spinal anesthesia.

A prophylactic intravenous antibiotic in the form of a third generation cephalosporin was administered 30 minutes before, during, and after the procedure, until the patients were discharged from the hospital.

**Group I (Stoppa technique)**

A midline incision extends from 2 cm below the umbilicus to 1 cm above the symphysis pubis. The preperitoneal space is entered with blunt dissection. The dissection includes the retropubic space of Retzius and Bogros, and continues laterally, progressing to the retroinguinal space and further exposing the iliopsoas muscle. Direct hernias are identified and reduced. Indirect defects can be divided and the proximal peritoneum over sewn, leaving the distal peritoneum in place undissected and attached to the cord. The spermatic cord and the gonadal vessels are parietalized by dissecting them from their peritoneal attachment to allow them to lay tension-free in the posterior pelvis. This step averts the need for mesh splitting. The polyproline mesh is fashioned as a chevron, and placed in the preperitoneal space with long clamps. The size of the prosthesis is measured on the patient. The width equals the distance between the anterior superior iliac spines, and vertically measures the distance between the umbilicus and the symphysis pubis plus 6 cm. In obese patients, the mesh should be several centimeters wider than the interspinous dimensions. The mesh is held in place without the need for fixation since the intra-abdominal pressure forces the mesh to lay flat between the peritoneum and the fascial layers. None of the hernial defects was repaired. Closed suction drainage was positioned only if improper Haemostasis and was removed after 24 to 48 hours (daily amount less than 30 ml).

**Group II (bilateral Lichtenstein hernia repairs)**

Lichtenstein technique was similar to the original tension-free technique described by Lichtenstein et al [1989][14]. The incisions were inguinal on each side without crossing the midline, leaving a 2cm gap between each end to minimize post-operative suprapubic edema. In this technique we used polyprolene mesh and tailored it according to the size of the inguinal region anchoring a polypropylene mesh to the inguinal ligament with a continuous suture and to the aponeurotic tissue over the pubic bone, the rectus sheath and the internal oblique m. or aponeurosis with interrupt-

ed sutures, reconstructing the deep inguinal ring by making a slit at the lateral end of the mesh, creating two tails that were sutured together to the inguinal ligament around the spermatic cord in a sling configuration.

The duration of operation, length of incision, postoperative complications, length of hospital stay and postoperative pain were recorded in each patient.

The patients using Visual Analogue Scale (VAS) Instruct the patient to point to the position on the line to indicate how much pain they are currently feeling evaluated postoperative pain. The far left end indicates 'No pain' and the far right end indicates 'Worst pain ever'. Chronic pain was defined as any pain sensation lasting longer than 3 months postoperatively, or when local injection of analgesia was necessary [15].

All patients were instructed to take tablets of NSAID as needed. Total analgesic consumption was recorded during the first postoperative week. Physical activity was permitted directly after the operation without any restriction. Outpatient physical examinations were performed on the seventh and 30th days postoperatively.The patients were asked to note the time they returned to pain-free daily activity and work, and their satisfaction with the operation.

Quality of life was examined using the version 2.0 of the short-form 36 healthy survey (SF-36v2) .

**Follow up**

Patients without co morbid conditions were discharged after 24 hours and return for follow-up in outpatient clinic until removal of stitches. All patients were not restricted in their activity but were encouraged to return to their previous lifestyle without limitation as soon as pain or discomfort permits. Later on, the patients examined every 6-month and answer a written questionnaire.

**Statistical analysis:**

The statistical analysis of data done by using excel program and SPSS program statistical package for social science version 16.

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 data, independent sample t-test was used to compare between two groups.

Chi square test was used for qualitative data

N.B: P is significant if < or = 0.05 at confidence interval 95%.

## Results

- Sixty patients were included in the study. Their mean ages in group I and Group II were (56.8 ± 11.2) and (53 ± 12.7) years respectively.

- There is no significant difference regarding age between two groups,

- Table (1) showing patient characteristics (ASA, BMI, type of anesthesia, recurrent cases).

Operative time in group I were done in a significantly shorter time than group II, 52 ± 20.7 minutes and 75 ± 16 minutes, respectively (p < 0.00l) , The length of incision in group I was (10.6 ± 2.7) which is significantly shorter than the sum of bilateral incisions in group II (15.5 ± 3.6) (p < 0.00l). While Mean Hospital study was( 2.6 ± 1.8) group I and in group II was (4.9 ± 1.3) (p < 0.00l) (Table 2).

According to the visual analogue Pain Score Scale, patients in the Stoppa reported less pain, and used fewer analgesics (P=0.02). (Table 3).

There were no intraoperative complications in all patients; five patients developed complications in group I (16.6%), while eleven patients developed complications in-group II (36.7%) (Table 4).

In group I, scrotal edema occurred in two patients (6.6%), seroma developed in one patient (3.3%), discovered 7 days postoperative and was aspirated twice at one week interval and resolved, one scrotal ecchymosis (3.3%) and one superficial wound haematoma (3.3%), all of them occurred in the first two days postoperatively and resolved few days later. no neu-

ropathies, chronic pain or testicular atrophies occurred.

In group II chronic inguinal pain, seen in three patients (10%). over the long term We detected a unilateral testicular atrophy in one case (3.3%). Two cases revealed superficial wound infection (6.6%) after 5 and 7 days postoperative and both of them were treated conservatively. four cases (13.3%) showed recurrence (3 unilateral and one bilateral) 5, 7, 8 and 12 months from the operation all of them were repaired by posterior preperitoneal approach. On the opposite, there was no recurrence in the Stoppa group.

**Quality of life :**

This study examines quality of life at 15th day and6th month of operation, there was improvement in the quality of life in both groups but group I was superior than group II. (Table 5) .

**Table (1) :** Patient's characters.

| | Group I | Group II | P value |
|---|---|---|---|
| NO | 30 | 30 | |
| Age | 56.8 ± 11.2 | 53 ± 12.7 | 0.83 |
| ASAI | 13 | 14 | 0.79 |
| ASA2 | 17 | 16 | 0.74 |
| BMI | 24.2 ± 4.6 | 25.1 ± 39 | |
| Recurrent Cases | 11 | 8 | 0.4 |
| Spinal | 25 | 26 | 0.71 |
| General | 5 | 4 | 0.71 |

No = Number ASA = Amarican Association of Anathesia BMI = Body Mass Index

**Table (2) :** Showing Operative time Incision length Hospital stay and Return to normal daily activity / day.

| | Group I | Group II | P value |
|---|---|---|---|
| Operative time | 52 ± 20.7 | 75 ± 16 | < 0.001 |
| Incision length | 10.6 ± 2.7 | 15.5 ± 3.6 | < 0.001 |
| Hospital stay | 2.6 ± 1.8 | 4.9 ± 1.3 | < 0.001 |
| Return to normal daily activity | 16.3 ± 4.2 | 23.5 ± 6.2 | < 0.001 |

**Table (3) :** Pain score scale according to visual analogue scale (VAS).

| | Group I | Group II | P value |
|---|---|---|---|
| P.O day 1 | 5.3 ± 0.29 | 5.4 ± 0.62 | 0.43 |
| P.O day 2 | 2.4 ± 0.4 | 3.5 ± 0.31 | 0.02 |
| P.O day 7 | 1.2 ± 0.11 | 1.43 ± 0.13 | 0.03 |
| Analgesia | | | |
| No of tablets | 7.66 ± 1.1 | 9.5 ± 0.9 | 0.03 |
| No of day taken | 2.5 ± 0.13 | 3.13 ± 0.27 | 0.02 |

P.O → post operative
No → Number

**Table (4) :** Complications .

| | Group I | Group II | P value |
|---|---|---|---|
| Total | 6 | 11 | 0.12 |
| Scrotal oedema | 2 | 0 | 0.15 |
| Scrotal ecchymosis | 1 | 0 | 0.31 |
| Seroma | 1 | 0 | 0.31 |
| Wound haematoma | 1 | 0 | 0.31 |
| Chronic pain | 0 | 3 | 0.07 |
| Testicular atrophy | 0 | 1 | 0.31 |
| Mesh infection | 0 | 2 | 0.15 |
| Wound infection | 1 | 1 | |
| Recurrence | 0 | 4 | 0.05* |

**Table (5) :** Outcome of quality of life assessed by SF - 36-health survey.

| | Group I | | | Group II | | | P Value |
|---|---|---|---|---|---|---|---|
| | Pre.op | Post.op | Variation | Pre.op | Post.op | Variation | |
| Physical functioning | 63 | 71 | 12.6 ± 2.1 | 61 | 68 | 11.4±2.4 | < 0.048 |
| Role physical | 44 | 61 | 38.6±8.4 | 45 | 53 | 26.6±7.8 | < 0.001 |
| Bodily pain | 70 | 63 | 10±2.8 | 69 | 66 | 4.3±0.8 | < 0.002 |
| General health | 55 | 68 | 23.6±5.4 | 55 | 62 | 12.7±3.2 | < 0.001 |
| Vitality | 59 | 64 | 8.4±1.05 | 58 | 61 | 5.17±0.9 | < 0.001 |
| Social functioning | 55 | 77 | 40±11.2 | 54 | 71 | 31.5±9.8 | < 0.002 |
| Role emotional | 76 | 81 | 6.6±0.7 | 75 | 78 | 4±0.2 | < 0.001 |
| Mental health | 69 | 72 | 4.3±07 | 68 | 71 | 4.4±06 | 0.12 |

## Discussion

Bilateral repair of inguinal hernias has been accepted as a two-stage procedure, and their simultaneous repair has been discouraged for years. Stoppa described his midline preperitoneal approach for the repair of bilateral inguinal hernias. Data obtained in the last 20 years has shown that bilateral hernias should be repaired simultaneously rather than sequentially[2,16] Amid et al using the Lichtenstein technique (1) later advocated .Simultaneous repair of bilateral hernias under local anesthesia. In their recent review, Fischer et al documented excellently the feasibility and tolerability of the simultaneous repair of bilateral inguinal hernias[17]. The advantages of laparoscopic repair of both hernias simultaneously were also recently reported[18,19]. In this study, we prospectively researched the advantages and disadvantages of the two open, tension-free mesh techniques in the repair of bilateral inguinal hernias.

The operative time was significantly shorter in The Stoppa group (52 Vs 75 min) than bilateral Lichtenstein repair (P<0.0001). The average is much less than the 115 min described in the literature[20,21,22], but greater than the 51 min described by Stoppa[23]. This is may be because of Lichtenstein repair requires extensive dissection in the inguinal canal and involves manipulation of at least one or two inguinal nerves[24] with the need for extensive suturing and long-term mesh related complications to the surrounding cord structures.This was one of the few significant differences between the two groups. However, our 52 min which is the mean operative time for the GPRVS is considerably shorter than that of Solorzano et al [1999][20] (115 min), and was comparable to those for unilateral repair[2,19].

The mean hospital stay was 2 days in the first group and 70% of the cases were discharged after 24 hours while the mean hospital stay was 3 days in the second group and 56% of the cases were discharged after 24 hours .This rate of discharge after 24 hours is higher than recorded in other studies by 23% [20].

Liem et al [25] reported the time for return to normal activity is l0 days as in open inguinal surgery for unilateral hernia. Champault et al.,[26] acknowledged that return to work after the Stoppa operation in their series was 35 days, a figure very high than we find in our study 16 and 23 day periods in first and second groups respectively. Serpell et al., found that the median time to return to normal activity following simultaneous bilateral open hernia repair was 21 days. 1n addition, the median time to return to work following bilateral laparoscopic hernia repair is 12-14 days which compares favorably to the 28 days reported after simultaneous bilateral open repair[21].

Complications In this series, the Stoppa group showed complications rate of 16.6 % (16.6 % minor and 0 % major) comparable to 14% complication rate with other reports.[20,21,27,28] while in the second group, complication rate was 23.3% compared to 18% in other studies[29,30].

In our study the Stoppa repair was less painful on the 2nd and 7th postoperative days than the simultaneous bilateral Lichtenstein repair with corresponding less analgesic requirement, we think this was an important difference in terms of patients' postoperative comfort. Less pain has always been well accepted by the patients. The Lichtenstein and other tension-free procedures have always had better pain scores than conventional methods of tissue repair[1,19] Although the newer laparoscopic repair techniques have favorably lower pain scores than conventional open methods[25], their superiority over open tension-free repair techniques has not been demonstrated[31,17] However, laparoscopic repair causes less pain than the Stoppa procedure [26].

Post surgical chronic pain represents a major, largely unrecognized Clinical problem[32]. After mesh-based inguinal hernia repair, 10% of patients suffer chronic pain [24]. Postoperative pain can be the consequence of ongoing inflammation, extensive dissection in the inguinal canal or, much more commonly, a manifestation of iatrogenic neuropathic pain due to injury to peripheral

nerves[32,33] A last issue that might be addressed considering pain, is the influence of fixation of the mesh,[30]. By placing the mesh in the preperitoneal space, a physiological place for the mesh with intraabdominal forces on one side and the oblique muscles on the other, fixation seems less mandatory, although maybe not completely to be abandoned.

In the experience of Stoppa et al and others with GPRVS, all recurrences occurred within 6 months and were ascribed to technical failures, namely, placement of mesh with small size or which develops fissures[34]. The mesh presents a shrinkage, which reduces its size by 20% during the first 6 months, this fact, and knowledge based on a study done by Amid et al.[35] proposes the need to place mesh with larger size to prevent recurrences. The mesh should not be smaller than 24·16 $cm^2$, which is the distance between both iliac spines and from the navel to the pubis[36]. In our study, there was no recurrence among the first group, whereas in other series, the 2% recurrence rate using the GPRVS technique[37,21,27].

The second group, four patients showed direct recurrences 5, 7, 8 and 12 months respectively, all of them were repaired using preperitoneal approach. In addition, two patients presented with a femoral hernia after 9 and 12 months, respectively, both of them were obese, and their new femoral hernias were repaired with a plug technique. The mesh might create tension by pulling up the inguinal ligament and facilitating a femoral hernia. In fact, Lichtenstein has stated that up to 45% of femoral hernias may be secondary to groin [38] repair to the inguinal ligament) In GPRVS, the replacement of the endoabdominal fascia seals the inguinal, femoral, and obturator canals as well as all other potential sites of weakness in the lower abdomen. For this reason, late recurrences are not reported.

## Conclusion

Stoppa procedure is a reliable technique to repair bilateral inguinal hernia with less postoperative pain, faster return to normal daily activity, low recurrence rate and

high patent satisfaction and should be offered to patients who are at risk for recurrence .

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# BENHA MEDICAL JOURNAL


## STOPPA REPAIR VERSUS LICHTENSTEIN TECHNIQUE IN TREATMENT OF BILATERAL INGUINAL HERNIAS (PROSPECTIVE RANDOMIZED STUDY)


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# FEATURES OF HORMONAL DISTURBANCES IN CIRRHOTIC PATIENTS WITH HEPATIC ENCEPHALOPATHY

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## Abstract

***Introduction:*** *Features of hormone disturbances in patients suffering from chronic hepatic failure have been documented for many years.* ***Objective:*** *To evaluate the possibility of diagnostic and prognostic values of cortisol, total T3, TSH and prolactin concentrations in cirrhotic patients to predict HE and severity of hepatic disease.* ***Materials and methods:*** *study was carried out on 75 (40 males, 35 females) cirrhotic patients (HCV positive) with HE and 50 (28 males, 22 females) cirrhotic without HE (HCV positive). Patients underwent clinical evaluation with determination of the degree of HE. The severity of cirrhosis was assessed according to Child-Pugh classification. Immulite 1000 was used for determination of cortisol and total T3 and chemiluminescent immunometric assay for determination of prolactin and TSH.* ***Results:*** *Cortisol, total T3 concentrations in cirrhotic patients with HE were highly significant decreased versus patients without HE. Cut-off value for cortisol was less than 18.3 µg/dl could predict HE in cirrhotic patients according to AUROC curve showing a sensitivity of 52% and a specificity of 61%. Cut-off value for total T3 was less than 45.5 ng/dl could predict HE in cirrhotic patients showing a sensitivity of 64% and a specificity of 80.6%. Whereas prolactin concentration in cirrhotic patients with HE was highly significant increased versus patients without HE and its cut-off was more than 18.85 ng/dl could predict HE in cirrhotic patients showing a sensitivity of 88% and a specificity of 90.3%. TSH concentration showed no significant difference in patients with HE versus patients without HE. Serum cortisol, total T3 concentrations were signifi-*

*cantly decreased with progression of liver disease according to Child–Pugh classification. Prolactin level was highly significant increased with progression of liver disease from Child A to Child C.* ***Conclusion:*** *Hormonal abnormalities of cortisol, total T3 and prolactin may represent risk factors and early indicators of impending hepatic encephalopathy and progression of liver disease severity.*

***Keywords :*** *Cirrhosis - Cortisol -Hepatic encephalopathy- Prolactin - Total T3 -TSH.*

## Introduction

Hepatic encephalopathy (HE), a major complication of cirrhosis, is a clinical syndrome characterized by mental status changes in patients with severe hepatic insufficiency [1]. Features of hormone disturbances in patients suffering from chronic hepatic failure have been documented for many years[2]. The hypothalamic - pituitary - gonadal axis is affected the most[3], but also other hormones are altered by hepatic insufficiency as described for thyroid hormones[4] and growth hormone [5].

Liver is the primary site of metabolism of adrenal steroid hormone and synthesis of cholesterol, which is the major precursor of steroid[6]. Therefore, preexisting liver dysfunction may further disturb the activation of the hypothalamic - pituitary - adrenal axis (HPA) axis during severe sepsis and septic shock. Moreover, adrenal insufficiency in severe sepsis and septic shock may aggravate hemodynamic impairment in critically ill patients with cirrhosis, leading to a poor prognosis [7].

The liver plays an important role in the metabolism of thyroid hormones, being involved in their conjugation, excretion and peripheral deiodination, and in synthesizing thyroid binding globulin (TBG)[8]. Evidence of an association between chronic diseases of the liver and thyroid alterations have often been reported, but limited information is available on thyroid function tests in non-alcoholic cirrhotic patients with hepatic encephalopathy [9].

In cirrhosis, hypogonadism can be primary or central, resulting from the inhibition of the hypothalamus- pituitary - gonadal

axis[10], with studies showing simultaneous (gonadal and central) damage[11]. Hyperprolactinemia is often present in these patients, as well as hyperestrogenemia, both responsible for the clinical characteristics of feminization. Hyperprolactinemia and hyperestrogenemia can contribute to the genesis of hypogonadism.[12].

The main goal of the present study was to evaluate the possibility of diagnostic and prognostic values of cortisol, total T3, TSH and prolactin concentrations in cirrhotic patients to predict hepatic encephalopathy (HE) and the severity of hepatic disease as measured by the Child-Pugh score.

## Materials and Methods

This study was carried out on 75 (40 males, 35 females) cirrhotic patients (positive anti-HCV antibodies) with hepatic encephalopathy and 50 (28 males, 22 females) cirrhotic without hepatic encephalopathy (positive anti-HCV antibodies) infection without hepatic encephalopathy. They were age and sex matched with their mean age (49.22±5.62years) recruited from inpatient and outpatient clinic of tropical medicine department and specialized medical hospital, Mansoura University. Cirrhosis was diagnosed on a clinical basis involving laboratory tests, endoscopic evidence, sonographic findings and/ or previously by liver biopsy, and regular follow-up in our Liver Outpatient Clinic. The exclusion criteria were the presence of significant comorbid illness such as heart, respiratory or renal failure; and history of any neurologic disease such as Alzheimer's disease, Parkinson's disease or non-hepatic metabolic encephalopathies, Patients on psychoactive drugs, such as antidepressants or sedatives and patients receiving drugs known to cause hyperprolactinaemia.

All study participants underwent comprehensive biochemical, clinical evaluation with determination of the degree of encephalopathy. The severity of cirrhosis was assessed according to Child–Pugh classification into Child A (n=40), B (n=40) and C (n=45). For the calculation of the CTP score we used the Pugh score modification [13]. The degree of

encephalopathy was defined on the basis of previously reported criteria[14] ranked between grade 1 and grade 4. Informed consent was taken and the protocol was approved by the hospital ethical committee in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

**Laboratory investigations :** Routine laboratory tests include serum creatinine and liver function tests (albumin, total bilirubin, ALT, AST and INR) were done. Special protocol investigations include serum cortisol, TSH, total T3 and prolactin. Seven mls venous blood sample was collected. 1.6 ml of each blood sample was added to 0.4 ml of 3.8% sodium citrate containing tube and after mixing and centrifugation the separated plasma was used for prothrombin time determination from which INR was detected. The remaining blood were left to clot and serum was separated and kept in a deep freeze at -70$^{o}$C till used for liver function tests, Cortisol, TSH, total T3 and prolactin.

- **Hormones:** Immulite 1000 (competitive chemiluminescent immunometric assay) was used for determination of serum cortisol [15] and total T3 [16] and (solid phase 2-site chemiluminescent immunometric assay) was used for determination of serum prolactin [17] and TSH [18].

**Statistical Analysis:** Data was analyzed using SPSS (Statistical Package for Social Sciences) version 10. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data was tested for normality by Kolmogrov-Smirnov test. Normally distributed data was presented as mean ± SD. Student t-test was used to compare between two groups. Non parametric data was presented as min - max and median. Mann-Whitney and Kruskal-Wallis tests were used for comparison between groups. Spearman's correlation coefficient was used to test correlation between variables. Receiver Operating Curve (ROC) was done to determine a cut off point; sensitivity and specificity were calculated for this cut off point. $P < 0.05$ was considered to be statistically significant.

## Results

This study was carried out on 75 (40 males, 35 females) cirrhotic patients (positive anti-HCV antibodies) with hepatic encephalopathy and 50 (28 males, 22 females) cirrhotic without hepatic encephalopathy (positive anti-HCV antibodies). They were age and sex matched with their mean age (49.22±5.62 years). All study participants underwent comprehensive biochemical, clinical assessment with evaluation of the degree of encephalopathy. The severity of cirrhosis was assessed according to Child-Pugh classification in all patients into Child A (n=40), B (n=40) and C (n=45). Patients with HE ranked between grade I [n=21 (28%)], grade II [n= 20 (26.7%)] grade III [n=19 (25.3%) ]and grade IV [n=15 (20%)] (Table 1).

Cortisol, total T3 concentrations in cirrhotic patients with HE were highly significant decreased versus patients without HE (P = 0.001, P = 0.002). Cut-off value for cortisol was less than 18.3 μg/dl could predict HE in cirrhotic patients according to AUROC curve showing a sensitivity of 52% and a specificity of 61% (fig.1). Cut-off value for total T3 was less than 45.5 ng/dl could predict HE in cirrhotic patients according to AUROC curve showing a sensitivity of 64% and a specificity of 80.6% (fig.2), whereas prolactin concentration in cirrhotic patients with HE was highly significant increased versus patients without HE (P =0.007) and its cut-off was more than 18.85 ng/dl could predict HE in cirrhotic patients according to AUROC curve showing a sensitivity of 88% and a specificity of 90.3% (fig.3). Moreover TSH concentration showed no significant difference in patients with HE versus patients without HE (P = 0.15) (Table 2 and Table 6).

Serum cortisol, total T3 concentrations were significantly decreased with progression of liver disease according to Child–Pugh classification (A versus B, A versus C and B versus C) (P = 0.014, P = 0.007 and P = 0.006 respectively) for cortisol & (P = 0.029, P=0.000 and P=0.002 respectively) for total T3. In contrast serum prolactin level was highly significant increased with progression of liver disease from Child A to Child C (P = 0.023, P=0.000 and P =

0.007 respectively). In addition no significant difference was observed on comparing serum TSH level among Child A, Child B and Child C groups (P = 0.063, P=0.081 and P = 0.073) (Table 3).

Cortisol, total T3 concentrations were significantly decreased with progression of HE from grade I to grade IV (P = 0.004 and P = 0.003). Whereas prolactin concentration in HE was increased with progression of HE from grade I to grade IV (P=0.000) and TSH concentration showed no significant difference with progression of HE from grade I to grade IV (P = 0.08) (table 4).

The present study demonstrated positive significant correlation between serum total T3, serum cortisol concentrations and serum albumin, prothrombin time. Whereas there was a negative significant correlation between serum prolactin concentration and serum albumin, prothrombin time. In addition there was no correlation between serum TSH concentration and serum albumin and prothrombin time (table 5).

**Table 1.** Patients' Demographic and Laboratory Data.

| | Patients with HE (n = 75) | | Patients without HE (n = 50 ) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Age (years) | 49.22 | 5.62 | 51.38 | 7.56 |
| Gender (M/F) | 40/35 | | 28/22 | |
| S.Albumin (g/dl) | 3.24 | 0.60 | 3.38 | 0.46 |
| S.bilirubin (mg/dl) | 2.73 | 1.57 | 1.58 | 0.53 |
| Creatinine (mg/dl) | 1.19 | 0.49 | 1.09 | 0.45 |
| INR | 1.48 | 0.29 | 1.43 | 0.42 |
| AST (U/L) | 65 | 12 | 71 | 14 |
| ALT (U/L) | 49 | 6 | 52 | 7 |
| Child Class<br>A<br>B<br>C | <br>25(33.3%)<br>23(30.7%)<br>27 (36%) | | <br>15 (30%)<br>17(34%)<br>18 (36%) | |
| HE<br>I<br>II<br>III<br>IV | <br>21(28%)<br>20(26.7%)<br>19(25.3%)<br>15(20%) | | <br>0<br>0<br>0<br>0 | |

**Table 2:** Serum cortisol, TSH, total T3, prolactin concentrations in cirrhotic patients with HE versus patients without HE

| | Patients with HE (n = 75) | | Patients without HE (n = 50 ) | | P value |
|---|---|---|---|---|---|
| | Median | Range | Median | Range | |
| Cortisol (µg/dl) | 11 | 4 – 33 | 23.15 | 21 – 37 | 0.001 |
| TSH (µIU/ml) | 2.17 | 0.27 – 5.27 | 2.05 | 0.49 – 4.49 | 0. 15 |
| T3 (ng/dl) | 42.5 | 35 – 57.8 | 88 | 72.4 – 112 | 0.002 |
| Prolactin (ng/ml) | 22.25 | 7.35 – 71.2 | 9.11 | 8 – 12.9 | 0.007 |

**Table 3:** Serum cortisol, TSH, total T3, prolactin concentrations in cirrhotic patients with HE according to the severity of hepatic disease as measured by the Child-Pugh score

| | Cortisol (µg/dl) | TSH (µIU/ml) | Total T3 (ng/dl) | Prolactin (ng/dl) |
|---|---|---|---|---|
| | Median (Range) | Median (Range) | Median (Range) | Median (Range) |
| Child A (n 25=) | 24 (13.4 – 33) | 1.63 (0.27 – 3.5) | 52 (44 – 57.8) | 13.2 (7.35 – 19) |
| Child B (n 23=) | 13.7 (9 – 23) | 2.42 (0.42 -4.38) | 43 (41 – 52) | 18 (13 – 28) |
| Child C (n 27=) | 9.85 (4 – 16) | 2.8 (1.56 –5.27) | 39.5 (35 – 45) | 28.6 (15 – 71.2) |
| A versus B<br>A versus C<br>B versus C | P=0.014<br>P=0.007<br>P=0.006 | P=0.063<br>P=0.081<br>P=0.073 | P=0.029<br>P <0.000<br>P=0.002 | P=0.023<br>P<0.000<br>P=0.007 |

**Table 4:** Serum cortisol, TSH, total T3, prolactin concentrations in cirrhotic patients with different grades of hepatic encephalopathy

| | Grade I (n = 19) Median (Range) | Grade II (n = 16) Median (Range) | Grade III (n = 23) Median (Range) | Grade IV (n = 17) Median (Range) | P value |
|---|---|---|---|---|---|
| Cortisol (µg/dl) | 19.5 (10.3 – 33) | 19.6 (10 – 25) | 11 (8.8 – 15.3) | 8.8 (4 – 9.7) | 0.004 |
| TSH (µIU/ml) | 0.99 (0.27 –2.24) | 1.85 (0.4 – 2.14) | 2.80 (0.97 –4.25) | 3.39 (4.1 – 5.27) | 0.08 |
| Total T3 (ng/dl) | 48.5 (43.8 – 57) | 52 (40 – 53.2) | 42 (37 – 44) | 39 (35 – 41) | 0.003 |
| Prolactin (ng/dl) | 14.55 (7.35 -20) | 14.2 (12 – 25.6) | 22.25 (15 – 33) | 35 (26.6 – 71.2) | 0.00 |

**Table 5:** Correlation between serum albumin and INR with (serum cortisol, TSH, total T3 and prolactin)

| | Albumin (g/dl) | | INR | |
|---|---|---|---|---|
| | r | P | r | P |
| **Cortisol (µg/dl)** | 0.445 | 0.006 | – 0.628 | 0.003* |
| **TSH (µIU/ml)** | – 0.244 | 0.151 | 0.250 | 0.06 |
| **Total T3 (ng/dl)** | 0.453 | 0.006* | – 0.657 | 0.002* |
| **Prolactin (ng/dl)** | – 0.565 | 0.000* | 0.658 | 0.000* |

**Table (6):** Diagnostic performance of cortisol (µg/dl), Total T3 (ng/dl) and Prolactin (ng/dl) in studied cases according to ROC curves to differentiate cirrhotic with HE from cirrhotic without HE cases.

| | Cut-off | Sensitivity | Specificity | Accuracy |
|---|---|---|---|---|
| **Cortisol(µg/dl)** | 18.3 | 52% | 61% | 56.2% |
| **Total T3(ng/dl)** | 45.5 | 64% | 80.6% | 78.5% |
| **Prolactin (ng/dl)** | 18.85 | 88% | 90.3% | 95.9% |

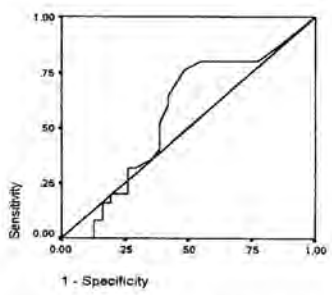


**Figure 1-ROC curve for cortisol**

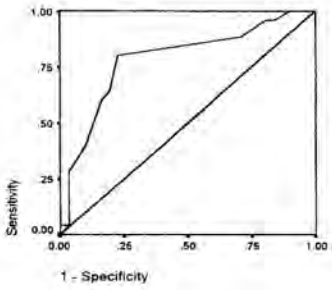


**Figure 2- ROC curve for total T3**

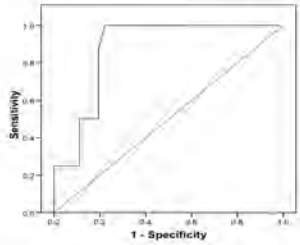


**Figure 3-ROC curve for prolactin**

## Discussion

Hepatic encephalopathy (HE) is a neurocognitive disorder in which brain function is impaired and is associated with both acute and chronic liver dysfunction. HE occurs in the presence of liver injury or when the liver is bypassed in the presence of a portosystemic shunt[19]. Features of hormone disturbances in patients suffering from chronic hepatic failure have been documented for many years [2].

Patients with cirrhosis are susceptible to bacterial infection,[20, 21] which can lead to circulatory dysfunction, renal failure, hepatic encephalopathy, and decreased survival. Actually, cirrhosis is associated with an increased risk of sepsis and sepsis-related death [22].

Patients with cirrhosis are characterized by hyperdynamic circulation, which is closely related to the complications of liver cirrhosis. The hemodynamic impairment can be made worse during sepsis, leading to multiple organ failure and mortality system dysfunction and may have contributed to mortality. In this regard our results showed that, serum level of cortisol was significantly decreased in patients with HE versus cirrhotic patient without HE and cortisol level was significantly decreased with progression of liver disease from Child A to Child C and decreased with severity of hepatic encephalopathy.

Consistent with these results from this study Tsai et al[7] reported that adrenal dysfunction may contribute to cardiovascular derangement and mortality in patients with cirrhosis and severe sepsis. Furthermore, Tsai et al found that adrenal dysfunction was related to the degree of liver failure and multiple organ system dysfunction and may have contributed to mortality. Fernández et al [23] found that Adrenal dysfunction was frequent in patients with advanced cirrhosis (Child C: 76% vs. Child B: 25%, P = .08). Harry et al.[24] recently showed that adrenal insufficiency is common and may contribute to hemodynamic instability and mortality in patients with acute liver failure.

Nevertheless, the impact adren-

al insufficiency has on the outcomes of patients with cirrhosis and severe sepsis and septic shock is unknown. It is plausible that liver disease per se may cause relative adrenal insufficiency (RAI) by a number of mechanisms. These include low levels of HDL cholesterol central to cortisol production [25], increased conversion of cortisol to the inactive cortisone and the negative effect of cytokines such as tumor necrosis factor (TNF-a) on hypothalamic function[26,27]. Our study differs from Sterczer et al[28] who concluded that there is a close relation between portosystemic encephalopathy and hypercortisolism in dogs with portosystemic shunts (PSS) and that both deviations resolve completely within four weeks of closure of the shunt and this difference could be attributed to that Sterczer et al investigated hypercortisolism in dogs with congenital portosystemic shunts without liver cell failure.

Thyroid dysfunction has been reported previously in a variety of non-thyroid illnesses including liver, pulmonary and renal neoplastic disease, severe systemic illness, fasting, malnutrition, postoperative state, physical trauma and acute infections. Low total and free T3 with normal total T4 and thyrotropin concentrations in the absence of clinical hypothyroidism have been frequently reported in patients with non-thyroidal illnesses [29- 31].

We found a significant reduction in serum total T3 in patients with HE than cirrhotic patient without HE, the lowest values being found in patients with grade IV hepatic encephalopathy and Child C. Whereas TSH showed no significant difference in patients with HE versus cirrhotic patient without HE. These phenomena can be explained by a reduced hepatic conversion of T4 to T3, also described as “low T3 syndrome” in liver cirrhosis [32, 33]. The mechanism being inversely related to the degree of hepatic dysfunction. It completely resolved after OLT in accordance with observations of Van Thiel et al.[34]. Hepner and Walfish reported a significant inverse correlation between serum T3 concentrations and the severity of liver dysfunction. A progressive decrease in T3 levels in chronic

liver diseases has been described as indicative of a poor prognosis [35,36]. Kayacetin et al [9] demonstrated a fall in FT3 and T3 parallel to severity of the disease. Borzio et al 1983[37] repored that despite clinical euthyroidism T3 was diminished in correlation with the severity of liver dysfunction, especially in Child C patients.

Prolactin secretion follows a pulsatile pattern, with a characteristic nocturnal rise, but cirrhosis is associated with elevated 24-h prolactin levels and loss of circadian prolactin rhythm [38-40]. These finding were in consistent with the present study that showed significantly increased serum level of prolactin in patients with HE more than cirrhotic patients without HE and its level was significantly increased with progression of liver disease from Child A to Child C(table 3) and increased with severity of hepatic encephalopathy (table 4). Koller et al[41] found that 16.7% of patients had elevated serum prolactin levels, and had significantly higher Child-Pugh and MELD scores as well as higher ascites and encephalopathy stage, they also found that ascites, higher INR, jaundice and higher Child-Pugh and MELD scores were more often with increasing prolactin concentrations.

Mukherjee et al[42] reported that majority of patients of cirrhosis with suspected portal-systemic encephalopathy had significantly higher serum prolactin than those without encephalopathy (p =0.05). Significantly higher values of serum prolactin on admission had positive correlation with mortality (p=0.01). Clinico-biochemical severity of hepatic dysfunction was directly correlated with level of serum prolactin.

The present study revealed a fall in serum total T3, serum cortisol parallel to severity of the disease and HE, and a good correlation between serum total T3, serum cortisol concentrations, serum albumin and prothrombin time. Whereas there was a significant negative correlation between serum prolactin concentration and serum albumin and prothrombin time. These results suggest that serum total T3, serum cortisol and serum prolactin concentrations may be considered a

sensitive index of hepatic function in liver disease and severity of HE.

Limitations of the study include the relatively small number of patients so results obtained from this study can't be generalized, further studies with larger sample size are required. On the other hand short Synacthen (corticotrophin) stimulation test (SST) was not used to evaluate the appropriateness of the adrenal response in this setting due to ethical consideration.

In conclusion, patients with liver cirrhosis complicated by hepatic encephalopathy were found to have low serum total T3, serum cortisol and high serum prolactin levels and are associated with disease severity and progressing grades of hepatic encephalopathy. Depressed serum total T3, serum cortisol and increasing serum prolactin levels, together with a prolonged prothrombin time and decreased serum albumin, therefore appear to be characteristic of cirrhotic patients prone to develop hepatic encephalopathy. So, hormonal abnormalities of cortisol, total T3 and prolactin may represent risk factors and early indicators of impending hepatic encephalopathy and progression of liver disease severity.

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# BENHA MEDICAL JOURNAL


## FEATURES OF HORMONAL DISTURBANCES IN CIRRHOTIC PATIENTS WITH HEPATIC ENCEPHALOPATHY


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# OUTCOME OF ADENOIDECTOMY IN RESOLUTION OF OTITIS MEDIA WITH EFFUSION AS A METHOD TO AVOID COMPLICATIONS OF EAR SURGERY

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## Abstract

***Objective:*** *To evaluate the outcome of adenoidectomy in improving patients aged 4-8 years complaining of otitis media with effusion (OME) as a method to keep ear drum intact with avoiding complications of ear surgery.*

***Patients & Methods:*** *This study included 100 patients aged 4-8 years diagnosed clinically and by pneumatic otoscopy, tympanometry and x-ray soft tissue neck lateral view, to have bilateral middle ear effusion (type B tympanogram) for at least 3 months without improvement with adenoid or adenotonsiller enlargement, patients were divided into two groups, Group A : included 50 children ,this group was operated upon by right side myringotomy and grommet tube insertion ,left ear drum kept intact and Group B: included 50 children ,this group was operated upon by right side myringotomy only without grommet insertion, left ear drum kept intact, adenoidectomy or adenotonsillectomy was done for the two groups.*

*For the intact drums follow up of the children included in group A and B by tympanogram was done after the $1^{st}$, $3^{rd}$, $7^{th}$, $9^{th}$, $12^{th}$ month. For the myringotomy and tube ears follow up by tympanogram was done after tube extrusion or tube removal and healing of tympamnic membrane after the $7^{th}$,$9^{th}$,$12^{th}$ month. For the myringotomy ears follow up by tympanogram was done after healing of tympamnic membrane after the $3^{rd}$, $7^{th}$,$9^{th}$,$12^{th}$ month. Ear drum changes such as perforation, retraction, myringosclerosis,and otorrhea were reported during the follow up period and all data were collected.*

***Results:*** *in group A and out of 50 patients with intact drums, patients with type A tympanogram were (24%) after 1 month, (44%) after 3*

*months raised to (68%) after 7 months and reached (78%) after 12 months and out of 50 myringotomy and tube ears patients with type A tympanogram were(74%) after 7 months and reached 82% after 12 months ,in group B and out of 50 patients with intact drums, patients with type A tympanogram were (20%) after 1 month (40% )after 3 months raised to (70%) after 7 months and reached 80% after 12 months and out of 50 myringotomy ears patients with type A tympanogram were (72%) after 3 months raised to (78%) after 7 months and reached 80% after 12 months.*

***Conclusion:*** *we concluded that inspite of marked improvement in patients operated by myringotomy or myringotomy and tube insertion soon after surgery, but on follow up patients with adenoidectomy without myringotomy or myringotomy and tube insertion with keeping ear drum intact, those patients improved near the same degree of myringotomy or myringotomy and tube insertion after about 7 months ,thus adenoidectomy alone in patients with age group 4-8 years may have a value in resolution of OME with keeping ear drum intact, with avoiding risk of complications of ear surgery.*

***Key words:*** *otitis media with effusion, myringotomy, adenoidectomy, myringotomy with tube insertion.*

## Introduction

Otits media with effusion (OME) (secretory otitis media) is a middle ear inflammation in which there is retro tympanic liquid collection without signs or symptoms of acute infection and integral tympanic membrane.[1] Otitis media with effusion (OME) is one of the most common disorders in childhood and the leading cause for conductive hearing loss in children.[2] The proper indication for treatment of otitis media with effusion by myringotomy or ventilation tube insertion remains controversial.[3] Complications in insertion of ventilation tubes include otorrhea, tympanosclerosis, tympanic membrane perforation, retraction and cholesteatoma.[4] Sequele once tympanostomy tubes have been extruded are not uncommon, these sequele include residual perforation of the tympanic membrane, tympanosclerosis, segmental atrophy, retraction or retraction pocket, cholesteatoma and hearing loss.[5] The rationale of adenoidectomy in the

treatment of OME has been to remove a potentially chronically infected focus in the pharynx[6]. The use of adenoidectomy in the treatment of OME include possible improvement of eustachian tube function as a large adenoid could cause eustachian tube dysfunction by blocking the nasopharyngeal orifice of the tube.[7] Adenoidectomy has been reported to be beneficial in treating children who are older than 4 years and have OME.[8] The resolution rate of OME was better after adenoidectomy in children with larger adenoid compared to children with smaller adenoid.[9] Benefits must be weighed against side effects of treatment as well as sequele induced by the treatment itself [3].

## Materials and Methods

This study was carried out in the otorhinolaryngology department of Benha university hospital from February 2007 to August 2009 to allow follow up period of one year for the last children operated upon, the protocol of this study was approved by the Local Ethical Committee, informed consent from the parents of every child was taken, children aged 4-8 years and suffering from nasal obstruction and hearing impairment with suspicion of otitis media with effusion (OME) were collected from outpatient clinic of otorhinolaryngology department of Benha university hospital, history was obtained for each child and after full Ear, Nose and Throat examination, patients with signs of OME such as fluid levels, air bubbles, absence of cone of light, foreshortened handle of malleus on otoscopic examination in addition to history of nasal obstruction and mouth breathing were collected, pneumatic otoscopy, tympanometry and x-ray soft tissue neck lateral view were done for every child and the children with bilateral middle ear effusion (type B tympanogram) for at least 3 months without improvement and adenoid or adenoid and tonsil enlargement were chosen for this study, patients with previous myringotomy with or without insertion of ventilation tube, previous adenoidectomy or tonsillectomy, history of ear surgery, cleft palate ,down syndrome, congenital malformation of the ear, cholesteatoma or chronic mastoiditis, tym-

panic membrane perforation, middle ear destructive changes, sensoneural hearing loss were excluded from the study.

100 patients included in this study were divided into two groups:

- **Group A :** included 50 children, this group was operated upon by right side myringotomy and grommet tube insertion ,left ear drum kept intact .

- **Group B :** included 50 children, this group was operated upon by right side myringotomy only without grommet insertion, left ear drum kept intact, adenoidectomy or adenotonsillectomy was done for the two groups.

For the intact drums follow up of the children included in group A and B by tympanogram was done after the 1st, 3rd, 7th, 9th, 12th month. For the myringotomy and tube ears follow up by tympanogram was done after tube extrusion or tube removal and healing of tympamnic membrane after the 7th, 9th, 12th month. For the myringotomy ears follow up by tympanogram was done after healing of tympamnic membrane after the 3rd, 7th, 9th,12th month. Ear drum changes such as perforation, retraction, myringosclerosis, and otorrhea were reported during the follow up period and all data were collected and analyzed for running this study, children with type B tympanogram after the follow up period received either reinsertion of a ventilation tube on the right side or a secondary ventilation tube on the left side or both.

## Results

In group A and after surgery out of 50 patients with intact drums, number of patients with type A tympanogram were 12 (24%) after 1 month, 22 (44%) after 3 months, 34 (68%) after 7 months, 37 (74%) after 9 months, 39 (78%) after 12 months and number of patients with type C tympanogram were 18 (36%) after 1 month, 14 (28%) after 3 months, 15 (30%) after 7 months, 7 (14%) after 9 months, 1 (2%) after 12 months and number of patients with type B tympanogram were 20 (40%) after 1 month, 14 (28%) after 3 months, 1 (2%) after 7 months, 6 (12%) after 9 months, 10 (20%) after 12 months (Table 1), also in group A after surgery

and out of the ears with myringotomy and tube insertion, number of patients with type A tympanogram were 37 (74%) after 7 months, 41(82%) after 9 months, 41 (82%) after 12 months, number of patients with type C tympanogram were 11 (22%) after 7 months, 6 (12%) after 9 months, 0 (0%) after 12 months and number of patients with type B tympanogram were 0 (0%) after 7 months 1 (2%) after 9 months, 7 (14%) after 12 months, (Table 1) (there were 2 patients who had persistent perforation after tube extrusion till end of the study).

In intact drums there was significant difference between improved patients (patients with type A tympanogram) after 3 months in comparison with who improved after 1 month ($p<0.05$), and high significant difference after 7, 9, 12 months in comparison with who improved after 1 month ($p<0.001$) (Table 2) also there was no significant difference between improved patients in intact drums (68%) or myringotomy and tube (74%) after 7 months ($p>0.05$) (Table 3).

In group B and after surgery, out of 50 patients with intact drums, number of patients with type A tympanogram were 10 (20%) after 1 month, 20 (40%) after 3 months, 35 (70%) after 7 months, 35 (70%) after 9 months, 40 (80%) after 12 months and number of patients with type C tympanogram were 20 (40%) after 1 month, 15 (30%) after 3 months, 14 (28%) after 7 months, 10 (20%) after 9 months, 1 (2%) after 12 month and number of patients with type B tympanogram were 20 (40%) after 1 month, 15 (30%) after 3 months, 1 (2%) after 7 months, 5 (10%) after 9 months, 9 (18%) after 12 months (Table 1).

In the ears with myringotomy, number of patients with type A tympanogram were 36 (72%) after 3 months, 39 (78%) after 7 months, 40 (80%) after 9 months, 40 (80%) after 12 months and number of patients with type C tympanogram were 13 (26%) after 3 months, 10 (20%) after 7 months, 5 (10%) after 9 months, 1(2%) after 12 months and number of patients with type B tympanogram were 0 (0%) after 3 months, 0 (0%) after 7 months 4

(8%) after 9 months 8 (16%) after 12 months. (Table 1), (there was 1 patient who had persistent perforation after myringotomy till end of the study).

In intact drums there was significant difference between improved patients (patients with type A tympanogram) after 3 months in comparison with who improved after 1 month ($p<0.05$), and high significant difference after 7,9,12 months in comparison with after 1 month ($p<0.001$), and in ears with myringotomy there was no significant difference between improved patients after 3,7,9,12 months ($p>0.05$) (Table 2).

On comparison intact drums with the myringotomy ears there was significant difference between improved patients in myringotomy ears after 3 months (72%) and improved patients in intact drums after 3 months (40%) ($p< 0.05$), (Table 3). but there was no significant difference between improved patients in intact drums (70%)or myringotomy (78%) after the 7th month ($p>0.05$). so there was no significant difference between improved patients in intact drums, myringotomy ears, myringotomy and tubed ears after the 7 month (Table 2).

During period of follow up otorrhea was (34%) in patients with tube insertion, (16%)in patients with myringotomy, (4%) in intact drums, perforation was (4%) in patients with tube insertion, (2%) in patients with myringotomy, (0%) in intact drums, retraction was (20%) in patients with tube insertion, (18%) in patients with myringotomy, (16%) in intact drums, tympanosclerosis was (30%) in patients with tube insertion, (10%) in patients with myringotomy, (4%) in intact drums, cholesteatoma was (0%) in all, patients with type B tymanogram at the end of follow up period were (14%) in patients with tube insertion, (16%) in patients with myringotomy, (20%) in intact drums (Table 4).

**Table 1:** list of study groups .

| Study group | Type of tympanogram | Before operation | 1 month after operation | 3months after operation | 7 months after operation | 9 months after operation | 12months after operation |
|---|---|---|---|---|---|---|---|
| intact drums in group A | A<br>C<br>B | 50 | 12<br>18<br>20 | 22<br>14<br>14 | 34<br>15<br>1 | 37<br>7<br>6 | 39<br>1<br>10 |
| intact drums in group B | A<br>C<br>B | 50 | 10<br>20<br>20 | 20<br>15<br>15 | 35<br>14<br>1 | 35<br>10<br>5 | 40<br>1<br>9 |
| ears with myringotomy in group B | A<br>C<br>B | 50 | | 36<br>13<br>0 | 39<br>10<br>0 | 40<br>5<br>4 | 40<br>1<br>8 |
| tubed ears in group A | A<br>C<br>B | 50 | | | 37<br>11<br>0 | 41<br>6<br>1 | 41<br>0<br>7 |

**Table (2)** :Comparison of the study groups

| Study group | Improved left intact drums in patients with myringotomy and tube insertion Group A | | | Improved left intact drums in patients with myringotomy only Group B | | | Improved right ears With myringotomy only Group B | | | Improved right ears with myringotomy and tube insertion Group A | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | No | % | Test of significant | No | % | Test of significant | No | % | Test of significant | No | % | Test of significant |
| 1 month | 12 | 24% | | 10 | 20% | | | | | | | |
| 3 months | 22 | 44% | Z1=1.71 P<0.05 | 20 | 40% | Z5=1.83 P<0.05 | 36 | 72% | | | | |
| 7 months | 34 | 68% | Z2=3.24 P<0.001 | 35 | 70% | Z6=3.73 P<0.001 | 39 | 78% | Z9=0.35 P>0.05 | 37 | 74% | |
| 9 months | 37 | 74% | Z3=3.57 P<0.001 | 35 | 70% | Z7=3.73 P<0.001 | 40 | 80% | Z10=0.46 p>0.05 | 41 | 82% | Z12=0.45 p>0.05 |
| 12 months | 39 | 78% | Z4=3.78 P<0.001 | 40 | 80% | Z8=4.24 P<0.001 | 40 | 80% | Z11=0.46 p>0.05 | 41 | 82% | Z13=0.45 p>0.05 |

Z1=% after 3M vs % after 1M
Z2=% after 7M vs % after 1M
Z3=% after 9M vs % after 1M
Z4=% after 12M vs %after 1M
Z5=% after 3M vs %after 1 M
Z6=% after 7M vs % after 1M
Z7=% after 9M vs % after 1M.
Z8=% after 12M vs % after 1M
Z9=% after 7M vs %after 3 M
Z10=% after 9M vs % after 3M
Z11=% after12M vs %after 3 M
Z12=% after 9M vs % after 7M
Z13=% after 12M vs %after 7 M
No=number
%=percentage
Z=z test
p=p value
M=month

**Table 3:** Comparison of the study groups.

| Comparison of improved ears | Z | P |
|---|---|---|
| 72% in Rt myringotomy ears vs 40 % in Lt intact drums after 3 ms | 2.14 | <0.05 |
| 78% in Rt myringotomy ears vs 70 % in Lt intact drums after 7ms | 0.46 | >0.05 |
| 74% in Rt tubed ears vs 68% in Lt intact drums after 7ms | 0.36 | >0.05 |
| 78% in myringotomy ears vs 74% in tubed ears after 7ms | 0.36 | >0.05 |

**Table 4 :** incidence of complications .

| Complication | Patients with tube insertion N=50 | Patients with myringotomy only N=50 | Intact drums N=100 |
|---|---|---|---|
| Otorrhea | 34% | 16% | 4% |
| Perforation | 4% | 2% | 0% |
| Retraction | 20% | 18% | 16% |
| Tympanosclerosis | 30% | 10% | 4% |
| Cholesteatoma | 0% | 0% | 0% |
| type B tymanogram at the end of follow up | 14% | 16% | 20% |

## Discussion

Bluestone et al.,[7] reported that a possible improvement of the eustachian tube function is considered as the main reason for including adenoidectomy as a part of the surgical treatment of otitis media with effusion.

This study included 2 groups , group A and group B, on studying patients with intact drums in group A, B there was significant difference between improved patients(patients with type A tympanogram) after 3 months in comparison with after 1 month ($p<0.05$), and high significant difference after 7, 9, 12 months in comparison with after 1 month ($p<0.001$), but in ears with myringotomy and tube there was no significant difference between improved patients after 7, 9, 12 months and in ears with myringotomy there was no significant difference between improved patients after 3, 7, 9, 12 months. ($p>0.05$).

On comparison intact drums with the ears with myringotomy and tube there was no significant difference between improved patients in intact drums (68%) or myringotomy and tube (74%) after 7 months

On the other hand there was significant difference between improved patients in myringotomy ears after 3 months (72%) in comparison with improved patients in intact drums after 3 months (40%) ($p<0.05$), but there was no significant difference between improved patients in intact drums (70%) or myringotomy (78%) after 7 months

(p>0.05), so there was no significant difference between improved patients in intact drums about (70%) or myringotomy (78%) or myringotomy and tube (74%) after 7 months (p>0.05), so inspite of marked improvement occurred in ears with myringotomy and tube &ears with myringotomy alone soon after surgery than drums kept intact but after about 7 months from surgery the improvement was near similar in all of them.

Caye- Thomasen et al.,[3] reported significant difference in the mean hearing thresholds only at the first postoperative audiometry test at 3rd month, all subsequent audiometry tests show no difference in the hearing thresholds for both groups. Popova et al.,[10] reported that on comparing myringotomized patients vs patients with ventilation tubes there was no difference in the mean hearing thresholds between the two groups for the whole follow up period.

Maw,[9] enrolled 103 children 2-11 years of age with bilateral chronic otitis media with effusion and followed them for one year, all underwent unilateral tympanostomy tube placement and assigned to adenoidectomy, adenotonsilletomy or no surgery, he mentioned that adenoidectomy was significantly more effective compared to control group as regard to resolution of fluid and better resolution was in older 6 years and up children Gates, et al.,[11] assigned 578 children 4-8 years with otitis media with effusion to four groups and followed them for 2 years, the myringotomy group had the greatest amount of time with fluid (51 weeks), the adenoidectomy and myringotomy group and the adenoidectomy with myringotomy and tube groups both had a lower percentage of time with effusion (31- and 27 weeks) respectively than myringotomy with tube alone( 36 weeks) and he recommended adenoidectomy and myringotomy as the first line procedure Coyte et al.,[12] reported that adenoidectomy or adenotonsillectomy at the time of initial tympanostomy tube insertion reduces the likelihood of reinsertion of tubes among children 2 years or older. Boston et al.,[13] reported that adenoidectomy reduces significantly the

probability of needing a second set of tubes. Kadhim et al.,[14] mentioned that proportion of children having a second tube insertion was 16% lower in children who had adenoidectomy and 17 % lower with adenotonsillectomy compared to children who had tube insertion alone.

On the other hand in the study carried by [15] and [16], both studies concluded that adenoidectomy can not be recommended as the primary treatment in very young children. Casselbrant et al.,[17] mentioned that in children aged 24-47 months adenoidectomy does not add any further benefit to myringotomy and tube for the treatment of middle ear effusion. Hammaren-Malmi et al.,[16] has shown that there is no association between the size of the adenoid and the outcome of the surgical procedure. In children 4 years or older adenoidectomy with myringotomy alone may be effective but tube insertion is advised for younger children[17]. Paradise et al.,[18] recommended that ventilation tubes as first line of surgical therapy as hearing loss in OME is the most troublesome and therefore important manifestation of the disease because it is a risk factor for subsequent linguistic, educational and developmental impairments.

In this study otorrhea was 34% in patients with tube insertion, 16% in patients with myringotomy, 4% in intact drums. Gates et al.,[11] reported that purulent otorrhea occurred one or more times in 11% of adenoidectomy and myringotomy patients and in 24% of adenoidectomy and tube insertion patients Popova et al.,[10] reported that none of adenoidectomy and myringotomy had otorrhea episodes while 40% of adenoidectomy and tube insertion occurred. Casselbrant et al.,[17] reported that otorrhea occurred in 10.1% in adenoidectomy and myringotomy and 40.9% in adenoidectomy and tube insertion.

In this study perforation was 4% in patients with tube insertion, 2% in patients with myringotomy, 0% in intact drums. Daly et al.,[4] reported that tympanic membrane perforation after tube extrusion was 3%. In the study of [5] was 2.2%. Johnston et

al.,[19] reported that perforation was 4.5%, this in short duration tubes raised to 16.6% in long duration tubes as reported by [20].

In this study retraction in pars tensa or pars flacida was 20% in patients with tube insertion, 18% in patients with myringotomy,16% in intact drums.Valtonenet al.,[21] reported 7.1% retraction of pars flacida and 9.6% retraction of pars tensa In children received tubes. Maw and Bawden,[22] reported that retraction rate of tympanic membrane was 26%. Kay et al.,[5] reported that retraction rate of tympanic membrane was 28.1%. Tos et al.,[23] reported that retraction rate of tympanic membrane was 37%. Daly et al.,[4] reported that retraction rate of tympanic membrane was 47% .

In this study, tympanosclerosis was 30% in patients with tube insertiom,10% in patients with myringotomy, 4% in intact drums.In patients with tympanostomy tubes tympanosclerosis was 32% in the study of [5] and was 40% in the study of [4]

In this study cholesteatoma was 0% in all, Kay et al.,[5] reported 0.8% of cholesteatoma out of 8321 ears that undergone myringotomy and tube.

In this study, patients with type B tympanogram after the end of follow up period were14%. in patients with tube insertion, 16% in patients with myringotomy and about 20% in intact drums Popova et al.,[10] reported that recurrence of OME in adenoidectomy and myringotomy group was 10% compared to adenoidectomy and tube insertion group that was 14%, recurrence occur in 19.9% in the study of [13] who reported that the need to reinsert ventilation tubes was almost twice higher in the group of younger children when they performed the first surgery reinforcing the role of immune maturation in the decrease of these diseases.

We concluded that inspite of marked improvement in patients operated by myringotomy or myringotomy and tube insertion soon after surgery, but on follow up patients with adenoidectomy without myringotomy or myringotomy and tube insertion those patients im-

proved near the same degree of myringotomy or myringotomy and tube insertion, thus adenoidectomy alone in patients with age group 4-8 years may have a value in resolution of OME with keeping ear drum intact, with avoiding risk of complications of ear surgery.

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# BENHA MEDICAL JOURNAL


## OUTCOME OF ADENOIDECTOMY IN RESOLUTION OF OTITIS MEDIA WITH EFFUSION AS A METHOD TO AVOID COMPLICATIONS OF EAR SURGERY


**Ahmed El-Shall MD**



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# EFFECT OF EXERCISE ON ADIPONECTIN LEVEL AND HEPATIC STEATOSIS IN RATS

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## Abstract

***Background:*** *Hepatic steatosis or fatty liver is a common cause of liver function tests abnormalities, and there is a growing attention on the role of adiponectin in the development of NAFLD and its relation to the severity of hepatic steatosis. The previous studies on the effect of exercise on adiponectin level and its relation to intrahepatic fat content gave conflicting results. Therefore this study was undertaken to determine the effect of exercise on adiponectin level in high fat diet (HFD) induced hepatic steatosis.* ***Methods:*** *Male Sprague-Dawley rats were given either standard diet or HFD with or without exercise.* ***Results:*** *The HFD fed group showed significant increase in the levels of plasma free fatty acid (FFA), serum triglycerides (TG), serum cholesterol, and serum LDL. Also the levels of ALT, AST and ALP were significantly increased. Conversely, the serum adiponectin level was significantly lowered. The liver tissues TG content and the histopathological score of hepatic steatosis were significantly increased. Exercise training significantly increased the serum adiponectin levels in both standard diet and HFD fed groups. While concurrent exercise with HFD ameliorated the development of hepatic steatosis, through the reduction of the liver TG content and decreased the histopathological score of steatosis. Also concurrent exercise with HFD improved the serum lipid profile and decreased the levels of FFA, ALT, AST and ALP. In* ***conclusion:*** *Exercise training increases the serum adiponectin levels, and reduces the development of hepatic steatosis through the reduction of plasma FFA and improvement of serum lipid profile.*

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome[1]. NAFLD is a common cause of liver function tests abnormalities among adults, with a prevalence of 10-24% in the general population[2]. Fatty liver or hepatic steatosis refers to a condition characterized by an excess accumulation of triglycerides within hepatocytes[3]. Although simple steatosis is a benign process, some cases develop steatohepatitis, which then progresses to hepatic fibrosis, cirrhosis and even liver failure.

Adipose tissue is an active player in the regulation of metabolism since the discovery of adipocyte-derived factors, known as adipokines[4]. These adipokines include several members as adiponectin, leptin, resistin, interlukin-6 (IL-6), tumour necrosis factor-α (TNF-α). Most of these adipokines are elevated in obese persons, whereas adiponectin is reduced in obesity[5]. There is a complex interaction between many adipocytokines and the pathogenesis of NAFLD[6]. There is a growing attention on the role of adiponectin in the development of NAFLD and its relation to the severity of hepatic steatosis passing to cirrhosis[7].

There is a controversy about the effect of exercise on the circulating level of adipocytokines. Limited data showed that exercise training does not influence adipokines expression and release. Several other studies found that increased physical activity decrease the circulating levels of proinflammatory cytokines as TNF-α and increase adiponectin level[8]. So, the exact effects of exercise training on adipokines still need clarification. Also there are controversial results about the effect of exercise on reduction of the intrahepatic fat content in fatty liver. Some studies failed to demonstrate effect, while others reported the presence of effect of exercise training on hepatic steatosis [9]. However Perseghin et al.,[10] demonstrated that a higher level of habitual physical activity is associated with a lower intrahepatic fat content.

The present study is conducted to clarify the following aspects in

relation to nonalcoholic fatty liver disease: 1) Possible role of adiponectin level changes in hepatic steatosis. 2) Effects of exercise training on the following: adiponectin levels in fatty liver, serum lipid profile in hepatic steatosis, serum levels of ALT, AST, alkaline phosphatase (ALP) and intrahepatic fat content.

## Materials and Methods

**Experimental animals :**

Fourty Sprague-Dawley male rats (weighing 100 - 120 gm) (about 6 weeks) were obtained from the animal house of medical experimental research center (MERC), faculty of medicine, Mansoura university. They were caged, 3 per cage, in a controlled temperature room at (22-25 C). They were maintained on standard rat chow diet and free access to water for 4 weeks until reached 150-200 gm.

**Experimental design:**

The animals were randomly assigned into 4 groups 10 rats in each group as the following: A) ***Standard diet fed groups (SD):*** that were subdivided into 2 subgroups: **Group I** (control group): Standard diet fed sedentary group (SD Sed) : these animals were maintained on standard rat chow for 12 weeks. The standard rat diet consists of : 12.5% lipids, 63.2% carbohydrate, and 24.3% protein[11]. **Group II:** Standard diet fed exercise trained group (SD ExT): these animals were maintained on standard rat chow for 12 weeks associated with daily exercise training by swimming in water pool starting by 5 min daily then gradually increased up to 15 min at the end of the study duration[12]. B) ***High fat diet fed groups (HFD):*** that were subdivided into 2 subgroups: **Group III:** High fat diet fed sedentary group (HFD Sed): these animals were maintained on HF diet for 12 weeks. The HF diet consisted of 42% lipid, 36% carbohydrate, and 22% protein[11]. **Group IV:** High fat diet fed exercise trained group (HFD ExT): these animals were maintained on HF diet for 12 weeks associated with daily exercise training by swimming in water pool starting by 5 min daily then gradually increased up to 15 min at the end of the study duration[12].

All animals were weighed twice per week and their food intake was monitored three times per week for 12 weeks. At the end of this period, blood and tissue samples were taken.

**Collection of samples**

At the end of the study period the animal was fasted over night (about 8hours) then at the morning the animal weighed and given thiopental (50 mg/kg) intraperitoneal. Then the animal fixed on the dissecting table and the abdominal cavity was opened longitudinally. The blood sample was collected by syringe from the heart and put in 2 sets of tubes one with anticoagulant (EDTA) for plasma samples and the other for serum samples.

**Harvesting Liver specimen**

The liver was infused with saline through the aorta to wash the blood content. Then the liver was dissected carefully and removed from the abdomen then washed with saline and dried by a piece of gauze. The liver weight was recorded then the liver was divided into 3 pieces by a scalpel, one piece was put into formaldehyde 10% for haematoxline and eosine (H&E) and Masson Trichrome stains, while the second piece was rapidly frozen for oil red O staining, for histopathological examination. The third piece was rapidly stored frozen below -70 Co for homogenization, and assay for liver triglyceride content.

Analytic procedures: Serum level of liver enzymes AST, ALT, alkaline phosphatase (ALP) was determined using the available kit from bioMerieux (Marcy-l Etoile, France). Adiponectin concentrations were measured with commercially available ELISA kits (RayBiotech). Serum lipid profile, serum triglyceride (TG), Cholesterol, LDL and HDL concentrations were measured using kits from Spinreact (Spain). Plasma free fatty acid (FFA) levels were measured by colorimeteric method already described for chloroform solutions of fatty acids [13].

**Liver tissue:** For quantitative analysis of liver TG content 200-mg fragments from the liver were homogenized in 20 volumes of isopropanol, and then the supernatant was assayed for TG using a

commercial triglycerides kit (Spinreact) (Spain).

For histological analysis: Liver specimens were embedded in paraffin blocks and sections of 5 µ thick were made then stained with H & E and Masson Trichrome for evaluation of the degree of liver fibrosis. Frozen-embedded liver sections were cut in a cryostat at 5 µm and mounted on microscope slides then fixed with formaldehyde (10%) and stained with oil red O. Lipid infiltration was assessed with light microscopy. This enabled a morphological quantification of liver lipid infiltration in terms of lipid vacuole size and number.

Statistical analysis: Values are expressed as means ± SD. Statistical analyses were performed by a one-way ANOVA for nonrepeated-measures design, with diet and exercise training as main effects. $P < 0.05$ was considered statistically significant.

## Results

**1) Effect of exercise on rat body weight, liver weight and liver weight/ body weight ratio.**

As shown in table (1), concurrent exercise with standard diet produced insignificant increase in the rat body weight from 238.5 ± 23.1 gm to 263 ± 39.4 gm, also high fat diet produced insignificant increase in the rat body weight from 238.5 ± 23.1 gm to 260 ± 35.3 gm as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused insignificant increase in the rat body weight from 260 ± 35.3 gm to 266.5 ± 23.9 gm, as compared with high fat diet fed sedentary group. As shown in table (1), concurrent exercise with standard diet caused significant increase in the rat liver weight from 6.15 ± 0.78 gm to 9.43 ± 1.85 gm ($P < 0.001$), also high fat diet caused significant increase in the rat liver weight from 6.15 ± 0.78 gm to 8.63 ± 1.96 gm ($P < 0.001$) as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused insignificant decrease in the rat liver weight from 8.63 ± 1.96 to 7.55 ± 1.41 gm, as compared with high fat diet fed sedentary group. But Concurrent exercise with standard diet increased the rat liver weight / body weight ratio significantly from 2.58 ± 0.25

to 3.59 ± 0.50 (P < 0.01), also high fat diet increased the rat liver weight / body weight ratio significantly from 2.58 ± 0.25 to 3.45 ± 0.41 (P<0.01) as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused significant decrease in the rat liver weight / body weight ratio from 3.45 ± 0.41 to 2.83 ± 0.48 (P<0.05) as compared with high fat diet fed sedentary group.

**2) Effect of exercise on serum adiponectin levels, plasma free fatty acids levels and hepatic lipid (triglyceride) content.**

As shown in table (1), concurrent exercise with standard diet caused significant increase in the serum adiponectin level from 387.74 ± 21.72 pgm/ml to 461.9 ± 36.81 pgm/ml (P<0.001), but HFD caused significant decrease in the serum adiponectin level from 387.74 ± 21.72 pgm/ml to 220.6 ± 20.28 pgm/ml (P<0.001) as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused significant increase in the serum adiponectin level, from 220.6 ± 20.28 pgm/ml to 333.96 ± 14.38 pgm/ml (P<0.001), as compared with high fat diet fed sedentary group. Also shown in table (1), concurrent exercise with standard diet caused insignificant decrease in plasma FFA from 18.77±1.55mg/dl to 18.11±1.57mg/dl, but HFD increased plasma FFA significantly from 18.77±1.55 mg/dl to 43.31 ±5.58mg/dl (P<0.001) as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused significant decrease in plasma FFA from 43.31±5.58 mg/dl to 31.14±2.5 mg/dl, as compared with high fat diet fed sedentary group. Also concurrent exercise with standard diet caused insignificant decrease in the liver triglyceride content from 12.67±0.79mg/gm tissue to 10.79±1.39 mg/gm tissue, but HFD increased the liver triglyceride content significantly (about 200%) from 12.67±0.79mg/gm tissue to 25.36 ±4.58 mg/gm tissue (P<0.001) as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused significant decrease in the liver triglyceride content (about 54%) from 25.36 ± 4.58 mg/gm tissue to 13.75 ± 0.51mg/gm tissue (P<0.001) as compared

with high fat diet fed sedentary group.

**3) Effect of exercise on serum liver enzymes levels.**

As shown in table (2), concurrent exercise with standard diet caused insignificant increase in serum liver enzymes levels (AST from 35.2±3.7IU/dl to 45.1±8.9 IU/dl, and ALP from 69.9±6.8 IU/dl to 87.9±7.2 IU/dl) but it caused significant increase ALT from 23.2±3.2IU/dl to 41.2±8.3IU/dl (P<0.001), While HFD caused significant increase in the serum liver enzymes levels (AST from 35.2±3.7IU/dl to 144±17.1IU/dl (P<0.001), ALT from 23.2±3.2 IU/dl to 87.1±8.0IU/dl (P<0.001), and ALP from 69.9±6.8IU/dl to 249.2± 27.6IU/dl (P<0.001) as compared with standard diet fed sedentary group. Whereas concurrent exercise with HFD caused significant decrease in the serum liver enzymes levels (AST from 144±17.1 IU/dl to 88.4±13.1IU/dl (P<0.001), ALT from 87.1±8.0IU/dl to 56± 6.3IU/dl (P<0.001), and ALP from 249.2±27.6IU/dl to 159.5±29.8IU/dl (P<0.001)), as compared with high fat diet fed sedentary group.

**4) Effect of exercise on serum lipid profile.**

As shown in figure (1), concurrent exercise with standard diet caused insignificant decrease in serum TG levels from 70.1±5.7 mg/dl to 62.7±8.7mg/dl, serum cholesterol from 85.1±7.2mg/dl to 83.1±11.5mg/dl, serum LDL from 49.84±8.2mg/dl to 45.83± 12.4mg/dl, but significantly increased serum HDL from 21.24± 2.04 mg/dl to 24.73±1.99 mg/dl (P < 0.001). While HFD caused significant increase in the serum TG levels from 70.1±5.7 mg/dl to 136.2±19.04 mg/dl (P<0.001), serum cholesterol from 85.1±7.2 mg/dl to 189.2±21.72mg/dl(P< 0.001), serum LDL from 49.84± 8.2 mg/dl to 148.01±20.07 mg/dl (P<0.001), but it caused significant decrease in serum HDL from 21.24±2.04 mg/dl to 13.95±1.24 mg/dl (P<0.001), as compared with standard diet fed sedentary group. Whereas concurrent exercise with HFD caused significant decrease in the serum TG levels from 136.2±19.04mg/dl to 90.9±11.19 mg/dl (P<0.001), serum cholesterol from 189.2±21.72 mg/dl to 116.2±21.86 mg/dl (P<0.001), serum LDL from 148.01±20.07 mg/dl

to 80.08±19.17mg/dl (P<0.001), but it significantly increased serum HDL from 13.95±1.24 mg/dl to 17.94±1.94 mg/dl (P<0.001), as compared with high fat diet fed sedentary group.

**5) Effect of exercise on liver Histopathological scoring and staging.**

As shown in table (2) and fig (2), concurrent exercise with standard diet caused insignificant increase in the histopathological score of hepatic steatosis from 0.8±0.42 to 1.3±0.95; also it caused insignificant decrease in the histopathological stage of hepatic fibrosis from 0.5±0.71 to 0.0 ± 0.0. But HFD caused significant increase in the histopathological score of hepatic steatosis from 0.8±0.42 to 4.5±0.71 (P<0.001) and also HFD significantly increased the histopathological stage of hepatic fibrosis from 0.5± 0.71 to 2.4±0.55 (P<0.01), as compared with standard diet fed sedentary group. While concurrent exercise with HFD caused significant decrease in the histopathological score of hepatic steatosis from 4.5±0.71 to 2.0±0.94 (P<0.001), also it significantly decreased the histopathological stage of hepatic fibrosis from 2.4 ± 0.55 to 1.2±0.45 (P<0.01), as compared with high fat diet fed sedentary group.

**Table (1):** Effect of exercise on body weight, liver weight, liver / body weight ratio, serum adiponectin (pgm/ml), plasma FFA (mg/dl), liver TG content (mg/gm)).

| | *Standard diet* | | *High fat diet* | |
|---|---|---|---|---|
| | **SD Sed** | **SD ExT** | **HFD Sed** | **HFD ExT** |
| **Body wt (gm)** | 238.5 ± 23.1 | 263 ± 39.4 | 260 ± 35.3 | 266.5 ± 23.9 |
| **Liver wt (gm)** | 6.15 ± 0.78 | 9.43 ± 1.85$^{c}$ | 8.63 ± 1.96$^{b}$ | 7.55 ± 1.41 |
| **L/B wt ratio** | 2.583 ± 0.25 | 3.59 ±0.50$^{c}$ | 3.45 ± 0.41$^{c}$ | 2.83 ± 0.48$^{a}$ |
| **Adiponectin** | 387.74 ± 21.72 | 461.9 ± 36.81$^{c,f}$ | 220.6 ± 20.28$^{c}$ | 333.96 ± 14.38$^{c,f}$ |
| **FFA (mg/dl)** | 18.77 ± 1.55 | 18.11 ± 1.57$^{f}$ | 43.31 ± 5.58$^{c}$ | 31.14 ± 2.50$^{c,f}$ |
| **Liver TG (mg/gm)** | 12.67 ± 0.79 | 10.79 ± 1.39$^{f}$ | 25.36 ± 4.58$^{c}$ | 13.75 ± 0.51$^{f}$ |

Values are means ± standard deviation; **SD**, Standard diet fed; **HFD**, High fat diet fed; **Sed**, sedentary state; **ExT**, Exercise trained state. **L/B wt ratio**: liver weight / body weight ratio; **FFA**, free fatty acids; **TG**, triglyceride. P values as compared with the SD Sed group: $^{a}$ P< 0.05, $^{b}$ P< 0.01, $^{c}$ P< 0.001. P values as compared with HFD Sed group: $^{d}$ P< 0.05, $^{e}$ P< 0.01, $^{f}$ P< 0.001.

**Table (2):** Effect of exercise on serum liver enzymes (IU/dl) and histopathological score of steatosis and stage of fibrosis

| | *Standard diet* | | *High fat diet* | |
|---|---|---|---|---|
| | **SD Sed** | **SD ExT** | **HFD Sed** | **HFD ExT** |
| **AST (IU/dl)** | 35.2 ± 3.7 | 45.1 ± 8.9[f] | 144 ± 17.1[c] | 88.4 ± 13.1[c,f] |
| **ALT (IU/dl)** | 23.2 ± 3.2 | 41.2 ± 8.3[c,f] | 87.1 ± 8.0[c] | 56 ± 6.3[c,f] |
| **ALP (IU/dl)** | 69.9 ± 6.8 | 87.9 ± 7.2[f] | 249.2 ± 27.6[c] | 159.5 ± 29.8[c,f] |
| **Steatosis Score** | 0.8 ± 0.42 | 1.3 ± 0.95[f] | 4.5 ± 0.71[c] | 2.0 ± 0.94[b,f] |
| **Fibrosis stage** | 0.5 ± 0.71 | 0.0 ± 0.0[f] | 2.4 ± 0.55[b] | 1.2 ± 0.45[b] |

Values are means ± standard deviation; SD, Standard diet fed; HFD, High fat diet fed; Sed, sedentary state; ExT, Exercise trained state. P values as compared with the SD Sed group: [a] P< 0.05, [b] P< 0.01, [c] P< 0.001. P values as compared with HFD Sed group: [d] P< 0.05, [e] P< 0.01, [f] P< 0.001.

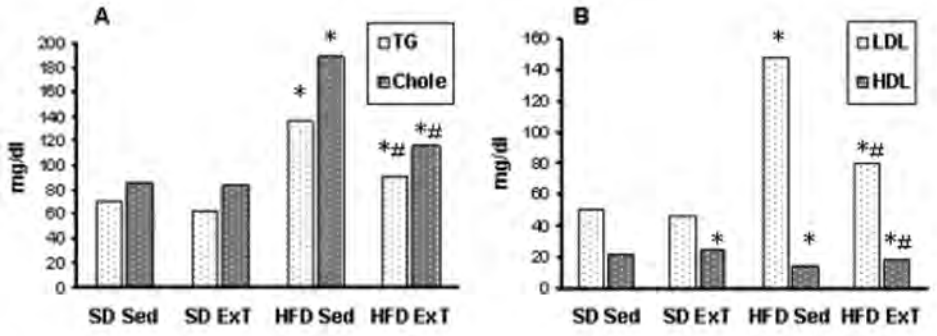


**Fig (1): Effect of exercise on serum lipid profile: A)** serum level of TG and cholesterol; **B)** serum level of LDL and HDL. **SD**, Standard diet fed; **HFD**, High fat diet fed; **Sed**, sedentary state; **ExT**, Exercise trained state. * P< 0.001 as compared with SD Sed group, # P< 0.001 as compared with HFD Sed group.

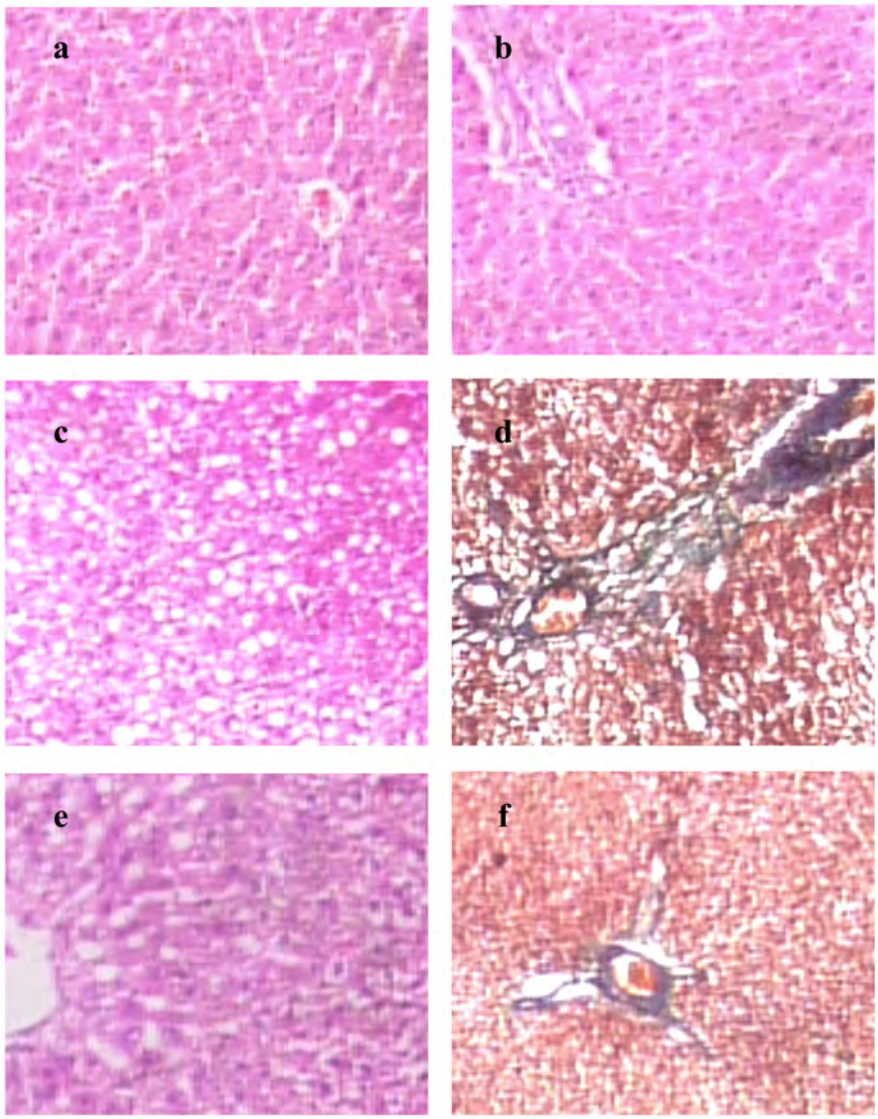


**Fig (2):** Liver histology a) normal liver histology of standard diet fed group b) liver histology of standard diet fed with exercise group, c) liver histology of high fat diet fed group H & E, d) liver histology of high fat diet fed group masson trichrome stain, e) liver histology of HFD fed with exercise group H & E, f) liver histology of HFD fed with exercise group masson trichrome stain.

## Discussion

There are controversial reports about the effect of exercise on adiponectin level and the reduction of the intrahepatic fat content in fatty liver. Some studies failed to demonstrate effect of exercise training on HF diet-induced hepatic steatosis in rats[14], while another study reported the presence of effect of exercise training on hepatic steatosis; however it is, to be most effective, exercise must be concurrent with the induction of fatty liver[9]. In the present study the exercise training program was conducted concurrently at the same time with the standard diet as well as the HF diet, to demonstrate the effect of exercise training program in physiological condition as well as in HFD.

Unlike expected, in the present study, concurrent exercise with standard diet and with HFD caused insignificant increase in the rat body weight as compared with the corresponding sedentary group (table 1). Some studies demonstrated that exercise with standard diet increased the body weight compared to the sedentary group, while concurrent exercise with HFD decreased the body weight compared to the HF diet sedentary group[9]. Others reported that, exercise decreased the body weight either with standard diet or with HFD compared with corresponding sedentary group[11]. This controversial effect of exercise on the body weight may be due to the different used dietary and exercise training program. In this study, these findings could be explained by, that the exercising animal may consume more calories than the sedentary, given that the energy expenditure increase with exercise should have been compensated by an increase in the food intake. Also in the present study, concurrent exercise with HFD, caused insignificant decrease in the liver weight but significantly decreased the liver weight / body weight ratio as compared with the HFD sedentary group (table 1). These findings partially agreed with that of Charbonneau et al.,[11], who demonstrated that exercise decreased the liver weight and liver weight / body weight ratio compared to both corresponding standard diet and HFD groups. While concurrent exercise with standard diet

caused significant increase in the liver weight and in the liver weight / body weight ratio as compared with the standard diet sedentary group (table 1). The increase in the liver weight in standard diet exercise trained group was in agreement with that of Murakami et al., [15], who demonstrated that exercise training with control diet for 12 weeks resulted in significant increase in the liver weight in spite of little increase in body weight. In that study the increase in the liver weight was associated with significant increase in the liver glycogen content that could be an explanation for the increase in the liver weight.

Studies on the effects of exercise on circulating adiponectin have been equivocal[16], many studies showing a beneficial (i.e., increasing) effect of fat loss, but not exercise per se [17], and some others showing additional benefits of vigorous exercise[18&19]. Quite surprisingly, in one study, exercise decreased circulating levels of adiponectin at 12 week of intervention[20]. In contrast, Kriketos et al.,[21] reported that adiponectin levels increased by about 260% after 2-3 bouts of low to moderate intensity exercise. In the present study, concurrent exercise with standard diet and with HFD caused significant increase in the serum adiponectin level as compared with the corresponding sedentary group (table 1). However several studies exploring the effects of exercise on the adiponectin levels have resulted in inconsistent findings, it has been shown that the basal adiponectin level was not altered after long term exercise[22&23]. However a number of training studies have been shown that chronic exercise will increase adiponectin levels if the training program is accompanied by weight/fat loss [24,25&26]. More research is necessary to definitively determine the unique effects of exercise on adiponectin as well as the implications and mechanisms behind these effects.

The evidence that exercise training reduces liver lipid accumulation induced by fat-rich diets is controversial. In previous studies, an effect and the absence of an effect of exercise training on hepatic steatosis have been reported. Therefore, exercise to be most effective, must be concurrent with the induction of fatty liver.

However, Straczkowski et al.,[14] failed to show an effect of concurrent exercise training on HF diet-induced hepatic steatosis, in which, rats were fed on a 59% fat diet; the metabolic alterations induced by the HF diet might have been too intense to be compensated by the exercise training program. In the present study concurrent exercise with standard diet produced no significant changes in plasma FFA and in the liver triglycerides content, while concurrent exercise with HFD caused significant decrease in the plasma FFA and the liver triglycerides content as compared with the corresponding sedentary group (table 1). This means that exercise training have the power to prevent the hepatic lipid accumulation, and prevent the development of hepatic steatosis. These results are consistent with that of Gauthier et al.,[9], who demonstrate that concurrent exercise prevents high-fat-diet-induced hepatic steatosis in rats. Also in humans it was demonstrated that higher level of habitual physical activity is associated with a lower intra-hepatic fat content and this may be due to the effect of exercise per se[10]. However interestingly, in spite of low fat diet led to significant improvements in hepatic steatosis, the combination of low fat diet and exercise did not have a greater effect, this could explained as there is a "threshold" hepatic TG level and behavioral interventions cannot lead to reductions beyond that point[27]. Also it was demonstrated that the HFD resulting in severe liver steatosis, and exercise significantly reduced liver steatosis in HFD. Exercise attenuated all the changes due to HFD, and improved the lipid profile and fatty liver, contributing to steatohepatitis control [28].

These results is supported by the histopathological examination, which reveal that concurrent exercise with standard diet caused no significant changes in the histopathological score of hepatic steatosis; and the stage of fibrosis as compared with standard diet fed group. While concurrent exercise with HFD caused significant decrease in the histopathological, score of hepatic steatosis and stage of fibrosis as compared with high fat diet fed group (table 2, figure 2). These results indicate that

exercise training reduce the degree of hepatic steatosis. These findings are in concordance with those of previous studies in which hepatic steatosis induced by HF diets in rodents was ameliorated by exercise training [9,28]. The importance of applying different protocols of exercise training associated with other therapies to control and reduce the high prevalence of NAFLD in the obese population is recognized[29]. As demonstrated here, the exercise training protocol used in the present study was effective in reducing fatty liver and suppressing HF diet-induced steatosis.

In the present study, concurrent exercise with standard diet caused insignificant decrease in the serum levels of TG, cholesterol and LDL but significantly increased serum HDL as compared with standard diet fed group. While concurrent exercise with HFD caused significant decrease in the serum levels TG, cholesterol and LDL, but it caused significant increase in serum HDL as compared with high fat diet fed group (figure 1). The present findings demonstrated that HFD intake led to dyslipidemia in sedentary rats, in agreement with the literature [30&31]. Thus, exercise was able to reduce serum triglycerides and cholesterol and to improve HDL-cholesterol concentrations. The activity of lipoprotein lipase increases through exercise and is responsible for releasing fatty acids from the lipoproteins by promoting an imbalance between HF diet and the effects of exercise[32]. Furthermore, during exercise and after glycogen depletion, fatty acid becomes the major fuel for the exercising metabolism in muscle; during the recovery period, glycerol is necessary for glycogen repletion [9,33&34].

In the present study, with 12 weeks HFD, the liver TG content was increased by ~ 100 %. Exercise training, in the form of swimming 15 minute / day for 12 weeks results in reduction of intrahepatic TG content. Although the modalities of application of the exercise regimen seem to play a role, the present results clearly indicate that a 40% HF diet-induced hepatic steatosis can be improved by a concurrent exercise training program. The precise mechanism by which hepatic steatosis can be prevented through exercise train-

ing remains speculative. Consistent with the above-discussion, the possible mechanisms through which exercise training could have prevented liver fat accumulation are: 1) a diminished delivery of lipids to the liver, and/or 2) an increased incorporation of TG into VLDL. Altogether, these results suggest that the preventing effect of exercise training on hepatic fat accumulation is a consequence of a diminished delivery of FFA to the liver rather than an increased VLDL synthesis. One of the mechanisms underlying the positive effects of exercise training is related to fat utilization by increased lipid oxidation during exercise[35]. Despite this, there is no firm consensus how exercise training can prevent the induction of hepatic steatosis. Exercise reduces the adverse effects of dietary fat in high-fat fed animals[36]. Moreover, during exercise, several adaptive mechanisms are established to supply fuel to skeletal muscle and accelerate glucose and fatty acid metabolism[37]. Exercise training can prevent the accumulation of fat in the liver of rats receiving a high-fat diet[38]. However, other studies using a similar high-fat diet-induced obesity model in rats have shown that exercise had no effect on liver lipid accumulation[30&31]. The reasons for this discrepancy are not clear, although differences in the dietary, the exercise training program and the techniques used for assessing liver lipid infiltration should all be considered.

In the present study, concurrent exercise with standard diet caused no significant change in the serum liver enzymes levels (AST and ALP) but it caused significant increase ALT, while concurrent exercise with HFD caused significant decrease in the serum liver enzymes levels (AST, ALT, and ALP) as compared with the corresponding sedentary group (table 2). Since AST and ALT exist primarily in hepatocytes, which are increased into serum during hepatic damage (39). Thus, these results suggest that the reduction of serum liver enzymes level by concurrent exercise with HFD means that exercise decrease hepatocyte damage that occur by HFD. These findings are in agreement with previous studies in human with NAFLD, which demon-

strated a combination of diet and exercise is associated with improved serum ALT and AST (40). Others have demonstrated that there was a significant improvement in serum aminotransferases in the NAFLD patients' using the diet and exercise regimen. Interestingly, patients who exercised and did not lose weight still had a significant improvement in aminotransferase levels(41). Also it was reported that the combined diet and exercise reduce intrahepatic fat content in obese older adults and that liver enzymes were within the normal range(42).

**In summary:** Exercise training produces an increase in the adiponectin levels in both standard diet and HFD fed animals. Also concurrent exercise training with HFD reduces the development of hepatic steatosis, through the reduction of plasma FFA level and improvement of serum lipid profile. The elevation of serum adiponectin concentration by exercise may play a hepatoprotective role against the development of hepatic steatosis.

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## EFFECT OF EXERCISE ON ADIPONECTIN LEVEL AND HEPATIC STEATOSIS IN RATS


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# DIFFERENT MORPHOLOGICAL PATTERNS OF DIABETIC MACULAR EDEMA USING SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY

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## Abstract

***Purpose:*** *To analyze prospectively the retinal morphology of different patterns of diabetic macular edema (DME) using spectral-domain optical coherence tomography (SD-OCT) and understand pathogenesis of subretinal detachment associated with DME.*

***Methods:*** *The study included 50 patients (64 eyes). The patients underwent complete ophthalmic examination including Goldmann fundus contact lens. SD-OCT was done to all of them. All OCT scans were examined for the presence of diffuse retinal thickening (DRT), cystoid macular edema (CME), subretinal detachment (SRD), posterior hyaloids traction (PHT), and tractional retinal detachment (TRD). The most frequent pattern was DRT seen in 38 eyes (59.4%) followed by CME seen in 36 eyes (56.3%). Both DRT and CME were seen in 10 eyes (15.6%). SRD was seen in 18 eyes (28.1%). PHT was seen in 11 eyes (17.1%). PVD was seen in 14 eyes (21.9%).*

***Conclusion:*** *SD- OCT provides us with valuable information on the retinal morphologic changes associated with DME. It is helpful to clarify the pathogenesis of DME and optimize the treatment for each type.*

## Introduction

Diabetic macular edema (DME) is common complication in diabetic patients seen in daily clinical routine causing significant visual. Impairment for the affected patients. The Wisconsin Epidemiologic study of Diabetic Retinopathy found a 10-year incidence of DME between 13.9% and 25.4%

with a poor long prognosis [1]. It is defined by retinal thickening involving or threatening the center of the macula [2]. Previously described methods of assessing DME include slit-lamp bio microscopy, indirect fundoscopy, fluorescein angiography and fundus stereo photography [3].

Since optical coherence tomography (OCT) became commercially available in 1995, it has provided useful information on morphologic changes associated with a variety of vitreomacular diseases including macular edema [4]. It provides cross-sectional images of the retina, which mimic the histologic sections of light microscopy [5]. Otani et al [6] were the first authors to publish three patterns of structural changes in diabetic macular edema investigated by OCT. namely sponge-like swelling, cystoid macular edema and serous retinal detachment. Kaiser et al [7] investigated macular edema associated detachment (PHD). The purpose of this study was to analyse prospectively the retinal thickness. Morphology of all the patterns including those associated with macular traction using spectral-domain (SD) OCT.

## Patients and Methods

This is a prospective case series of consecutive diabetics who were referred to Mansoura ophthalmology Center. Inclusion criteria were patients with confirmed diagnosis of diabetes mellitus showing clinically significant macular edema as defined by early Treatment diabetic Retinopathy study [8]. Patients with poor quality OCT due to media opacities. Macular edema from etiologies other than diabetes were excluded from the study.

The study included 50 patients (18 males and 32 females; 64 eyes). The patients underwent complete ophthalmic examination including Goldmann fundus contact lens. OCT was done using SD-OCT (3D OCT 1000 version 2.00; Topcon, Tokoyo, Japan). The system acquires 128 horizontal B-scan images, each containing 512 A-scans, covering a 6 mm horizontal 6 mm vertical 1.7 mm axial vol. 26 no 3 sept. 2009.

Volume in less than 3.7 seconds. A color fundus photograph is taken with an integrated non-

mydriatic camera. The B-scan images can be used to form a projection image of fundus that has pint-to-point registration with any A-scan. The B-scans within the 6 _ 6-mm block can be used construct 3-dimental (3D) images.

Central macular thickening was measured automatically with OCT retinal mapping software when DME was associated with subretinal detachment (SRD). The height of the SRD at its thickness above were measured manually using the caliper tool built into the OCT software. All OCT scans were examined for the presence of diffuse retinal thickening (PHT), and tractional retinal detachment (TRD).

These various patens were scored based on their unique appearance on OCT images: 1) DRT was identified as increased retinal thickening, 200πm with reduced intraretinal reflectivity. 2) CME was identified by the localization of intraretianl cystoids like spaces that appeared as round or oval areas of low reflectivity with highly reflective septa separating the cystoid like cavities. 3) SRD was defined as dome - shaped elevation of the posterior surface of the retina over a non-reflective cavity with minimal shadowing of the underlying tissue. 4) PHT was identified was defined as peak-shaped elevation of inner retinal surface and extending towards the optic nerve or periphererally 5) TRD was defined as peak-shaped detachment of the retina.

The posterior hyaloid was visualized as linear signal of low reflectivity above the retina. Separated from it by a clear space. Posterior vitreous detachment PVD was diagnosed when the posterior hyaloid was detached from macula but remained visible in front of it in all OCT scans.

For descriptive statistics of the quantitative variables the mean. Range and standard deviation (SD) were used to describe central tendency and dispersion. Differences between the means of the independent samples were analyzed by the Mann-Whitnney test. It was considered significant if ($p < 0.05$).

## Results

The study included 50 patients. 48 eyes (75%) had non proliferative diabetic retinopathy the mean age of the patients was 55 ± 7.3 years (range 41 to 71). At time of initial examination, no patient was suspected of having serous macular detachment. Mean retinal thickness ± SD was 429.34 ± 174.16 μm (range 230 to 806).

The most frequent pattern was DRT seen in 38 eyes (59.4%) followed by CME seen in 36 eyes (56.3%). Both DRT and CME were seen in 10 eyes (15.6%). Macular cysts was located in the outer retinal layers in all of the cases (100%). Each cystoid cavity was walled off with septae. The cystoid cavities expanded in a round or oval configurations=. Two eyes (3.1%) had a single perifoveal cyst. 14 eyes (21.9%) showed both outer intraretinal cystoid and inner intraretinal cystoids. One eye with CME (2.9% of cases with CME) had smaller hole.

SRD was seen in 18 eyes (28.1%) (8 eyes were associated with CMO. 6 eyes with DRT while 4 eyes were associated with both DRT and CMO). There was no statistically difference in retinal thickness comparing eyes patients with SRD with those without (P=0.254).

PHT was seen in 11 eyes (17.1%). Only one eye had both PHT and SRD. Epiretinal membrane was seen in 12 eyes (18.8%). 50% of them had cystoid macular edeme while 50% had DRT. None of them had SRD. TRD was seen 3 eyes (4.7%). Retinal detachment alone either serous or tractionalwas not observed in any eye.

PVD was seen in 14 eyes (21.9%). There was no statistically significant difference in retinal thickness comparing eyes with PVD with those without (P=0.824). Two eyes of them (14.3% of eyes with PVD) had SRD.

## Discussion

OCT is a noninvasive quick and reproducible method of producing high-resolution cross-sectional images of the retina [9]. OCT has spe-cifically been utilized for morpho-logic analysis of DME [6,10,11]. Collectively, these

papers identified and de¬scribed five OCT patterns of DME : DRT. CME, SRT and foveolar detachment with and without traction.

In DME, OCT images showed increased retinal thickness and the presence of low internal reflec-tivity characterized by Otani et al [6] in two distinct features: (1) outer retinal swelling represented by an ill-defined widespread hypo-reflective area of thickening and (2) cystic hyporeflective spaces. Similar to other reports, [6,10] the most common subtype seen in our study was DRT seen in 59.4%. The second most frequent type was CME seen in 56.3% of our cases. Kim et al [12] reported while Otani et al [6] reported 47%.

Although the hyporeflectivity on OCT corresponding to accumulation of fluid was mainly located in the outer retinal layer, intraretinal cysts were located in the inner retinal layers in 21.9% of our cases using spectral-domain OCT. It has been reported in only 4% of cases of diabetic retinopathy using Humphrey 2000 system [13] while il was seen in 21.5% in another study using OCT 3 (Stratus OCT) [14]. Cystic spaces are supposed to be more accurately visualized with OCT devices of greater resolution than the first-generation devices [13]. Morphologically DME starts at the outer nuclear layer/ Henle's layer. As edema progress-es, cysts arc seen in the fovea and the DME spreads further into the inner nuclear layer [14].

OCT is particularly useful for analyzing vitreoretinal relationship and visualizing vitreoretinal adhesions [15]. 35.9 % of patients had macular traction (Vitreomacular traction and epiretinal membrane] which is consistent with a previous.

Report of macular traction in 31.5% of eyes with DME [16]. PVD was seen in 21.9%. SRD detachment was seen in 28.1% of cases which is consistent with other investiga-tors reporting 31% [17]. SRD was not detected on biomicroseopy in any of the studied eyes in the present study. It is obvious that DME prevents the detection of serous macular

detec-tion by clinical examination. Be-cause of its improved resolution and image quality, spectral-domain OCT allows an in vivo cross-sectional observation of very subtle serous macular detachment that is difficult to diagnose clinically.

The pathogenesis of serous retinal detachment is still being debated. Leakage from retinal or choroidal circulation into the subretinal space exceeding it's drainage capacity was thought to be the main mechanism [18]. In DME, the in¬creasing flow of fluid is probably not the only cause of SRD. be-cause there was no statistically significant difference of the retinal thickness in eyes with SRD andthose without was seen in this study.

The deterioration of retinal pigment epithelium function from ischemia may play a role in SRD as well as the rapidity of the increase in intraretinal Quid [13]. Subretinal fluid was not tractional in origin as. in this study. SRD was seen in one eye out of 11 eyes with PHT while it was not seen in any eye with epi-retinal membrane, thus eliminat¬ing any obvious involvement of traction in the pathogenic mecha-nism of SRD.

## Conclusion

Spectral-domain OCT provides us with valuable information on the retinal morphologic changes associated with DME. It is particularly useful for analyzing the vireomacular relationship and detecting SRD undetectable on biomicroseopy. Further prospec-tive studies are required to explain the pathogenesis of SRD and establish its prognostic value. The OCT pattern of diabetic macular edema could be helpful in either making a diagnosis or in determining the prognosis of DME. It is helpful to clarify the patho-genesis of DME and optimize the treatment for each type.

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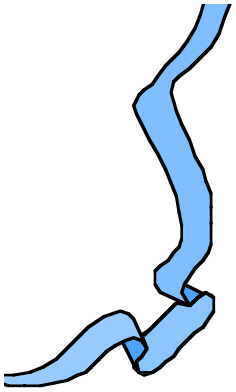

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Two copies of the manuscript and illustrations should be sent. Manuscripts should be typed double spaced on one side of the paper, with wide margins. The names of author (s) and their appointment (s) and addresses at the time of preparation of the manuscript should appear at the head of the paper; present addresses, if different, will be given as a footnote.

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Abbreviations are used sparingly

and only if a lengthy namy or expression is repeated frequently throughout the manuscript. Words must appear in full on first appearance in both summary and text, followed by the abbreviation in parenthesis.

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Measurements should be expressed in SI units with the exception of haemoglobin (g/dl) and blood pressure (mmHg).

**The accepted abbreviations for units**

| | | | |
|---|---|---|---|
| Kilogram (s) | kg | Hour (s) | h |
| gram (s) | g | minute (s) | min |
| 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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Authors should describc the plan of their investigation indicating the number of expcrimental units (e. g. subjects; blood samples). For repeated observations, their numbers and timing should be specified. Control subjects should be described as completely as the experimental subjects .

Measures of location (e.g. mean, median) should be accompanied by an appropriate measure of variability, e.g. standard deviation (SD) or standard error of the mean (SEM). Methods of statistical analysis should be indicated, but details are not required unless relevant to the discussion. In multivariate analysis, an indication of the goodness of fit of the model should be given (e.g.multiple correlation coefficient). When a result is claimed to be statistically significant the test used and the level of probability should be specified (e.g. paired t-test $p<0.01$) When only one type of statistical test is used in the paper, it should be stated in the scction for methods to avoid repetition. Tables should be self explanatory .

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