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CONTENTS

Effect of Ginger Extract on Deltamethrin Induced Histomorphological and Immunohistochemical Changes in Testes of Albino Rats

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Abstract: The current study investigated the effect of ginger{*Zingiberofficinale* Roscoe(*Zofficinale* R.}extract on deltamethrin induced testicular damage in albino rats. Treating animals with deltamethrin at a dose level of $1/_{10}$ LD_{50}, 3 days weekly for 6 weeks caused a decrease in body and testes weights. Remarkable decreases were also noted in sperm cell concentrations and sperm motility. In addition histopathological results revealed degeneration of spermatogenic cells, congestion of blood vessels and destruction of Leydige cells.The diameters of the seminiferous tubules and heights of their germinal epithelium were significantly reduced. Immunohistochemical results showed that Bax expression was increased in Leydige cells and p53 expression increased in spermatocytes of testes of deltamethrin treated rats. According to these results, deltamethrin induced oxidative stress and caused apoptosis in testes of albino rats. Treating animals with deltamethrin and ginger revealed an improvement in the histological changes observed in animals treated with deltamethrin and increased sperm concentration and motility. Moreover, ginger treatment leads to a decrease in the expression of p53 and bax. This effect of ginger extract may be attributed to its antioxidant activity.

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Keywords: Deltamethrin, testes, ginger, apoptosis, p53,Bax.

1. Introduction

Insecticides are frequently used in agriculture for the eradication of insects and the heavy use of chemical insecticides resulted in lethal effects on non-target organisms inagroecosystem, and has direct toxicity to users **(Ansari and Kumar 1988, Kalavathyet al., 2001)**. Synthetic Pyrethroids are manufactured analogues of naturally occurring pyrethrins found in the flowers of *Chrysanthemumcinerariaefolium***(Lutyet al., 2000).**These insecticides are commonly divided into two types: Type I compounds or (T-syndrome pyrethroids), which lack an alphacyano substituent, and Type II compounds or (CS-syndrome pyrethroids), which contain an alpha-cyanophenoxybenzyl substituent (**Naumann, 1990**). Moreover, the use of pyrethroid insecticides has been documented since 1970s, preliminary evidence suggested that its usage has been increasing and the pyrethroid insecticides are replacing the organophosphorus insecticides for residential control **(Sudakine, 2006)**. So, human exposure to the pyrethroid insecticides was increased (**Khan et al., 2008**). Deltamethrin is a synthetic pyrethroid with potent insecticidal property. The technical grade deltamethrin comprises of eight stereomeric esters (four cis and four trans isomers) of the dibromo analogue of chrysanthemic acid, 2,2- dimethyl-3-cyclopropanecarboxylicacids.Deltamethrin is extensively used as an ectoparasiticide in animals and as insecticide in crop production and public health programme**(Tuet al., 2007).** Deltamethrin was found to cause various adverse effects in experimental animals. Treating pregnant rats from day 6 to day 15 of pregnancy with deltamethrin caused retardation of growth, hypoplasia of the lungs, dilation of the renal pelvis and increase in placental weight **(Abdel-Khaliket al.,1993). Lukowicz-Ratajczak and Krechniak (1992**) reported that deltamethrin suppress immune system in Balb/c mice. It inhibited the mitotic index and increased the frequency of chromosomal aberrations in the bone marrow of rats **(Agarwalet al., 1994**). Reproductive toxicity and endocrine disruption, effects related to deltamethrin exposure have been reported in numerous studies **(Abdallahet al., 2010).**

Herbal and natural products represent one of the most common forms of complementary and alternative medicines. Many natural product extracts have been found to have a variety of pharmacological effects. Ginger (*Zingiberofficinale* Roscoe) is example of plants which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally and wlinary useful part **(Mascoloet al.,1989).** Ginger was used in popular to relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy **(Gilani and Rahman,2005)** .Many

pharmacological effects were reported on ginger and its pungent conistituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platelet, antioxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti-arthritic effect (**Fisher-Rasmussen et al.,1991, Sharma et al., 1991, Kamtchovinget al., 2002). Khaki et al.,, (2009)** reported that ginger extract possess a protective effect against DNA damage induced by H_2O_2 and enhanced sperm healthy parameters in rats. The effect of ginger on male reproduction was studied by some investigators **(Hafez 2010, Zahediet al.,, 2010, Sakr and Badawy, 2011**). The current study was designed to investigate the effects of ginger extract on histomorphological and immunohistochemical changes induced in testes of albino rats by deltamethrin.

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2. Materials and Methods

Preparation of ginger aqueous extract

Ginger (*Z. officinale*R.) rhizome was purchased from the local market. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and powdered. 125 g of this powder were macerated in 1000 ml of distilled water for 12 h at room temperature and were then filtered. The concentration of the extract is 24 mg/ml. Each experimental animal in the present study was orally given 1 ml of the final aqueous extract (**Kamtchovinget al.,, 2002).**

Animals and treatments

Adult (150±10 g body weight) male rats of Wistar strain were housed in groups of two per cage, maintained under controlled conditions of temperature (22 ± 2°C) and light (12 :12L : D) and provided with rodent food and water *ad libitium.* Animals were divided into 4 groups:

Group1: Animals of this group (10 rats) were considered as control and were given 0.5 ml corn oil.

Group 2: Each animal of this group (20 rats) was orally given 0.5 ml of final aqueous extract of ginger (24 mg/ml) 3 days weekly for 6 weeks.

Group 3: Animal of this group (20 rats) were orally given deltamethrin at a dose level of $1/_{10}$ LD_{50} (0.6 mg/kg body weight) **(Odaet al., 2011**) in corn oil, 3 days weekly for 6 weeks.

Group 4: Animals in this group (20 rats) were given the same dose of deltamethrin given to animals of group 2 followed by .05 ml of final aqueous extract of ginger (24 mg/ml) 3 days weekly for 6 weeks.

Epididymal sperm concentration and motility

The left epididymis of each rat was used for the determination of epididymal sperm concentration using the Neubauerhaemocytometer, while % sperm motility was determined as described by **Sönmezet al., (2005).** Fluid was obtained from the epididymis with a pipette and diluted to 2ml with tris-buffer solution. The percentage of motility was evaluated at ×1000 magnification.

Histological Study

Immediately after decapitation animals were dissected after 3 and 6 weeks , testis were removed from treated and control groups and fixed in Bouin's solution. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histological examination, sections were stained with Ehrlich's hematoxylin and counterstained with eosin. Seminiferous tubules diameter and germinal epithelial height were measured from the spermatogenic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules.

Immunohistochemical Study

From each testis block, 4 microns thick sections were cut on Neoprene-coated slides. The immunostaining was performed using the avidin-biotin complex (ABC) method and an automatic autostainer (CODE-ON Immuno/DNA slide stainer: Biotek solution, Santa Barbara, CA). Slides were deparaffinized and blocked for endogenous peroxidase with 1 .75% hydrogen peroxide in methanol for 20 mm, antigen retrieval for 15 mm using Biogenex Antigen Retrieval Citra solution in 90°C water bath for 30 mm. The slides were allowed to cool for 20 min before continuing. Slides were then blocked by normal horse serum for 5 mm at 37°C. The monoclonal antibody was applied overnight in humid medium at room temperature followed by the biotinylated secondary antibody for 15 min at 37°C and the ABC complex for 15 min at 37°C (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA). Diaminobenzidine (DAB) was applied for 20 min at room temperature as chromogenic slides were counterstained with hematoxylin, dehydrated, and covered by coverslips. In negative control slides, the same system was applied with replacement of the monoclonal antibody by diluted normal bovine serum. Baximmunostaining was performed using polyclonal rabbit-anti-human (A3533 Ig fraction; DAKO, Glostrup, Denmark) at a dilution of 1:50. Monoclonal antibody which recognizes both wild type and mutant p53 was used

(code no: M7001, dilution 1:50, DAKO) for p53 detection.

Statistical Analysis

Data were expressed as mean values ± SD and statistical analysis was 1 performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® 4 Inc., USA).

3. Results

Morphmetrical results

Results in table 1 revealed that rats intoxicated with deltamethrin showed significant decrease in the body and testes weights after 6 weeks of treatment. Treatment with ginger caused apparent increase in body and testes weights (table1). Epididymal sperm concentration of 4.50±0.5 million/ml obtained in the control rats was significantly higher ($p<0.05$) than those of rats treated with deltamethrin (2.3±0.4) after 6 weeks. Sperm motility in the control group (60.2±3.5%) was significantly decreased in deltamethrin group. On the other hand, sperm concentration and motility increased in rats given deltamethrin and ginger extract (table2). Data in table 3 showed that treatment with deltamethrin caused atrophy of the seminiferous tubules. The diameter of seminiferous tubules was significantly decreased in deltamethrin treated rats. A decrease in epithelial height of seminiferous tubules is also recorded in compare with control ones. However, treatment with ginger extract caused an increase in diameters and epithelial heights of the seminiferous tubules.

Histological observations

Histological examination of testis of control rat showed normal appearance of seminiferous tubules and interstitial tissue. Sertoli cells and spermatogenic cells (Spermatogonia, 1ry and 2ry spermatocytes and sperm) appeared normal. Interstitial tissue and Leydige cells can be recognized (Fig.1A).Animals intoxicated with deltamethrin showing many histomorphological changes. After 3 weeks, the seminiferous tubules lost its shape and appeared with irregular outline and widely separated from each other (Fig.1B). The gem cells were degenerated and exfoliated in the lumen center (Fig.1C). Intertubular blood hemorrhage was observed with abnormal appearance of germ cells which showed pyknotic nuclei (Fig.2A). These alterations became severe after 6 weeks. In these specimens, the intertubular tissue was degenerated and showed many vacuoles with blood hemorrhage and most of the seminiferous tubules were devoid of germ cells (Fig.2B). Examination of testes of animals treated with deltamethrin and ginger revealed less prominent histopathological changes when compared with deltamethrin group. Most of the seminiferous tubules appeared with increase of spermatogenic cells and an increase in the number of sperm bundles was seen (Fig.2C).

Immunohistochemical results

Immunohistochemical examination of testes of control rats revealed that p53 was expressed in germ cells (spermatogonia, primary and secondary spermatocytes) (Fig.3A). Animals treated with deltamethrin showed an increase of p53 expression in these cells (Fig.3B). Treating animals with deltamethrin and ginger showed a decrease of p53 expression. The percentage of p53 expression was 14% in control rats compared with 46% and 22% in rats intoxicated with deltamethrin and deltamethrin plus ginger, respectively (Fig.4). Figure 5 (A & B) showed the expression of Bax in Leydige cells. The number of the Bax positive staining cells increased in Leydige cells of rats treated with deltamethrin compared with control and decreased after treatment with deltamethrin and ginger (Fig.6).

Table 1.Change inmean value of the body and testes weights in rats of different groups after 6 weeks.

Animal group	Body weight(g)	Testes weight(g)
Control	167 ±3.8	2.52 ± 0.5
Ginger extract	171 ± 4.5	2.80± 0.3
Deltamethrin	101 ± 2.4*	1.67± 0.2*
Deltamethrin+ ginger	152± 1.2	2.0 ± 0.1

(*). Significant at $P < 0.05$

Table 2. Mean sperm concentration and % sperm motility in rats of different groups.

Animal group	Sperm concentration ($\times 10^9$)	Sperm motility (%)
Control	4.5 ±0.5	60.2 ± 3.5
Ginger extract	4.1 ± 0.2	58.5± 2.2
Deltamethrin	2.3 ± 0.4*	22.6± 3.7*
Deltamethrin+ ginger	3.5 ±0.2	41.5± 2.3

(*). Significant at $P < 0.05$

Table 3. Mean value of the diameter and epithelial height of seminiferous tubules in rats of different groups.

Animal group	Diameter of tubules	Germinal epithelial height
Control	223 ±5.6	99.5 ± 6.2
Ginger extract	211 ± 3.7	86± 3.2
Deltamethrin	144 ± 3.4*	56± 4.2*
Deltamethrin+ ginger	189.6± 2.8	72 ±4.2

(*). Significant at $P < 0.05$

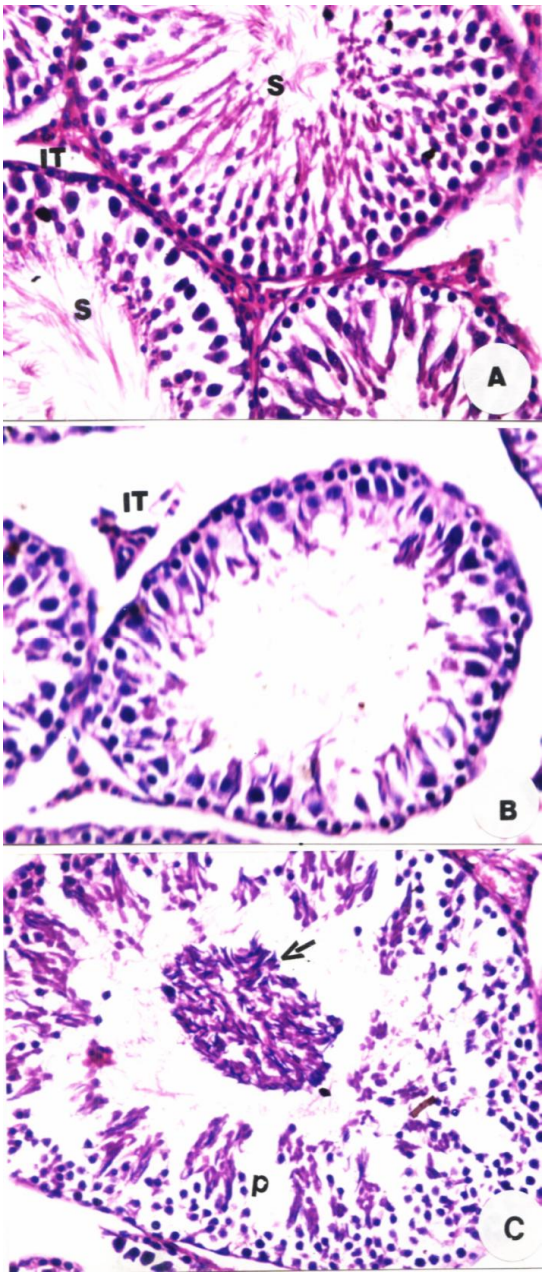

Fig.1.(A): Section in testis of a control rat showing seminiferous tubules with active spermatogenesis, S: sperm, IT: interstitial tissue, (B): After 3 weeks of treatment with deltamethrin showing irregular seminiferous tubules with reduced spermatogenic cells and damaged interstitial tissue (IT), (C): exfoliated degenerated spermatogenic cells in the lumen (arrow), P: pyknotic nuclei, X 300.

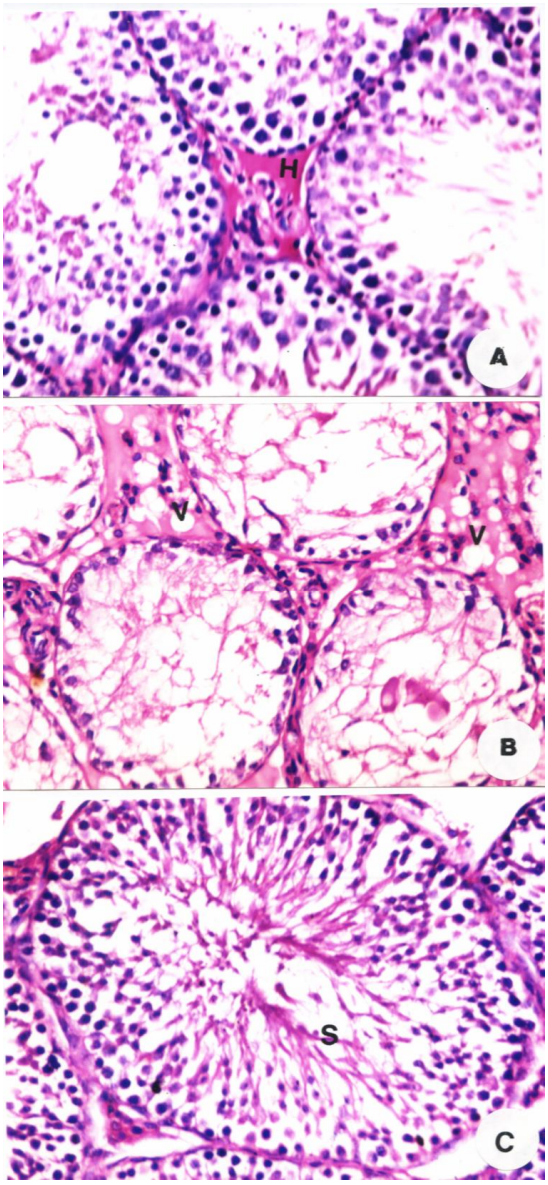

Fig.2.(A): Section in testis of a rat after 3 weeks of treatment with deltamethrin showing degenerated germ cells and intertubular hemorrhage (H), (B): Section in testis of a rat after 6 weeks of treatment with deltamethrin showing highly degenerated interstitial tissue with vacuoles (V) and blood hemorrhage. The seminiferous tubules appeared with complete absence of germ cells, (C): Section in testis of a rat after treatment with deltamethrin and ginger showing increase of germ cells and sperm bundles (S), X 300.

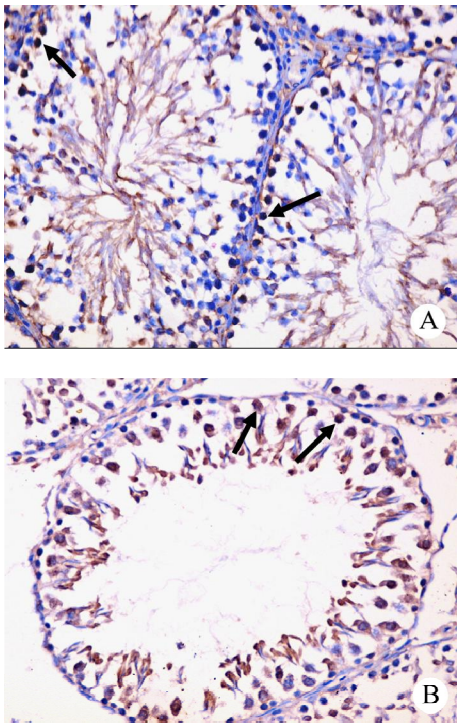

Fig.3. Seminiferous tubules of (A): a control rat, (B): deltamethrin-treated ratshowing p53 expression in germ cells (arrows), X 300

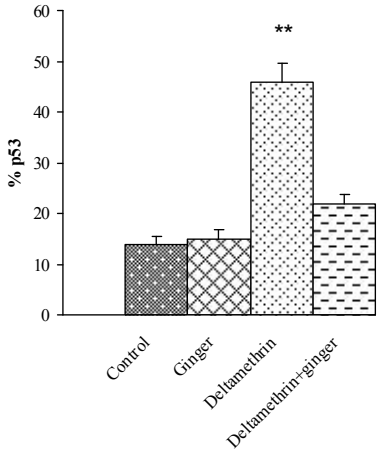

Fig.4. Percentage of p53 positive staining germ cells in different animalgroups, (**) significant at P<0.05

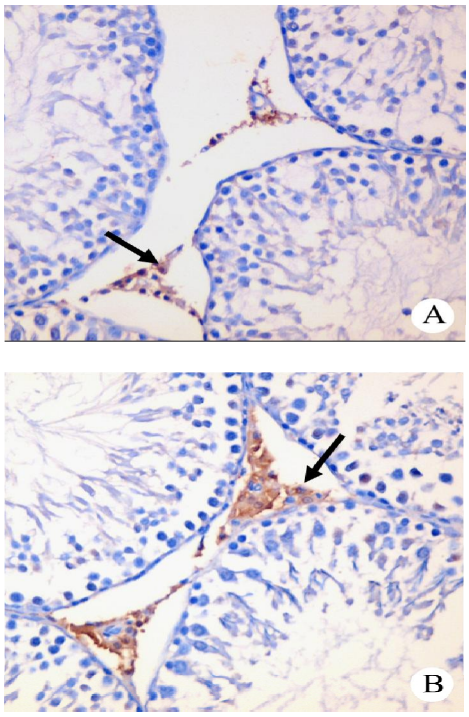

Fig.5. Seminiferous tubules of (A): a control rat, (B): deltamethrin-treated ratshowing Bax-positive staining Leydige cells (arrows), X 300

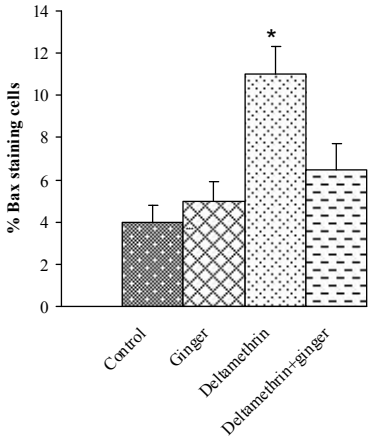

Fig.6. Percentage of Bax positive staining cells indifferent animal groups, (*) significant at P<0.05

4. Discussion

Administration of deltamethrin caused a decrease in body as well as testes weights of rats. The sperm concentration and sperm motility was also decreased. Similarly, **Abdel-Khaliket al., (1993)** found that maternal weight gain was reduced in Sprague-Dawley rats intoxicated with deltamethrin**. Abd el-Aziz et al., (1994)** reported that deltamethrin significantly decreased the weight of testes, seminal vesicle, and prostate glands. Significant decreases were also noted in sperm cell concentrations, percentage of live cells and sperm motility. Male rats administered with deltamethrin for 65 days at doses of 1 or 2 mg/kg showed significantly lower testicular, prostate gland, and seminal vesicle weight. The mating success of treated rats was reduced by 50% during the study and for two months afterwards at both doses **(Bradberryet al., 2005).Takahashi and Oishi,(2001)** mentioned that the weight of the testis is basically dependent on the mass of the differentiated spermatogenic cells; the reduction in the weight of the testis may be due to decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity. The obtained results support this speculation; deltamethrin induced histopathological alterations in the testes of treated rats and lead to inhibition of spermatogenesis. The effect of deltamethrin on male reproduction was studied by (**Abdallahet al., 2010; Oda and El-Maddawy, 2011).**

Immunohistochemical results revealed that deltamethrin increased expression of p53 and Bax in testes of rats. Several investigators reported that p53 expression is confined to the primary spermatocytes within the testicular seminiferous tubules **(Almonet al., 1993;** Sjöblom**and** Lähdetie **1996; Stephan et al., 1996)**. These studies support the present results in which P53 was detected within the seminiferous epithelium and not in the Leydigee cells or other interstitial cells. However, Bax protein was detected in interstitial cells suggesting that Bax gene expression is not under the exclusive control of p53 (**Taylor et al., 1998**). There are two main pathways known to cause apoptosis (**Fadeel and Orrenius 2005).** One is intrinsic (mitochondrial) and the other is extrinsic pathway. Intrinsic pathway triggers by stress-caused reasons in the cell as irradiation, toxins and oxidative stress. These stress sources can affect the members of Bcl-2 family, which stabilizes or destabilizes the mitochondrial membrane by proapoptotic or antiapoptotic (Bax and Bcl-XL) factors. Extrinsic pathway triggers by extracellular ligands(Fas-ligand [FasL]). Apoptosis of germ cells may be induced with both extrinsic and intrinsic pathways.

It is known that Bax, Bad, Bcl-xl, and Bcl-2 are expressed in rodent testes **(Krajewskiet al., 1996).** The tumor suppressor p53 is a potent inducer of apoptosis (**Symonds et al.,1994)** and is found in unusually high concentration in germ cells (**Almonet al., 1993).** During spermatogenesis, apoptosis in testicular germ cells is recognized as an important physiologic mechanism to limit the germ cell population to numbers that the Sertoli cells can support **(Billiget al.,, 1995).** Regulation of germ cell apoptosis in the normal testis is controlled by the Bcl-2 family, p53 and Fas-signaling pathway **(Woolveridge and Morris, 2000).** Many toxicants were reported to induce germ cells apoptosis such as MEHP, 2,5-hexandione, nitrobenzene, deltamethrin, and hydroxyurea**(Shinodaet al., 1998; El-Goharyet al., 1999; Shin et al., 1999**). Increased apoptosis of testicular cells coincided with increased expression of the apoptosis-promoting proteins Bax and p53 was recorded in ratsfollowing combined exposure to Pyridostigmine Bromide, N,N-diethyl m-toluamide and permethrin (**Abou-Doniaet al., 2003).** Excessive or inadequate apoptosis of testicular cells result in abnormal spermatogenesis, azoospermia and severe oligozoospermia**(Lin et al., 1997).**

The production of ROS is a normal physiological event in various organs including the testis. On the other hand, overproduction of ROS can be harmful to sperm and subsequently to male fertility **(Akiyama, 1999**). Pyrethroids are known to generate reactive oxygen species (ROS) and result in oxidative stress in intoxicated animals (**Kale et al., 1999). Oda and El-Maddawy (201**1) has reported that deltamethrin induced lipid peroxidation in testes of rats. Lipid peroxidation is a marker of oxidative damage, which plays an important role in the toxicity of many pesticides. **El- Goharyet al., (1999**) reported the deltamethrin induced lipid peroxidation and nitric oxide production in plasma of rats. **Aitken et al., (1989)** has shown that increase of lipid peroxidation can lead to oxidative damage to sperms DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatozoa. The link between oxidative stress and apoptosis was recorded **(Buttke and Sandstorm, 1994**). Thus, deltamethrin may induce oxidative stress and resulted in the recorded histomorphological alterations and apoptosis in testes of albino rats which involved expression of p53 and Bax.

The current results showed that ginger extract ameliorates the histopathological alterations caused by deltamethrin in testes of albino rats. Moreover, it caused decrease of apoptosis as indicated by decrease of expression of p53 and bax. Similarly, **Sakr and Badawy (2011)** reported that ginger improve the

histological alterations and reduce apoptosis in testis of mice treated with metiram fungicide. **Amin and Hamza (2006)** demonstrated that *Z. officinal* extract reduced the extent of cisplatin-induce sperm abnormality, enhanced sperm motility and testicular damage by increase the activities of testicular antioxidants. Ginger rhizome was found to overcome reproductive toxicity of gentamicin and induced spermatogenesis through the elevation of testosterone levels **(Zahediet al.,, 2010).Hafez (2010)** reported that intake of ginger roots as a drink may be beneficial for diabetic patients who suffer from sexual impotency as their extracts induce antidiabetic activity and enhance male fertility in diabetic rats. **Amin et al.,, (2008)** reported that ginger attenuated the testicular damage and decreased apoptotic damage both in testes and sperms. It also retained the control value of p53 protein expression in the testicular tissue. **Morakinyoet al.,, (2010)** reported that co-administration of aqueous ginger extract with arsenite was found to protect against adverse change in the reproductive organ weight, attenuate the decrease in sperm functions, enhance plasma reproductive hormones level along with increased antioxidants activities and reduced peroxidation **Qureshiet al.,, (1989**) reported that ginger extract significantly increased the sperm mortality and sperm contents in the epididymis and vas deference without producing any spermatotoxic effect. Aaqueous extract of *Z. officinal* was found to increaseweight of testes, the serum testosterone level and epididymal α-glucosidase activity. in male rats **(.Kamtchovinget al.,2002). Khaki et al.,, (2009)** reported that administration of ginger significantly increased sperm percentage, viability, motility and serum total testosterones in rats.

The effect of ginger and its extracts were attributed to antioxidant activityof its major ingredients namely Zingerone, gingerdiol, Zingiberene, gingerols and shogoals**(Zancanet al.,, 2002).** It is concluded from the obtained results that one or more constituents of the used ginger extract may ameliorate the testicular abnormalities induced by deltamethrin in rats.

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Protective Effect of Rosemary (*Rosmarinus Officinalis*) Leaves Extract on Carbon Tetrachloride - Induced Nephrotoxicity in Albino Rats

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Abstract: Carbon tetrachloride (CCl_4) is an environmental pollutant that showed toxicity in different organs. Exposure to CCl_4 is known to induce the formation of reactive oxygen species (ROS). Rosemary *(Rosmarinus officinalis*) is a herb commonly used as spice and flavoring agents in food processing and is useful in treatment of many diseases. The purpose of present study was to investigate the protective role of rosemary on CCl_4-induced renal damage. Treating rats with 1.0ml /kg body weight of 10% CCl_4 twice a week for 6 weeks induced many histological changes in the kidney cortex .The renal tubules lost their characteristic appearance and their lining epithelial cells were degenerated. The glomeruli were atrophied and the renal blood vessels were congested. The intertubular spaces were infiltrated by inflammatory leucocytic cells. An increase in interstitial expression of α-SMA was observed compared with control group. CCl_4 also caused marked elevation in serum creatinine and urea. Treating animals with CCl_4 and aqueous extract of rosemary led to an improvement, in both biochemical and histopathological pictures. It is concluded that rosemary extract had a protective effect against kidney injury induced by CCl_4 and this effect may be attributed to its antioxidant activity.

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1. Introduction

Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. Rosemary *(Rosmarinus officinalis*) is a herb commonly used as spice and flavoring agents in food processing (Ho *et al.,*1994). Rosemary composed of dried leaves and flowers constitutes a particularly interesting source of biologically active phytochemicals as it contains a variety of phenolic compounds including carnosol, carnosic acid, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial and caffeic acid, with substantial *in vitro* antioxidant activity (Aruoma *et al.,* 1992). Leaves of rosemary possess a variety of bioactivities including antitumour (Singletary *et al.,* 1996) and anti-inflammatory actions (Altinier *et al.,* 2007). It is also useful in treatment or prevention of bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory diseases (Al-Sereiti *et al.,* 1999), hepatotoxicity, atherosclerosis biliary upsets, as well as for tension headache, renal colic ,heart disease, and poor sperm motility **(**Rampart *et al.,* 1986; Al-Sereiti *et al.,* 1999).The antioxidant potential of rosemary and its constituents has predominantly been derived from *in vitro* and *in vivo* studies (Richerimer *et al.,* 1996 ; Plouzek *et al.,* 1999). When rosemary extract was supplemented to chicken, it slows down effectively the lipid peroxidation (Serdaroglu and Yildiz-Trup, 2004). Lo *et al.* (2002) reported that carnosol, a naturally occurring polyphenol found in rosemary leaves, showed a potent antioxidative activity against α-diphenyl-B-picryldrazyl free radicals produced from Fenton reaction

Carbon tetrachloride (CCl_4) intoxication in animals is an experimental model that mimics oxidative stress in many pathophysiological situations (Mc Gregor and Lang, 1996). Carbon tetrachloride toxicity has resulted in many cases of poisoning by inhalation, ingestion or absorption. Prolonged exposure to carbon tetrachloride induced histopathological features such as inflammatory leucocytic infiltration, necrosis, fibrosis, cirrhosis and sometimes may lead to tumors (Qiu *et al.,* 2005). Jaramillo-Juárez *et al.* (2008) found that poisoning by CCl_4 induced toxic injury to both liver and kidney. Hepatic damage may be overshadowed by acute renal tubular necrosis, leading to renal oliguric of many species. Various studies demonstrated that CCl_4 intoxication caused free radical generation in many tissues such as liver, kidney, heart, lung, brain and blood (Dashti *et al.,* 1989). Ogeturk *et al.* (2005) reported that exposure to CCl_4 causes acute and chronic renal injuries. In addition, report on various documented case studies established that CCl_4 produces renal diseases in human (Ruprah *et al.,* 1985). The aim of the present study was to examine the protective effect of aqueous extract of rosemary in kidney in carbon tetrachloride intoxicated rats.

2. Materials and Methods

2.1. Preparation of rosemary extract

Extraction of rosemary was carried out according to the method of Dorman *et al.* (2003). Briefly, 50 g fine powdered herb were mixed with 500 ml distilled water in a quick fit flask round-bottom flask which connected to a hydrodistillation apparatus and the water was left to boil slowly for 120 minutes. The water from the flask was removed and another 300 ml of fresh distilled water were added and was boiled another 60 minutes. Water fractions were combined and filtered through qualitative Whatman filter. The filtrate was then subjected to lyopholyzation process through freeze drier under pressure, 0.1 to 0.5 mbar and temperature -35 to -41°C conditions. The dry extract was stored at 4°C until used. The used dose was 220mg/kg body weight.

2.2. Animals and treatments

Male albino Wistar rats weighting 100 ± 5 g were kept in the laboratory under constant conditions of temperature (24 ± 2 ^{o}C) for at least one week before and through the experimental work, being maintained on a standard diet and water were available *ad-libitum*. The animals were maintained in accordance with the guidelines prescribed by the Faculty of Science and the study was approved by the Animal Ethics Committee of the University of Menoufia, Egypt. The experimental rats were divided into four groups:

Group1: Animals were fed on the standard diet and were served as control group.

Group2: Rats were injected intraperitonealy with 1.0ml /kg b.w of 10% CCl_4 dissolved in olive oil twice a week for six weeks (Sakr *et al.*, 2007).

Group3: Animals were orally given 220mg/kg b.w. aqueous extract of rosemary**,** twice weekly for six weeks.

Group4: Rats were injected with CCl_4 followed by oral administration of rosemary extract, twice a week for six weeks.

2.3.Histological and immunohistochemical examinations

The treated animals and their controls were sacrificed by decapitation after 4 and 6 weeks of treatment. Their kidneys were removed and fixed in 10% neutral formalin. Fixed materials were embedded in paraffin wax and sections of 5 micrometer thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. For immunohistochemical localization of α-SMA, fixed wax sections were stained using the avidin-biotin peroxidase method. Formalin fixed paraffin-embedded tissue sections were deparaffinized and endogenous peroxidase activity was blocked with PBS, 0.3% H_2O_2, and 10% methanol for 45 min. To prevent nonspecific binding, the sections were incubated for 60 min in PBS containing 0.3% Triton X-100, 1% BSA, 4% goat serum (GS), and 4% horse serum (block solution). The sections were then incubated overnight at 4 °C with mouse monoclonal α-SMA primary antibody: Actin, Smooth Ab -1(1A4) mouse MAb MS- 113 -PO (1:100; lot: 113P101, Neo Markers Fremont,CA, USA. Thereafter, the sections were incubated for 1 h with Biotinylated Horse Anti-Mouse/Rabbit IgG secondary antibody (Vector Laboratories).Sections were then incubated with avidin–biotin-conjugated peroxidase or 45 min. Finally, the sections were washed and stained with 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma) containing 0.01% H_2O_2 in 0.05M Tris-buffered saline (pH 7.6) for 3–5 min. After the enzyme reaction, the sections were washed in tap water, counterstained with hematoxylin then dehydrated in alcohol, cleared in xylene, and mounted in DPX (Merck, Darmstadt, Germany). Area of α-SMA positive staining was assessed in predetermined high power field (40X) of the cortex (10 fields) then was captured by a digital camera (Kawai *et al.*, 2009).

2.4. Biochemical assays

For biochemical study sera were obtained by centrifugation of the blood samples and stored at 2O°C until assayed for the biochemical parameters. Creatinine and urea were estimated using the methods of Henry (1974) and Patton and Crouch (1977), respectively.

2.5. Statistical analysis:

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, PA). $P<0.05$ values were considered significant.

3. Results

3.1. Histological results

Histological examination of the kidney of control rat or rosemary- treated ones revealed entirely normal histological features, illustrated in figure (1a). The administration of CCl_4 caused significant histological damage to the kidneys, especially to the renal cortex. Examination of the kidney sections of animals after treatment with CCl_4 for 4 weeks, revealed enlargement and congestion of renal blood vessels (Fig.1b). Most of the renal tubules were damaged and lost their characteristic appearance and their lining epithelial cells became undistinguished. Intertubular leucocytic infiltrations were observed (Fig.1c). Marked alterations were observed after 6 weeks of treatment with CCl_4. A number of glomerular capillaries were suffering from severe signs of glomerular congestion, while others were completely damaged. The epithelial lining cells of the glomerular capillaries and of the Bowman's capsules were desquamated into the urinary spaces in the form of granular eosinophilic materials

(Fig.2a). The epithelial lining cells of the renal tubules were revealed nuclear pyknosis and variable forms of cellular rupture and damage. Tubular casts and flocculent materials were also noticed in the lumina of many tubules (Fig.2b). Treating animals with CCl_4 and rosemary revealed an improvement in the histological appearance of the kidney. Most of the renal tubules appeared normal but few tubules were damaged (Fig.2c). Comparison of changes in renal structures of different groups is summarized in table 1.

3.2. Immunohistochemical results

Kidney of control or rosemary-treated rats showed expression of α-SMA in the smooth muscle cells of renal arterioles (Fig.3a). An expression of α-SMA positive fibroblastic cells was recorded in the kidneys of CCl_4-treated rats (Fig.3b). Treatment with rosemary reduced interstitial expression of α SMA (Fig.3 c). Data in figure 4 showed that the percentage of α-SMA positive staining area was significantly ($P<0.05$) decrease in CCl_4- treated rats and the percentage decreased after treatment with rosemary.

3.3. Biochemical results

Treating animals with CCl_4 caused significant elevation in creatinine in the sera. On the other hand, a significant decrease was recorded after treatment with CCl_4 and rosemary (Fig.5). Similarly, blood urea exhibited a significant increase in the treated animals. Co-administration of rosemary lead to a decrease of blood urea (Fig.6).No significant change was recorded in values of creatinine and urea between rosemary and control group.

Table 1 Quantitative assessment of renal histological changes in different animal groups.

Animal Group	Tubular degeneration		Tubular cast		Leucocytic infiltrations		Glomeruli Atrophy	
	3 w	6 w	3 w	6 w	3 w	6 w	3 w	6 w
Control	-	-	-	-	-	+	-	-
Rosemary	-	-	-	-	+	+	-	-
CCl_4	+ +	+ + +	+ +	+ + +	+ + +	+ + +	+ +	+ + +
CCl_4+ rosemary	+	+	+	+	+ +	+	+	+

+ Mild (5–10% severity), + + Moderate (10–25% severity), + + +Severe (25–50% severity)

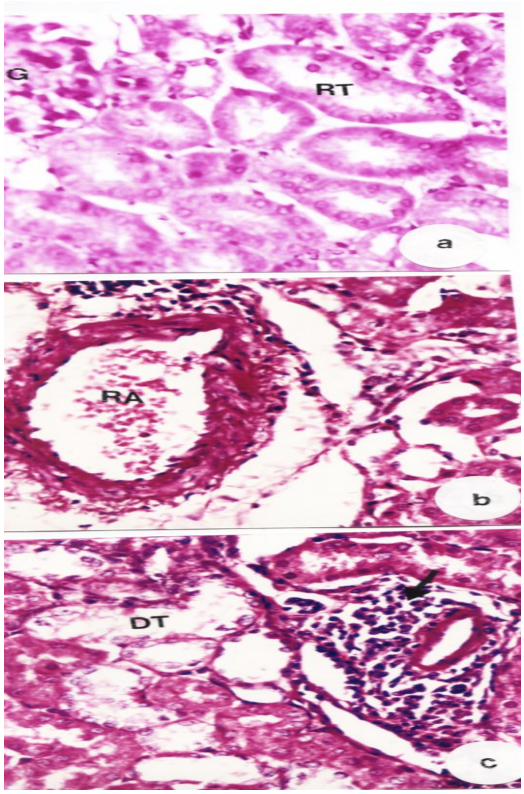

Fig.1. Sections in the kidney cortex of (a) control rat showing glomeruli (G) and renal tubules (RT); (b-c) 4 weeks after CCl_4 treatment showing enlarged and congested renal vein (RV), leucocytic infiltrations (arrow) and degenerated tubules (DT), (X300)

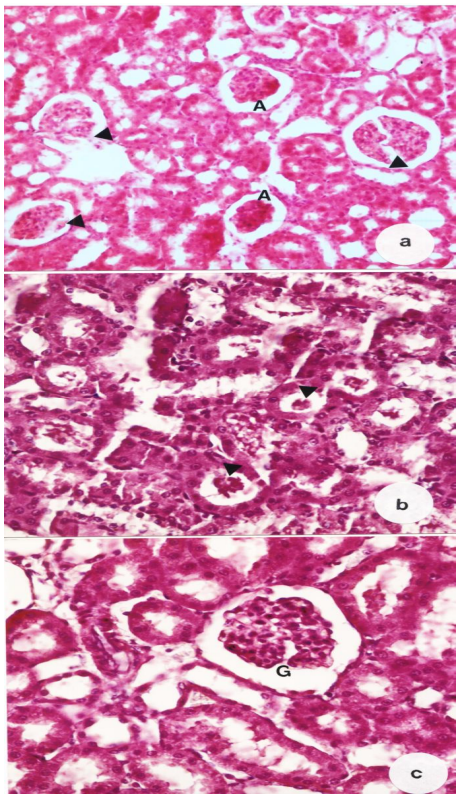

Fig.2. (a) Kidney cortex 6 weeks after CCl_4 treatment showing atrophied (A) and fragmented (arrow heads) glomeruli (X120), (b) proteinaceous casts in the lumen of renal tubules (arrow head) (X300), (c) after treatment with CCL_4 + rosemary showing normal renal tubules and glomeruli (G) (X300).

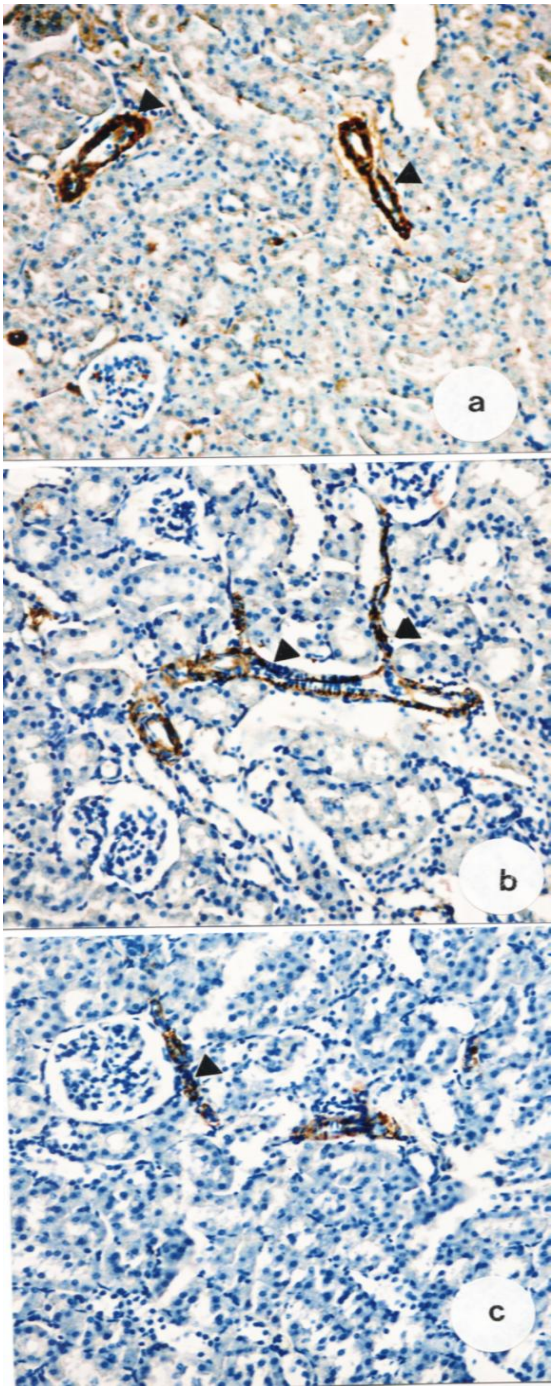

Fig.3. Immunohistochemical staining for *α*-SMA; (a) control kidney showing expression of *α*-SMA in renal arteriols (arrow head); (b) six weeks after treatment with CCl_4, showing increase in expression of *α*-SMA positive fibroblastic cells (arrow heads); (c) after treatment with CCl_4 and rosemary showing decrease of *α*-SMA expression (X 120).

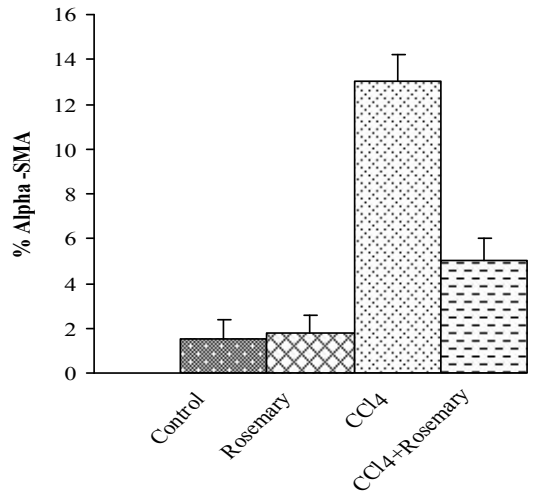

Fig.4. *%* *α*-SMA expression in different animal groups

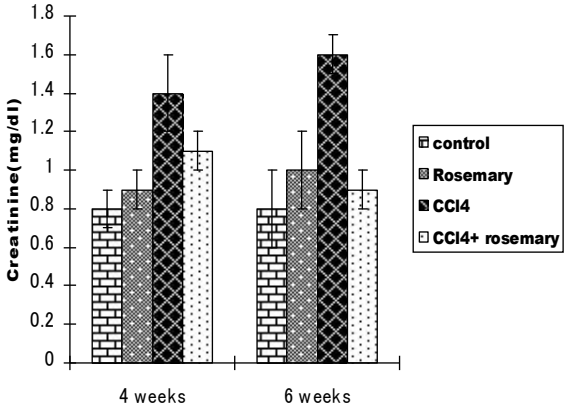

Fig.5. Change in creatinine in different animal groups.

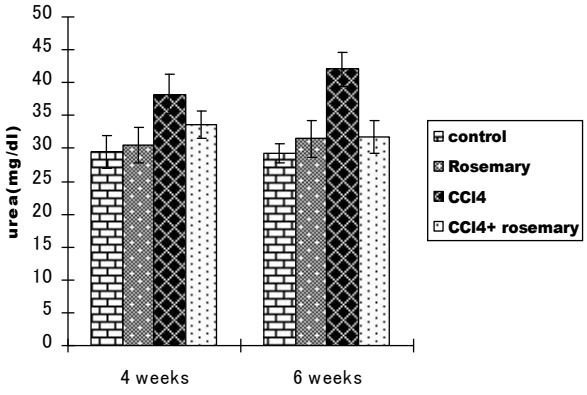

Fig.6. Change in urea in different animal groups.

4. Discussion

The elevation in creatinine and blood urea, and the observed histopathological alterations recorded in this work indicated that CCl_4 caused renal toxicity in rats. Ozturk *et al.* (2003) recorded similar histopathological alterations in rats kidney treated with CCl4 characterized by tubular epithelial cells alterations including vacuolization, atrophy, detachment of epithelial cells and tubular necrosis. With these histopathological changes, the capacity of tubular absorption may have been altered and functional overloading of nephrons with subsequent renal dysfunction was observed (Khan *et al.*, 2010). In addition to its hepatic toxicity, a number of reports clearly demonstrated that CCl_4 also causes disorders in kidneys, lungs, testes as well as in blood (Ahmad *et al.*, 1987; Ozturk *et al.*, 2003). Ogeturk *et al.* (2005) reported that exposure to this solvent causes acute and chronic renal injuries. Ruprah *et al.* (1985) established that CCl_4 produces renal diseases in human.

It was reported that CCl_4 metabolized by cytochrome p- 450 generates a highly reactive free radical, and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species can cause oxidative damage in DNA, proteins and lipids (Melin *et al.*, 2000). Various studies have demonstrated that CCl_4 causes free radical generation in many tissues including kidney. Olagunjua *et al.* (2009) suggested a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CCl_4 nephrotoxicity. Noguchi *et al.* (1982) reported that CCl_4 resulted in enhanced generation of trichloromethylperoxyl radical hydrogen peroxide in cultured hepatocytes as well as mesangial cells. *In vitro* and *in vivo* studies indicate that CCl_4 enhances lipid peroxidation, reduces renal microsomal NADPH cytochrome P450, and renal reduced/oxidiced glutathione ratio (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria (Rungby and Ernst, 1992).

Concerning the immunohistochemical results, an increase in expression of □-SMA was recorded in kidneys of CCl_4-treated rats. α-SMA expression is a typical molecular marker of myofibroblasts in many nephropathies (Kramer *et al.*, 2009). Renal fibrosis is the principal process involved in the progression of chronic kidney disease (Pradère *et al.*, 2008), ureteral obstruction, malignant hypertension, severe diabetic condition or chronic exposure to heavy metals (Cohen, 1995). The development of renal fibrosis involves the progressive appearance of glomerulosclerosis, tubulointerstitial fibrosis and changes in renal vasculature, and at a molecular level, fibrosis can be defined as an excessive accumulation of extracellular matrix such as collagen and fibronectins (Al-Bayati *et al.*, 2002).

The current study revealed that rosemary aqueous extract alleviated the renal toxicity of CCl_4. This was manifested by normal appearance of kidney tissues and decreased levels of creatinine and urea. Similarly, Ahmed and Abdella (2010) reported that rosemary prevented histopathological lesions and oxidative stress induced by doxorubicin in liver, kidney and heart of mice. Rosemary was also found to have a therapeutic potential in treatment or prevention of inflammatory diseases hepatotoxicity, renal toxicity and heart diseases (Babu, 1996, Válenzuela *et al.*, 2004).

Abdel-Wahhab *et al.* (2011) reported that administration of rats with rosemary extract alleviated the deleterious effect of CCl_4 on liver. They added that rosemary may act as a co-factor in the synthesis of biological endogenous antioxidant material such as glutathione-s-transferase and quinine reductase. Interstitial fibrosis was decreased in kidney of rats treated with CCl_4 and rosemary as indicated by decrease of expression of α-SMA. In agreement with this result, Yahuaca *et al.* (2005) reported the protective effect of rosemary on liver fibrosis and cirrhosis induced by CCl_4.

Rosemary is rich in phytochemical derivatives such as triterpenes, flavonoids or polyphenols. Many studies reported that the preventive effects of rosemary and its extracts are attributed to its antioxidant activity. Schwarz *et al.* (1992) and Zeng and Wang (2001) reported that carnosol, rosmanol and epirosmanol phenolic diterpenes of rosemary inhibit lipid peroxidation. Ursolic acid, a constant constituent of *Rosmarinus officinalis* extracts, has been shown to have antioxidant and anticarcinogenic properties (Huang *et al.*, 1994). Rosmarinic acid exhibits antioxidant and antiinflammatory effects (Halliwell, 1996). Rosemary extracts are able to donate electrons to reactive radicals, converting them to more stable and on reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems. Also, it was concluded that rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals (Moreno *et al.*, 2006).

In conclusion, the present results showed that rosemary aqueous extract alleviates the nephrotoxicity induced by CCl_4 in albino rats. This effect of rosemary may be attributed to the antioxidative activity of one or more of its constituents.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Echocardiographic Evaluation of Cardiac Structural and Functional Changes in Hepatitis C Positive Non-Alcoholic Liver Cirrhosis Patients and Their Plasma NT-ProBNP Levels

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Abstract: Background: Cirrhotic cardiomyopathy (CC) refers to cardiac structural, functional, and electrophysiological changes in liver cirrhosis (LC) patients. Emerging role of natruretic peptides in screening LC patients for development of CC was suggested. The aim of this study was to assess structural and functional cardiac changes in hepatitis C positive nonalcoholic (LC) patients and N- terminal pro brain natriuretic peptides (NT-proBNP) blood levels in these patients. **Methods:** Forty hepatitis C positive LC patients classified according to their child - Pugh score underwent transthorathic echocardiographic assessment of cardiac chambers dimensions, and left ventricular functions. Estimation of plasma NT-proBNP levels of these patients and 10 healthy age and gender matched healthy control subjects was done. **Results:** Child-Pugh C LC patients have significantly higher mean left ventricular end diastolic (LVEDD) and end systolic dimensions (LVESD) compared to those of Child- Pugh A and B LC patients. LV systolic function was preserved in the three Child- Pugh groups, while diastolic dysfunction was detected in 78.5% of Child- Pugh C patients. Mean plasma NT-proBNP level was significantly higher in Child-Pugh C patients compared to mean plasma levels in Child- Pugh A and B patients. ProBNP plasma levels correlated significantly with serum albumin, bilirubin, creatinine, international normalized ratio, Child-Pugh score, LVESD, LVEDD, left atrial and right ventricular diameters. **Conclusion:** Child-Pugh C LC patients suffered of cardiac structural changes associated with diastolic dysfunction and raised mean ProBNP plasma level but preserved systolic function. NT-ProBNP levels correlated significantly with echocardiographic changes and Child- Pugh score.
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Key words: cirrhosis- cardiomyopathy-echocardiography- NT-proBNP-Hepatitis

1. Introduction:

Cirrhotic cardiomyopathy is a recently recognized condition in cirrhosis [(1)]. This term denotes a chronic cardiac dysfunction, characterized by blunted contractile responsiveness to stress and altered diastolic relaxation with electrophysiological abnormalities, such as prolongation of the QT interval, all occurring in the absence of any other cardiac disease [(2,3)].These changes were previously thought to be related to latent alcoholic cardiomyopathy in alcoholic liver cirrhosis patients but latter clinical and experimental studies showed that these cardiac changes are seen in those with nonalcoholic cirrhosis [(1)].

This cardiac dysfunction may affect the prognosis of the patients and aggravate the course during invasive procedures such as surgery, insertion of a transjugular intrahepatic porto systemic shunts (TIPS), and liver transplantation [(4)].

Many patients chronically infected by hepatitis C virus (HCV) experience symptoms like fatigue, dyspnea and reduced physical activity. However, in many patients, these symptoms are not proportional to the liver involvement and could resemble symptoms of chronic heart failure [(5)]. Several studies have shown increased plasma levels of brain natriuretic peptide (BNP) and NT-proBNP in some patients with cirrhosis, and these findings may suggest cardiac dysfunction [(6)].NT-proBNP has been recently suggested to be an even better indicator of early cardiac dysfunction than BNP because of its stability and longer biological half-life [(7)]. Several studies have shown that plasma levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) are reliable diagnostic and prognostic markers for cardiac disease; furthermore, they correlate with symptoms of heart failure and with the severity of systolic and diastolic dysfunction [(8)].

The aim of this study was to evaluate echocardiographic structural and functional changes in non alcoholic HCV positive liver cirrhosis patients and plasma levels of NT-ProBNP in these patients.

2. Subjects and Methods:

The protocol of this study was first approved by the scientific board of Internal Medicine department and Committee of Research Ethics; Faculty of Medicine – Cairo University – Egypt and Informed consents were obtained from all participants.

The study included 40 Hepatitis C positive liver cirrhosis (LC) patients and 10 age and gender matched normal healthy controls. LC diagnosis was based on established clinical, biochemical, and ultrasonography criteria none were proven by biopsy. HCV infection was diagnosed with positive serum HCV - RNA by polymerase chain reaction. Participants were recruited from patients admitted to Internal Medicine departments in Kasr El Eini Hospital- Cairo University during the period between December 2010 and December 2011.Those with history of alcohol consumption, diabetes mellitus, hypertension, cardiac, renal or pulmonary diseases or previous cardiac surgery were excluded. Those suffering from valvular heart disease and those with a poor pericardial window were also excluded.

Patients underwent thorough clinical evaluation, chest X-ray, and electrocardiography (ECG). Blood sampling for hemoglobin, prothrombin time, serum sodium, potassium, urea, creatinine, albumin and bilirubin estimation and abdominal ultrasound imaging were done . Blood samples for analysis of NT-proBNP were centrifuged and plasma stored at −80 °C until analysis. Plasma concentrations of NT-proBNP in LC patients and healthy control subjects were measured by a sandwich immunoassay technique (Roche Diagnostics, Mannheim, Germany).

Patients were classified according to Child-Pugh criteria [serum bilirubin, prothrombin time, serum albumin, ascites, and encephalopathy].Chronic liver disease was classified according to point score into 5-6 points for Child -Pugh A score,7-9 points for child - Pugh B score and 10-15 points for child - PughC score[9].

Two-dimensional and M-mode echocardiography were performed to all patients by physician who was blinded to the results of the plasma NT-ProBNP levels, imaging was performed with Vivid 3N (General electric) equipped with 2.5 MHz and 3.5 MHz phased pulsed array transducers. Two-dimensional imaging examinations were performed in the standard fashion in parasternal long- and short-axis views and apical 4- and 2-chamber views. Cardiac chambers dimensions were measured according to the guidelines of the American Society of Echocardiography using M-mode method [10].

LV systolic and diastolic diameters and volumes , ejection fraction and fraction shortening were derived from biplane apical (2- and 4-chamber) views with a modified Simpson's rule algorithm[11].

Pulsed Doppler spectral recordings were obtained in the apical 4-chamber view from a 4x4-mm sample volume positioned at the tips of the mitral leaflets. The transmitral pulsed Doppler velocity recordings from 3 consecutive cardiac cycles were used to derive measurements as follows: E and A velocities were the peak values reached in early diastole and after atrial contraction, respectively, and deceleration time (DT) was the interval from the E-wave peak to the decline of the velocity to baseline. In those cases in which velocity did not return to baseline, extrapolation of the deceleration signal was performed[12].

LV functions were classified into 3 major categories:[12]

a)Normal LV Function

Normal ventricular function was defined by normal LV end-diastolic (3.5 to 5.5 cm) and end-systolic (2.5 to 3.6 cm) dimensions, no major wall motion abnormalities, an ejection fraction >50%, and no evidence of impaired or restrictive like relaxation abnormalities as described below.

b)LV Systolic Dysfunction

Systolic dysfunction was defined by an ejection fraction <50% .

C)LV Diastolic Dysfunction

Diastolic dysfunction was classified in 3 categories.

Impaired Relaxation

Impaired relaxation was defined as an E/A ratio <1 or DT >240 ms in patients <55 years of age and an E/A ratio <0.8 and DT >240 ms in patients >55 years of age.

Pseudonormal

Pseudonormal was defined as an E/A ratio of 1 to 1.5 and DT >240 ms. Confirmation reversal of the E/A ratio (to <1.0) by **Valsalva** when possible.

Restrictive

Restrictive like filling patterns were defined as DT <160 ms with >1 of the following: left atrial size >5 cm, or E/A >1.5.

Statistical analysis

Statistical Package of Social Science (SPSS) program version 15.0 was used for analysis of data. Data was summarized as mean , SD. T-test was used for analysis of 2 quantitative data, while Non parametric test (Mann Whitney U test) was used when data was not symmetrically distributed. One way ANOVA test was used for analysis of more than 2 quantitative data followed by post HOCC test for detection of significant. Pearson's correlation was also done. r was considered weak if < 0.25, mild if $r \geq 0.25 < 0.5$, moderate if $r \geq 0.5 < 0.75$ and strong if $r \geq 0.75$. P-value was consider significant if ≤ 0.05*.

3. Results:

The clinical and laboratory data of the studied liver cirrhosis patients classified according to their Child- Pugh score into 3 groups (A, B and C). Child- Pugh A group was composed of 9 patients (7men and 2 women) , Child- PughB group was composed of 16 patients(9men and 7 women) and Child- Pugh C patients was composed of 15 patients (10 men and 5 women). Child- Pugh C patients had significantly lower mean haemoglobin and significantly higher mean AST, ALT, INR, bilirubin and NT- proBNP blood levels compared to those of Child- Pugh A and Child- Pugh B patients (table 1).

Table (1) Comparison between laboratory data of patients in relation to child classification

	Child –Pugh A		Child –Pugh B		Child–Pugh C		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	52.90	8.65	57.07	9.96	49.08	5.98	0.06
Hb (g/dl)	10.78[a]	1.36	10.51[a]	0.57	9.32[b]	0.63	0.0001*
AST(IU/L)	60.70[a]	35.74	63.73[a]	19.51	89.47[b]	31.28	0.02*
ALT (IU/L)	58.60[a]	23.45	61.80[a]	23.90	87.53[b]	33.75	0.02*
Urea (mg/dl)	39.90	9.54	39.60	10.81	43.47	12.65	0.6
Creatinine (mg/dl)	0.95[a]	0.21	0.97[a]	0.18	1.12[b]	0.15	0.03*
Total bilirubin (mg/dl)	1.01[a]	0.37	1.95[b]	0.37	3.00[c]	0.78	0.0001*
Albumin (gm/dl)	3.0	0.82	2.8	0.48	2.7	0.8	0.9
INR	1.42[a]	0.28	1.52[a]	0.19	2.00[b]	0.50	0.0001*
NT-proBNP(pg/ml)	59.20[a]	6.08	72.40[a]	15.57	343.13[b]	117.90	0.0001*

Different symbol indicates significant.

The echocardiographic data of the studied liver cirrhosis patients showed that mean left ventricular end systolic diameter (LVESD) and mean left ventricular end diastolic diameter (LVEDD) were significantly higher among Child - Pugh C patients compared to those of Child- Pugh A and Child- Pugh B patients . Mean Child - Pugh C patients left atrial diameter was higher than the mean values of the other Child groups but only mean LA diameter of Child- Pugh B was significantly different from that of Child- Pugh Cpatients. Right ventricle diameter of Child- Pugh C patients was higher than those of Child - Pugh A and Child- Pugh B patients, although that difference did not reach statistical significance (Table 2).

Table 2. Echocardiographic data of liver cirrhosis patients according to child- Pugh classification

	Child A		Child B		Child C		P-value
	Mean	SD	Mean	SD	Mean	SD	
LA(cm)	3.51[ab]	0.70	3.18[a]	0.54	3.80[b]	0.62	0.03*
LVEDd(cm)	4.38[a]	0.51	4.59[a]	0.43	5.27[b]	0.51	0.0001*
LVESd(cm)	2.73[a]	0.50	2.65[b]	0.34	3.14[b]	0.45	0.009*
IVSDd(cm)	1.07	0.22	1.04	0.22	1.06	0.18	0.9
LVPWD(cm)	1.10	0.22	1.08	0.18	1.15	0.22	0.6
EF(%)	68.60	5.89	72.80	6.16	70.73	6.64	0.3
FS(%)	38.50	4.45	42.20	5.40	40.80	5.86	0.3
RVDd(cm)	2.91	0.44	2.70	0.45	3.16	0.59	0.06
E(m/sec)	0.65	0.16	0.70	0.29	0.75	0.16	0.6
A(m/sec)	0.70	0.14	0.82	0.22	0.72	0.13	0.2
E/A	0.95	0.27	.86	0.20	1.06	0.29	0.1
DECT(msec)	190.60	45.46	169.87	18.37	184.86	35.71	0.3

LA: left atrium, LVEDd: left ventricle end diastolic diameter, LVESd: left ventricle end systolic diameter, IVSDd: interventricular septal diameter in diastole, LVPWD:left ventricle posterior wall diameter in diastole,EF:ejection fraction,FS: fractional shortening, RVDd: right ventricle diameter in diastole, E : peak transmitral velocity reached in early diastole, A :peak transmitral velocity after atrial contraction, E/A ratio, ratio of velocity of E wave to velocity of A wave of Doppler mitral valve inflow and DECT: deceleration time. Different symbol indicate significant

Although mean E wave, A wave velocities, E/A values and deceleration time were not statistically different among patients in the 3 Child- Pugh groups (Table2), diastolic function classification according to the previously described echocardiographic classification showed that 50% of Child- Pugh A patients, 66.66% of Child- Pugh B patients and 78.5% of Child- Pugh C patients suffered of diastolic dysfunction (Table 3).

Table 3. Diastolic function of liver cirrhosis patients grouped according to Child classification

	Child- Pugh A	Child - Pugh B	Child- Pugh C
Normal diastolic function	5(50%)	5(33.33%)	3(21.5%)
Impared relaxation	4(40%)	10(66.66%)	8(57.1%)
Pseudonomalization	0(0%)	0(0%)	0(0%)
Restrictive pattern	1(10%)	0(0%)	3(21.4%)

Mean NT-pro BNP blood levels among studied liver cirrhosis patients according to their diastolic function were 140.84±144.14 pg/ml , 176.86±153.13 pg/ml and 231.5± 200.92pg/ml among those with normal diastolic function, impaired relaxation and restrictive pattern respectively (Figure 1).

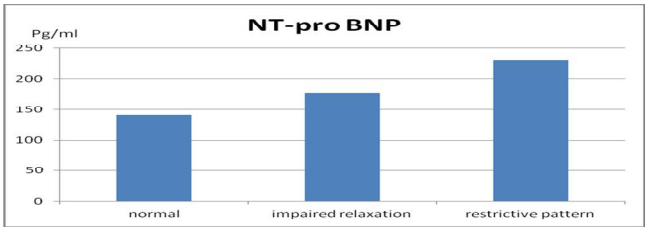

Figure1.NT-proBNP levels in liver cirrhosis according to the diastolic function of liver Cirrhosis patients.

Mean NT-pro BNP blood levels among liver cirrhosis patients in any Child- Pugh group were significantly higher than those of healthy control subjects. Mean NT-proBNP blood levels were significantly higher among Child- Pugh C patients compared to those of Child- Pugh A and Child- Pugh B patients (Figure 2).

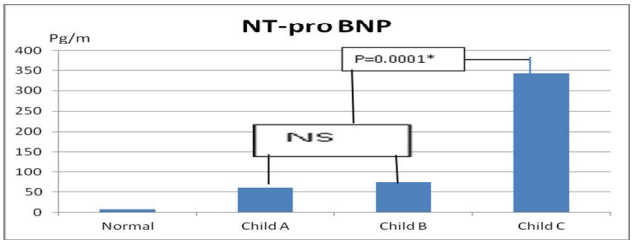

Figure 2. ProBNP levels in healthy control (normal) and liver cirrhosis patients according to Child-Pugh classificatio

There was a significant Correlation between blood NT-ProBNP blood levels and left atrial,LVESD,LVEDD, and right ventricular diameters as well as serum creatinine, bilirubin, albumin, INR and Child- Pugh score(Table 4).

Table4. Pearson Correlation between proBNP blood levels and echocardiographic , laboratory data and child score of liver cirrhosis patients

Variables	Correlation coefficient	proBNP
Left atrium diamater	r	0.3
	P-value	0.04
Left ventricle end diastolic diamater	r	0.7
	P -value	0.0001*
Left ventricle end systolic diamater	r	0.6
	P -value	0.0001*
Right ventricle diamater	r	0.3
	P -value	0.02*
Serum bilirubin	r	0.5
	P -value	0.002*
Serum albumin	r	0.01
	P -value	0.8
International normalized ratio	r	0.5
	P -value	0.0001
Child- Pugh score	r	0.8
	P -value	0.0001*
Creatinine (mg/dl)	r	0.4
	P -value	0.02*

4. Discussion:

In this study cardiac structural and functional changes in nonalcoholic liver cirrhosis patients who were not diabetic or hypertensive, with no history of cardiac disease and no valvular heart disease (that could affect cardiac structure and function) were explored using conventional trans thoracic echocardiography.

The study results showed that Child- Pugh C group of patients suffered of structural cardiac changes, in the form of significantly higher mean LVESD and LVEDD compared to those of Child-Pugh A and Child- Pugh B patients. In addition they have a larger mean right ventricular diameter dimensions compared to those of Child- Pugh A and Child- Pugh B patients but that difference didn't reach statistical significance. Mean left ventricular systolic function parameters (ejection fraction and fractional shortening) were within normal range, among the patients of the three Child groups. Diastolic dysfunction was more prevalent with advancing Child- Pugh score. 50% of Child- Pugh A patients , 66.66% of Child- Pugh B patients and 78.5% of Child- Pugh C patients suffered from diastolic dysfunction.

Similar to our results, Moller and Henriksen in 2002 reported an increase in both systolic and diastolic volumes of the left ventricle in cirrhotic patients, while changes in the right cardiac chambers are less prominent, and mean right ventricle diameters were normal in most studies[13]. Baik *et al.* reported that systolic function is preserved in liver cirrhosis patients with normal or even increased ejection fraction at rest [14].

Finucci et al. reported that cirrhotic patients in addition to increased left ventricular end-diastolic , left atrial , stroke volumes, they showed increased late diastolic flow velocity compared to normal controls; their results indicated an impaired left ventricular relaxation in these patients[15].Raedle, et al. 2008 used tissue Doppler imaging to assess the diastolic function in chronic liver disease patients . Their results showed that left ventricular diastolic dysfunction was found in 25 of 31(80.6%) patients with severe liver fibrosis/cirrhosis versus 2 of 8(25.0%) patients with moderate and 6 of 25(24.0%) patients with mild liver fibrosis. Their results agree with results of the present study regarding prevalence of diastolic dysfunction in patients with advanced liver cirrhosis [16].

In the present study mean NT-proBNP plasma levels were significantly higher in cirrhosis patients compared to healthy controls. In addition mean NT-proBNP plasma levels in Child- Pugh C patients were significantly higher than those of Child- Pugh A and Child- Pugh B patients. PlasmaNT-proBNP levels were positively correlated to Child- Pugh score, bilirubin, INR, serum creatinine, left atrial, right ventricular , LVESd and LVEDd. The previous results are similar to those of Henriksen, et al. in 2003 who showed for the first time that NT-proBNP concentrations are significantly increased in patients with advanced cirrhosis despite no signs of diminished hepatic degradation of NT-proBNP in these patients, in addition they reported that elevated levels of NT-proBNP in these patients are related to markers of advanced liver cirrhosis (Child- Pugh score and coagulation factors) [17].

Results of this study have shown that NT-proBNP blood levels correlates with left atrial, left ventricular, right ventricular dimensions and left ventricular diastolic dysfunction (NT-proBNP) blood levels being highest in those with restrictive filling) .These results are in concordance with results of the following studies.

Lim et al., noticed that BNP and NT-proBNP levels reflect left atrial size, correlating positively with left atrial volume, particularly in the general population and in patients with heart failure with preserved systolic function[18].Daniel and Maisel., reported that BNP and NT-proBNP levels also correlated positively with LV dimensions, volumes, and mass in a variety of settings and populations[19].Mariano-Goulart et al., reported that the right ventricle (RV) contributes to plasma levels of BNP or NT-proBNP, with either normal or impaired LVEF. Levels of both peptides correlate with measures of RV size, increasing with greater dilatation[20].Tschope et al. noticed that narturetic peptides levels increase with greater severity of overall diastolic dysfunction, independent of LVEF, age, sex, body mass index, and renal function, and the highest levels are seen in subjects with restrictive filling patterns[21].

Few studies, like our study was done to assess nonalcoholic hepatitis C liver cirrhosis patients with low cardiovascular risk , no history of cardiac disease and no valvular heart disease which makes data of this study valuable to the medical literature but it got few limitations. First: the limited number of patients included in this study was due to the vast exclusion criteria. Second: the use of conventional transthoracic echo-Doppler instead of tissue Doppler echocardiography might have limited our ability to differentiate normal from pseudonormal diastolic function in this study. Third: although cirrhotic patients with history or documented IHD were excluded from the study and cirrhotic patients in this study were of low cardiovascular risk, a recent study shed light on the fact that end stage liver disease patients have high prevalence of coronary artery disease and that non invasive assessment has limited

diagnostic accuracy in these patients. IHD is a silent disease that can only be accurately excluded invasively, that might be responsible for some of structural and functional changes seen in these patients[22].Fourth limitation is that even in stable subjects, natruretic peptides levels vary with repeat testing as a consequence of assay characteristics and biological variation however relative variation is greater in normal subjects, in whom absolute levels are low, but when absolute levels are higher as seen in our patients, the relative variation is lower [23].

5. Conclusion,

We noticed that the heart is another organ affected in liver cirrhosis. Not only those with alcoholic cirrhosis suffer from cardiac abnormalities, structural and functional changes are seen in advanced hepatitis C positive non-alcoholic liver cirrhosis patients. These changes correlated with increased blood levels of NT-proBNP. These changes were detected in a group of them with low cardiovascular risk, no history or documented previous cardiac disease or valvular heart disease. There is still no consensus regarding the diagnosis of cirrhotic cardiomyopathy , it is an interesting topic that merits further research to reach clear diagnostic criteria , gold standard diagnostic tests and a value of naturetic peptides in screening, diagnosis and prognostic assessment of that condition.

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2/21/2012

Model of occupational stress, that take of organizational commitment and normal personality type in staff of Banks.

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This research has been conducted to determine a model of occupational stress regarding people personality types, norm of working community and their organizational commitment. Method of this research is descriptive correlation method; statistical society of this research includes all staff members of Shahr Bank of Iran, among them 300 people were selected by random cluster sampling approach from Tehran. In this research some questionnaires of personality types Myers - Briggs, Philip L. Rice occupational stress, Alan - Mayer organizational commitment have been used to determine the normal people personality types, occupational stress, organizational commitment. The findings from step by step regression analysis showed that the most important predictors of occupational stress are emotional and introversive types of personality that have lower organizational commitment in emotional, normative, and continuous dimensions and by using these findings a model is presented to predict the occupational stress.

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Key words: model of occupational stress, personality types, organizational commitment

Social psychology is the science studies individuals' behavior in groups and society; because people have different personalities and they have different functions according to these personal differences. There are different definitions of personality and theorists introduce different views about the nature of the human character. In a general definition personality can be defined as an enduring and unique collection of individual traits that may be changed in different situations (Schultz, Dune P. (2006). Each person's personality is unique, that is in addition of the similarities that exist between people, and each person has unique features that make him distinct from others. Different understandings of personality concept clearly shows that by passing the time personality meaning has been more extensive than its concept that was a social and apparent image, now personality refers to basic and stable trend of each person (Schultz, D. P. (1990). In Jung's opinion lots of our conscious sensing and reaction to the environment is determined by contrasting extraversion and introversion mental attitudes. After knowing kinds of extraversion and introversion, he considered another distinction among people that are based on what he called them psychological functions. These functions refer to our different and conflicting ways of understanding the real outer and inner world of our mind. Jung considers four mental functions as: sensing, intuition, thinking and feeling(Jung, C. G. (1927). . Having different personality types requires different job requirements, on the other hand having a job and income source is one of needs, plans and concerns of a person that has completed childhood, having a job is a sign of adultness by which a person shows his abilities and skills and discovers his restrictions. Halland has based his theory on two important principals 1) Choosing profession depends on the type of personality. 2) Choosing profession is in a direct relationship with individual attitudes and trends; if a person cannot be in his place according to his capability and the type of his personality, he will have numerous problems Carsten, J (2006).

Occupational stress is a kind of stress that a given individual undergoes it in a certain job. Both individual and occupational features have considered involved in this definition. National institute for occupational safety and health defines occupational stress as lack of coordination between working needs with abilities, capabilities and aspirations of the individual (Pascale Carayon& etal ,1999). Special conditions of work, expanding the work pressure, job training cause increasing mental and physical illnesses. HSE defines stress as: some reactions that people show because of excessive pressures or demands that are contrary to their expectations (Health and Safety Executive (HSE) (2001)Psychologists of Texas University have considered two groups of consequences for occupational stress include physiological and psychological consequences (Kingama & Mireille, 2002), Cooper and David sons 1987 consider four main factors of occupational stress including internal factors, organizational role, communication in work

place and organizational atmosphere (Miller, David (2001). Nowadays occupational stress is one of the important problems in organizations that are considered in this research.

One of the most important capitals of each organization is having employees with high organizational commitment, in recent years organizational commitment has been important part of organizational studies and study center, because its relation with organization quality has been proved. Research show that staff commitment is an effective and strong force (Culverson DE,2002). Organizational commitment is an attitude about staff loyalty to the organization and is an ongoing process through participation in corporate decisions causes considering people, organization, success and prosperity of the organization (Seeratdoost Z,2005). Efficient manpower is a main index of excellence of an organization to other organizations. Committed manpower raises an organization in society and prepares the field for developing and expanding the organization (Saghafi N.2006). Researcher provides theoretical definitions of personality types, occupational stress and organizational commitment by considering mentioned items.

Method and Material

This research is a fundamental research and its purpose is to explore relationships between variables and research method is correlation. To analysis data descriptive statistics method has been used like Frequency tables, graphs, calculating means, SD and also statistical indices have been used to test hypothesis by correlative method and multi variables regression. To determine type of bank staff personality, Mayers- Brigs questionnaire has been used that is inferential from Gustav Jung theory and this questionnaire is one of the most famous questionnaires in world and has been populated in Iran by Jahanian (2006). Mayers-Brigs questionnaire , sixth edition European-English volume, is an instrument with 88 questions and includes 25 questions in extroversion – introversion dimension, 19 questions in sensory- intuition, 24 questions in thinking-feeling and 19 questions in judging-perceiver dimension and it has one extra question that doesn't include test scoring (Yiannakis, C. & Taylor, N.(2009). Philip. L.Rice questionnaire (1991) has been used to determine occupational stress level of bank staff(124). This test has 57 items with some information about occupational stress. It has been translated and standardized for the first time by Hatami (1999). Allen and mayer Organizational commitment questionnaire (1993) has been used to evaluate occupational commitment of staff. This questionnaire has been made by Allen, Mayer and Smith in 1993 to measure and evaluate three dimensions of occupational commitment includes feeling, continuous and normative commitment with 18 questions.

The statistical society in present research includes: all employees of City Bank in Tehran who are working in 2011 that selected cluster randomly

Descriptive Statistics

In Table 2- 8, the average of standard deviation, minimum and maximum values of the variables of the study are presented after data analysis and removal of incomplete and disrupted values for 300 subjects. It should be noted that firstly, occupational stress alongside its first three subscales, after that, organizational commitment besides its subscales and eventually, descriptive statistics of eight subscales of various Myers - Briggs indicators are offered. Furthermore, since the extreme high and low values for each variable of organizational commitment and occupational stress and personality types that have followed are the amounts to be determined, points 25% and 75% of the first and third quarter were calculated as well that will be provided in the following section.

Table 1: average, standard deviation, minimum and maximum values (n=300)

Statistic is Variables	Average	Standard deviation	Minimum	Maximum
Job Stress	161.46	19.64	113	220
Interpersonal relations	66.38	7.76	45	95
Physical health	56.38	11.48	25	91
Career interests	39.24	6.22	26	63
Organizational commitment	82.64	10.90	53	107
Commitment to continuous	29.79	5.94	15	40
Emotional commitment	25.82	4.34	15	38
Normative commitment	27.02	3.60	16	37
Extraversion	18.19	5.29	3	31
Introversion	15.07	5.44	2	31
Sensing	14.98	3.92	5	24
Intuition	10.25	3.42	2	19
Thinking	17.17	4.49	3	27
Feeling	7.30	3.64	0	16
Judgment	18.98	4.42	3	28
Perception	9.06	4.93	0	27

To verify this hypothesis, Durbin - Watson statistic for prediction of stress resulted in 1.77 and for the prediction of organizational commitment was

1.89 which is proper and stands for no correlation between the residual values. Given the verification of basic assumptions of regression analysis, its implementation for predicting occupational stress is permitted. The following table presents the results of regression analysis to predict the stress by the help of personality types.

According to Table 2, it can be seen that five models are extracted from the stepwise regression process and eventually, the 5 variables with the highest rate in predicting occupational stress have remained in the fifth model. The 3 variables of these variables are sub-scales of organizational commitment.

Table 2: standardized coefficients, squared correlation coefficient shifts in the multivariate stepwise model to predict stress

Model	Variable	beta	R^2	ΔR^2	t	F
1	Continuous Commitment	-0.57	0.33	0.33	-12.02**	144.42 **
2	Normative commitment	-0.45 -0.31	0.41	0.08	-9.41** -6.41**	102.51 **
3	Continuous Commitment Normative commitment Emotional commitment	-0.34 -0.27 -0.26	0.46	0.05	-6.73** -5.86** -5.24**	83.57 **
4	Normative commitment Emotional commitment Feeling	-0.33 -0.26 -0.24 0.17	0.49	0.03	-6.56** -5.79** -4.92** 3.98 **	69.77 **
5	Commitment to continuous Normative commitment Emotional commitment Feeling Extraversion	-0.30 -0.26 -0.22 0.19 -0.13	0.50	0.01	-5.93** -5.79** -4.49** 4.41 ** -2.95**	59.02 **

**P < 0.01

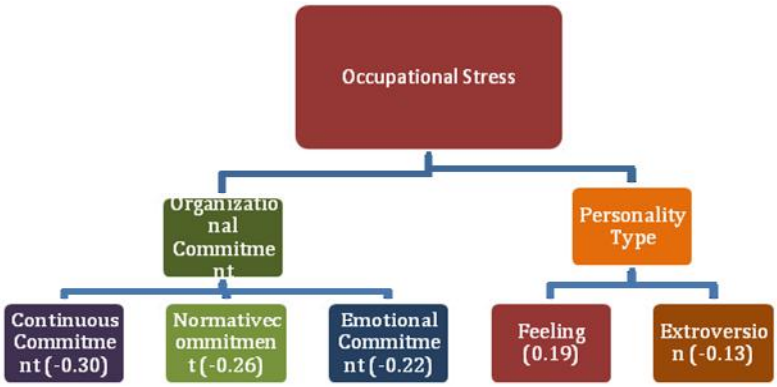
According to the above table, it can be observed that the first model to predict the beta prediction coefficient (-0.57) for the continuous commitment is significant (t = - 12.02, p <0.01). Therefore, the regression coefficient demonstrates that each of the standard deviations varies and an increase in continuous commitment with a standard deviation of 0.57 is aligned with a reduction in occupational stress. The power of prediction for the first model is about 33% (R^2=0.33).

In the second model, with the addition of normative commitment, the prediction power in the model almost increases by about 8 percents and reaches 41 percents. A significant predictive factor for the continuous commitment in this model shows that each deviation change in continuous commitment with a standard deviation of 0.45 is associated changes in occupational stress (t = - 9.41, p <0.01). Furthermore, a significant factor for normative commitment demonstrates that any deviation in the normative commitment to reduce the stress is associated with a standard deviation of 0.31 (t = - 6.41, p <0.01).

In the third model, with the addition of the emotional commitment, the prediction power increases by about 5 percents and reaches 46 percents. A significant predictive factor for the continuous commitment in this model shows that any deviation change in continuous commitment is associated with a standard deviation of 0.34 in occupational stress (t = - 6.73, p <0.01). Moreover, a significant factor for normative commitment represents that any deviation in normative commitment is aligned with a reduction in stress associated with a standard deviation of 0.27 (t = - 5.86, p <0.01). Similarly, the beta coefficient indicates that the emotional commitment to each standard deviation increase in this variable is associated with a 0.26 standard deviation decrease in stress levels (t = - 5.24, p <0.01).

In the fourth model, with the addition of feeling function from the eight Myers - Briggs functions, it can be seen that this function has a regression coefficient that is statistically significant (t = 3.98, p <0.01). The beta coefficient of which is 0.17 and prediction power through this function comparative to the third model have risen by about 3 percents (ΔR^2 = 0.49-0.46 = 0.03).

In the last model, with the addition of extraversion function, the prediction power via stepwise model has risen by one percent reaching to 0.50. The prediction coefficient of extraversion (-0.13) alongside other variables is significant (t = - 2.95, p <0.01). The regression coefficients for the feeling function (0.19) is positive and significant (t = 4.41, p <0.01). The regression coefficients for continuous commitment (t = - 5.93, p <0.01), normative (t = - 5.79, p <0.01) and emotional (t = - 4.49, p <0.01) are also significantly inversely correlated. Amongst these coefficients, the best predictive factor belongs to the continuous commitment and the final regression equation is as follows; Introversion (-0.13) + Feeling (0.19) + Emotional Commitment (-0.22) + Normative Commitment (-0.26) + Continuous Commitment (-0.30) = Occupational Stress



Research model: Predicting occupational stress by organizational commitment and personality types

Conclusion

By using research findings predicting model of occupational stress can be explained in this way that this model has been made based on differences of personality types and their relation with organizational commitment and commitment stress and result of its analysis by using regression states that organization commitment is one of predictors of occupational stress, in other word people with low organizational commitment tend to leave organization, they are sensitive to stress and they adhere less to their pledges, they prefer introversion and are mostly following personal emotions, in encountering problems they tend to illogical behavior and these results were obtained by findings of correlation between personality types with organizational commitment and occupational stress.

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2/20/2012

The influence of cognitive restructuring training on reducing Non organic sexual problems of couples

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Abstract: The aim of research is to determine the effect of cognitive restructuring training on reducing nonorganic sexual problems of couples in Isfahan. And increasing its dimensions (sexual satisfaction, sexual motivation, sexual confidence, sexual pleasure, and sexual health). In this research experimental methods with preparing pretest and posttest with case and control group were used. The samples of this research were 120 couples that were selected randomly from the research community and were classified in two groups of test and control. The research instrument was Hooper sexual problems and Cognitive Restructuring learning. In this study case group was trained for 10 sessions, each session 2 hours, and at the end of this term, again the questionnaire was completed by both groups. And by using analysis of data covariance, were analyzed. Research results showed that educating cognitive restructuring on reducing the total score of couples' sexual problems and all its dimensions. (P < 05/0). By reducing sexual problems, marital and sexual satisfaction will be increased and it will have a positive effect on couples' life . Because the cause of many psychological distress and marital conflicts is resulting from lack of sexual satisfaction and years of ignoring the sexual instinct in humans has had irreversible effects on social and marital relationships and has collapsed many families, finding some solutions for resolving sexual problems seems necessary. This study showed that cognitive restructuring can be taught alongside other methods of therapy for marital problems and disputes arising from sexual problems.

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Key words: Cognitive restructuring, sexual problems, sexual satisfaction, couples.

1-Introduction:

Several factors may be involved in the development and emergence of sexual dysfunction. Some people are suffering from organic diseases that affect their sexual activity and satisfaction. Inorganic factors such as barriers to social –religious beliefs, bad sexual experiences and sexual damage can also affect normal sexual function . (Hulbert,1994). The causes of sexual problems 1 - Physical causes 2- psychological causes 3 - Information (knowledge) causes. (eshghi,2007)

Sexual dysfunction created in any way, have many negative consequences. Existing studies show that sexual deficits are closely associated with social problems such as sexual offenses, sexual rape, mental illness and divorce (Hulbert,1994) Nervousness, abdominal and back pain, inability to concentrate and even inability to perform common tasks are some other consequences of failure to satisfy the sexual instinct, while ideal sexual function for establishing family basis and a basis to obtain an established culture .(Jahanfar &et al ,2006)

Behavioral - Cognitive approach in addition to cognitive factors considers sex, marital relations in general that can be treated with consideration. Not considering interaction aspect, sexual behavior, couple relation and effects of family and cultural – social environment likely causes not paying attention to some important effective factors in function and because of this they reduce effectiveness of remedy. Cognitive treatment can be defined as interventional methods that their purpose is changing cognitive and obvious behavior that is done by direct concentration on changing cognitive skills, thoughts and attitudes. (Spense, 1991). Many treating approaches in treating psychological problems of people, couples and families correct cognition and thinking by this hypothesis that main reason of creating disorder is irrational thinking. In fact, some individuals and couples trying to have cognitive reconstruction. (Firuzbakht, 2001).

Epstein and Bacum (1990), decide that in individual remedy the patient is a person and in couple therapy patient is a couple because of this cognitive therapists during working with couples consider a collection of individual conflicts and also with common factor that each person disorder effects on his/her partner. Cognitive therapists consider marital conflict as a result of quality of relation. So in evaluating couples problem both cognitions and their effects on behaviors and emotions of each other can be studied.(Besharat,2006).

Cognitive reconstruction is based on the assumption that some emotions are caused by unrealistic expectations. According to Ellis (1997)

research in cognitive reconstruction, people can learn rebuilding their irrational beliefs and learn these lessons well, and so they can remove something that is well- learned but incompatible behavior. Elis has reported some cases that emotional – rational treating has been successful in solving problems like cold natured, sexual dysfunction, marital complaints, and psychosis. (olia, 2007) If the person's mind is occupied with illogical reasoning and incorrect interpretations, s/he will be blind and deaf to reality and will annoy himself and his wife by bad judgment and improper action. (Rafiee Bandari,2006)

Educating cognitive reconstruction provides learning opportunities for cognitive, sexual problems, and prevents many problems. People for many reasons, including inadequate knowledge of each other, sex and marital life experience several problems. Cognitive reconstruction can help people for getting information, ideas and skills. Also it gives some sexual information and knowledge for a common goal and satisfying the sexual needs and the balance in personal, family and society life. (Brryman et al, 2002). Researchers have found that an irrational belief in marital sex life is a strong predictor for distress of marital life. Therefore they suggest that treatment programs for the ineffectiveness of relation should be based cognitive reconstruction of the spouses. (Eidelson & Epstein, 1982).

Cognitive restructuring and sex education are some methods in treating sex disorders that increase person's knowledge about sexuality, attitudes and cultural values associated with that and also it improves and promotes the effective communication on sexual issues (Tabrizi, 2007). By providing training, advice and information about human sexuality, problems gradually disappeared and ignorance will be replaced with knowledge for couples to know the effective and successful steps in dealing with sexual problems and even marital disputes (Jahanfar &et al, 2006).

Ritzr Research (2000) in the context of marital problems showed that sexual problems and dissatisfaction in the first year of life is associated with increased likelihood of divorce in the second year . Studies conducted by Christopher and Esperechr (2000) also indicate that sexual satisfaction is associated with high levels of marital satisfaction. Overall marital happiness is associated with sexual satisfaction. Happy couples are more satisfied with their sexual lives than unhappy couples. (Hunt quoted by Sapington, 2005)

Ogbern and Mayer quoted Olia 2007, concluded that sexual relations are not main reason for separations but 30% of couples' dissatisfaction is because of their sexual problems and if it continues, it can damage marriage. Since couples' conflicts cause inconsistency and lack of compatibility between them.

Those with marital satisfaction are satisfied in other relations too. Sexual satisfaction is dependent on relational satisfaction and emotional satisfaction in relations. (Christopher & et al 2000)

Shame of talking about sexual affairs, lack of proper understanding of sexual problems and lack of enough information in this field are some of effective reasons in creating sexual problems in primary experiences of marital life. In this regard, the Jahanfar believes that the type of a partner's behavior is an important tool in communicating deep emotional relation with him/her and sexual adequacy is necessity to obtain the full physical experience love. Continuous and regular sexual behavior that occurs in couples helps the couples to be able to establish mutual love.

The results Esere, M.O. & Idowu, A.I. (2000) showed that Cognitive Restructuring Training Program was effective in marital conflict resolution. It was also found that the treatment was not affected by gender.

The results Esere, (2010) showed that NEGOST and CORTP were effective in resolving the participants' marital conflicts thereby leading them to optimal marital relationship. CORTP, however, they found to be more effective in resolving marital conflicts.

Bishay (1988) treat women with abnormal sex using cognitive restructuring, and both were treated successfully in two single-case studies.

Whatever an above this article with aim to determine the effect of cognitive reconstruction training on Sexual Problems of couples in Isfahan were done.

2-Methods and Materials

Methods in this study are experimental pretest – post test with control group. The study population consisted of all couples in Isfahan (IRAN) in 2011. The sampling method was a random sampling; number of samples in this study was 120 couples (240 individuals) in Isfahan chosen randomly and were randomly assigned to two groups of 60 couples (120 people) in the case group and 60 couples (120 people) were in the control group.

In the first group (case group) independent variables (cognitive restructuring) were applied and the second group (control group) independent variable was not there.

Research variables consisted of independent variables (cognitive restructuring) and a dependent variable (Sexual Problems), respectively. In Both groups the evaluations were twice, which includes pre-test and post-test respectively and then test group will be trained for 10 sessions in 2 hours.

This study analyzed data from the descriptive statistics such as frequency tables, graphs, calculate averages, standard deviations, and inferential statistical indicators and for hypothesis testing factor analysis of covariance method is used. Tools used: 1 – Sexual Problems Questionnaire 2 - Sexual cognitive restructuring education.

A- Six-fold Hooper questionnaire of sexual problems

This questionnaire was provided by Annie Hooper (1995) in a training multimedia program (the ultimate sex guide) and analyses different aspects of sexual problems. There are 6 scales which provide one sexual profile, although, the questionnaires can be used separately.

There are six questionnaires deal with different aspect of your sexuality each of the following questionnaires is concerned with some aspects of sex. There are a total of 80 questions.

Table 1: Chronbach's alpha of 6-fold sex Hooper questionnaire

Hooper 6 fold sex aspects	Sexual Knowledge	Sexual confidence	Sexual Well-Binge	Sexual motivation	Sensuality	Sexual satisfaction	Total
Chronbach's Alpha	.957	0.915	0.960	0.906	0.745	0.801	.982
# of Items	20	15	15	10	10	10	80

This questionnaire has been fully standardized by researcher in Iran, and is used in this study and previously just two scales of it have been used only by Ms. Eshghi in her research. Research of Eshghi (2008) used questionnaire of sexual knowledge and sexual confidence and the given alpha were 0.70 and 0.72 respectively which is 0.95 and 0.91 in this research.

Chronbach's alpha coefficient was used in internal consistency of the entire questionnaire and the coefficient equals to 0.98. The overall credit is acceptable. Content validity of the questionnaire was confirmed by five counseling and psychology professionals.

B - Cognitive Restructuring training

Include: couples' awareness of kinds of illogical and spontaneous thoughts, training of A-B-C principles, and confrontation methods to illogical believes, training discussion method for correcting illogical believes.

Improvement sexual relationship training and Educating cognitive restructuring sexually dysfunctional couples' thoughts, improving sexual though Include: expressing the importance of sexual relationship, expressing the cycle of sexual problems, preventive factors of a correct sexual relationship, determining incorrect sexual myths. preventive factors in correct sexual relation - detecting incorrect sexual myths - to eliminate the negative sexual beliefs and myths - familiarity with the correct attitudes and misconceptions of couples about sex- describing the impact of negative thoughts and attitudes on establishing sex - Cognitive restructuring of dysfunctional sexual thoughts of couples**.**

3-Result

The findings suggest that the average age for case group is 38 years and for mean scores for married time 13.81 years for education 10 people below high school - 33 people diploma - 12 people bachelor – 50 people master of science - 15 people had PhD. Degrees and about children number 38 with one child - 62 with two children - 20 with three children. For the average age in control group is 37.43 years and for mean scores for married time 12.58 years for education 10 people below diploma - 26 people high school - 9 people bachelor – 58 people master of science - 14 people had PhD. Degrees and about children number 42 with one child - 66 with two children - 10 people with three children and 2 people with four children. The marriage duration was between 3 to 15 years.

In this research, considering resulted data of both experimental and control groups (each one 60 couples), the mean and standard deviation values of subscales of sexual problems before and after the implementation of cognitive rehabilitation methods are presented in the table below. After verifying the assumptions of covariance analysis, to study the effects of cognitive restructuring on sexual problems after subtracting the pre-test effects of values of the post-tests of variables and obtaining adjusted averages, the obtained values are compared in the research groups in Table 2.

Table 2: the mean and standard deviation of the pre test and post test variables in separate research groups

		variables											
		Sex knowledge		Sex Confidence		Sex Motivation		Sex Wellbeing		Sensuality		Sex Satisfaction	
gender	groups	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Female	Experiment												
	Pre test	2.62	1.45	14.63	5.14	8.93	4.34	12.03	2.99	15.83	2.45	5.48	1.73
	Post test	5.73	1.19	26.93	4.28	16.45	3.40	25.65	5.42	27.35	2.45	9.07	1.46
	Control												
	Pre test	2.73	1.60	14.02	3.84	8.11	3.07	11.67	3.24	15.22	2.45	5.45	1.45
	Post test	3.05	1.73	15.67	5.41	9.57	4.30	13.00	5.76	15.93	5.00	5.63	1.82
Male	Experiment												
	Pre test	3.05	1.67	16.85	5.11	11.43	4.05	11.68	3.37	14.58	2.74	5.35	1.74
	Post test	6.01	1.18	26.17	4.09	15.40	4.61	20.23	6.20	26.63	2.22	8.25	1.99
	Control												
	Pre test	2.83	1.42	16.33	5.12	10.93	3.68	10.68	3.64	15.38	2.64	5.63	1.54
	Post test	2.87	1.45	16.60	5.51	11.15	3.78	10.90	4.84	16.85	5.07	5.78	1.75

The following table presents the results of one variable covariance analysis in comparison of adjusted post test mean of sexual problems subscales in two experimental groups, two gender groups and gender and group interaction.

Table3: results of single-variable analysis of variance for sexual problems subscales

	ANCOVA											
	Sex knowledge		Sex Confidence		Sex Motivation		Sex Wellbeing		Sensuality		Sex Satisfaction	
Source	$F_{(1,235)}$	η^2	$F_{(1,235)}$	η^2	$F_{(1,235)}$	η^2	$F_{(1,235)}$	η^2	$F_{(1,235)}$	η^2	$F_{(1,235)}$	η^2
Sex	0.15	0.00	0.73	0.00	0.16	0.00	5.06*	0.02	3.07	0.01	0.00	0.00
Groups	320.30**	0.58	285.57**	0.55	110.42**	0.32	318.14**	0.57	231.75**	0.60	444.28**	0.65
S * G	1.04	0.00	1.90	0.04	6.22*	0.03	0.15	0.00	4.01*	0.01	3.76	0.02

*p≤0.05, **p≤0.01

Considering results of above table for sexual information variable it is observed that there is no significant difference between men and women in terms of adjusted means of this variable ($F_{(1,235)}$=0.15, η^2=0.00, p>0.05).But there is significant difference between the two groups of cognitive restructuring and control in terms of sexual information that demonstrates the effectiveness of cognitive rehabilitation training in the experimental group and increasing their sexual information ($F_{(1,235)}$ = 320.30, η2 = 0.58, p <0.01). In contrast effect of gender interaction and experimental groups is not significant (F (1,235) = 1.04, η2 = 0.00, p> 0.05).According to the results listed in Table 2 it can be seen that the average of sexual confidence doesn't differ significantly in the two gender groups (F (1,235) = 0.73, η2 = 0.00, p> 0.05) and there is a significant difference between the two experimental groups in terms of a modified post test of sexual confidence, (F (1,235) = 285.57, η2 = 0.55, p <0.01). For variable of sexual confidence, gender and groups interaction effect is not significant (F (1,235) = 1.90, η2 = 0.01, p> 0.05). Also the adjusted mean of sexual motivation in both men and women has no significant difference (F (1,235) = 0.16, η2 = 0.00, p> 0.05). There is significant difference between the two experimental groups in terms of average sexual motivation, (F (1,235) = 110.42, η2 = 0.32, p <0.01). So according to Table 1, this average is higher in the cognitive restructuring group. Significant difference can be seen in terms of motivation for effects of gender interaction and experimental groups (F (1,235) = 6.22, η2 = 0.03, p <0.05) but according to Scheffe post hoc test this effect is not significant. It can be seen that there are significant differences for sexual health mean between the two groups of sexual (F (1,235) = 5.06, η2 = 0.02, p <0.05) and experimental (F (1,235) = 318.14, η2 = 0.57, p <0.01). But the effect of gender and group interaction is not significant. Also considering results of table 2 it is observed that there is no significant difference between modified mean of sexual pleasure in men and women ($F_{(1,235)}$=3.07, η^2=0.01, p>0.05).

But this mean between the two experimental groups (F (1,235) = 231.75, η2 = 0.50, p <0.01) and interaction effects of gender and group (F (1,235) = 4.01, η2 = 0.02, p <0.05) has significant differences but results of Scheffe post hoc test for interaction is not significant. Also it can be seen that there is no significant difference for gender (F (1,235) = 0.00 η2 = 0.00, p <0.05) and interaction effects of gender and group (F (1,235) = 3.76, η2 = 0.02, p <0.05) is not significant. But there is significant difference between the two experimental groups in terms of

adjusted mean of sexual satisfaction subscales and cognitive restructuring has affected on improving couples' satisfaction in experimental group comparing to control group ($F(1,235) = 444.28$, $\eta 2 = 0.65$ $p < 0.01$).

According to the tables' data we see that educating cognitive restructuring has been effective in reducing sexual problems and it has also affected positively increasing sexual confidence, sexual motivation, sexual health, sexual pleasure, and sexual satisfaction.

In this study, confounding variables including age, sex, education, marriage, children and pre-test were controlled.

The results of this hypothesis are consistent with the following research:

Burleson (1997), Christopher and Spritcher (2000), Cooper, P. J. (2006), Eshghi (2008), Hasanzadeh R,et al (2006), Hartmann et al (2002), Hulbert et al (1993), Jacobson (1979) Khazae ,M& et al (2011) , Masters&Johnson (1979), McCabe (1997) , Nabipour, A. (2006), Pak Gohar &et al (2008), Sasanpour&et al (2012), Sasanpour (2007) Shams Moffarahe &et al. (2003), Rizer (2000).

4- Discussion:

It can be said in conclusion that, in order to reduce sexual problems and increase sexual and marital satisfaction, cognitive restructuring was a successful program. Participation in these groups has had a significant impact on improving the couple's marital life. During the holding period of ten training sessions, even a couple were not deterred from continuing to attend meetings, and group members remained constant until the end of the session, which suggests the usefulness and attractiveness of education for couples. Changes in sexual and marital satisfaction scores of subjects in this study, like other similar studies, is noteworthy. Relying on and citing the findings of previous studies, the hypothesis can be sure of the usefulness of cognitive restructuring in reducing sexual problems and improving sexual satisfaction of couples.

The results also showed that sexual cognitive restructuring training increase sexual knowledge, sexual satisfaction, sexual confidence, sexual desire, sexual pleasure, sexual health, marital satisfaction and general health. Sexual partners can discuss positive and negative feelings with each other through the process of communication by sexual cognitive restructuring training.

The effect of sex education is not different in male and female students. Result of this hypothesis is consistent with the results of some studies, but is not consistent with some findings. However, reports indicate that because sex education is necessary and essential for all people, male or female, therefore, a sex education program should be a comprehensive model. Such trainings should be tailored to gender, age, level of cognition, social – cultural context, and should be provided at the right time. Sexual activity and gender is an important part of life. Perhaps in the past, such behavior occurred by trial and error, and not based on correct information and knowledge. But today sex education is an important determinant for a successful marriage. With the spread of sexual diseases, increasing sexual disorders, increased rates of diseases transmitted through sexual activity, AIDS, etc, the emphasis on sex education is a necessity.

5- Conclusion

Cognitive restructuring training of sex partners helps to become familiar with a cuddling and learn a variety of cognitive restructuring techniques and the two enjoy a sexual behavior. Learning their attitudes and misconceptions about sexual issues that prevent couples from the getting closer to each other will help. It provides a healthy and safe environment to express sexual intimacy. There are some trainings and exercises to increase caring behaviors and reduce the mental, emotional and sexual distance of the partners. These skills lead to improved marital life.

This method let the person to speak and express his/her emotions freely that this freely and comfortable communication reduces anxiety moods and facilitates each person's feelings in relation. Removing unconscious feelings of guilt or fear of prosperity and enjoyment of replacing right cognitions instead of preventive and wrong cognitions can justify the effect of therapeutic intervention. This education is in such a way that the patient obtains necessary motivation and ability to accept and express sexual interests in attractive and pleasing conditions without former sexual tension.

Educating couples to proper understand each others' character and tendencies, correction of beliefs and attitudes, teaching appropriate and reasonable methods to fulfilling physical and emotional needs; can be one of the main paths of consultancy sessions before marriage. In fact, detection and elimination of sexual disorders, identifying concerns and help couples to improve the quality of marital relationships, has a significant effect on increasing sexual satisfaction and it plays a significant role in preventing family disputes and their resulted consequences.

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Knowledge and Practices of Working Mother about Breastfeeding and Weaning in Assiut City, Egypt

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Abstract: The importance of breastfeeding, especially exclusive breastfeeding (EBF) is well established for the infant, the mother and the family. The aim of this study is to assess the knowledge and practices of working mothers regarding breastfeeding and weaning using quantitative and qualitative approaches. The study was conducted in four MCH centers in Assiut city during 2010. Direct interviews were done with 43 working mothers had children aged 4 to 24 months as well as seven FGDs. 69.8% of the mothers were at the age 30 years or less. All the studied mothers knew that the breastfeeding is the best nutritional source for the baby. The majority of the mothers had good knowledge about the advantages of breastfeeding for the child and the mother. 67.4% initiated breastfeeding within the first 30 minutes after delivery. The participants in general were less knowledgeable about exclusive breastfeeding practices. There were some fault practices reported by the participants. There is a need for health education programs, which support and encourage breastfeeding particularly at a primary care level, focusing more on working mothers.

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Key words: Knowledge- practices- breastfeeding- weaning

1. Introduction:

Breastfeeding confers crucial health benefits to both mothers and their babies (Kramer et al., 2001). Breast milk is the natural first food for babies, it provides all the energy and nutrients that the infant needs for the first months of life, and it continues to provide up to half or more of a child's nutritional needs during the second half of the first year, and up to one-third during the second year of life (WHO, 2011).

Breastfeeding is critical for sustaining newborn infant health and wellbeing. Infants who are properly breast-fed grow better and experience less sickness and fewer deaths than other infants who are not breast-fed (John, 2005).

Each year more than 10 million children under the age of five years die, mainly from one of a short list of causes, and the majority live in low-income countries (Black et al., 2003). Millennium development goal number 4 is to reduce child mortality by two thirds by 2015 (United Nations Statistics Division, 2005). Under-nutrition is estimated to be the under lying cause of 53% of under five mortality (Bryce et al., 2005). Appropriate feeding practices are of the fundamental importance for the survival, growth, development and health of infants and young children (Jones et al., 2003). Fault feeding practices including lack of breastfeeding and early introduction of solid foods have been reported as health risks (Uany and Solmons, 2005).

Breastfeeding is an essential measure for the prevention of malnutrition and protection against infection in infancy (Livingstone et al., 2000). Breastfeeding is one of the oldest practices recommended by all religions and it is the universally endorsed solution in the prevention of early malnutrition (Dana, 1979). It is estimated that the lives of one million infants can be saved in the developing world by promoting breastfeeding (Heining and Dewey, 1996; Moreland and Coombs, 2000).

World Health Organization and the American Academy of pediatrics recommends that an infant should be breast- fed without supplemental foods or liquids for the first 6 months of age, known as exclusive breastfeeding and thereafter continued breastfeeding until two years of age along with complementary foods (WHO, 2011).

The prevalence of breastfeeding differs from one country to another and from one society to another, this of course is due to cultural and religious believes (Li et al., 2003).

In many developing countries, labor force participation by women in the childbearing years has increased rapidly. Social and economic changes present new challenges for women attempting to combine their roles as workers and mothers. Employed mothers perceived some contradictions messages on breastfeeding and most of them preferred to leave work after birth to exclusively care of their babies and others wished to have more institutional support (Barona-Vilar et al., 2009). In Egypt, many of women who are employed when they become pregnant return to the work by the time their children are three month old. Little is known about how these challenges affect infant feeding choices.

Aim of the study:

The present study aims to give an overview on and assess current knowledge and practices of working breast-fed mothers related to the feeding of their young children up to two years.

Subjects and methods

This study was performed by using cross sectional design and Focus Group Discussions (FGDs) during 2010. Four Maternal and Child Health Care (MCH) centers (El-Willidia, El-Arbaeen, Weast-Elbalad and Kolta) in Assiut city were be chosen randomly from total eight MCH centers located in different socio-economic areas in Assiut city. The target population was working mothers of children aged from 4 to 24 months. Verbal consent was obtained after the participants had been informed about the study objectives.

Formal administrative approvals were taken before the start of the fieldwork. These included approval by the Ethical and Technical Review Committee of the Assiut Faculty of Nursing for the study proposal and permission of Assiut Health Directorate.

The researchers recruit 50 working mothers visiting the MCH centers to participate in the study. Although all women agreed to participate 7 women did not complete their participation after a period of time because they were busy so the response rate is 86 %.

Data were collected by using both quantitative and qualitative approaches from mothers. For quantitative data collection, a semi-structured questionnaire was used for direct interview. The questionnaire included sociodemographic data, questions to assess mother knowledge and practices regarding breastfeeding. A pilot study was performed prior to the study and all necessary modifications were done.

The focus group discussion guide explored participants' knowledge, experiences, preferences and assumptions about breastfeeding. The guide was tested with a group of women not included in the study. The focus group moderator and observer received previous training on this type of qualitative data collection.

Seven focus group discussions were carried out in the nurses' room or in the clients waiting place of the MCH centers. Groups ranged in size from 5 to 7 mothers and each session lasted 45-60 minutes. Moderator used the protocol to ask open-ended questions and probe response. Prior to each discussion the researcher ensured the issue of confidentiality and they also made sure that there was a relaxed atmosphere before the discussion started. Topics covered were initiation of breastfeeding, practices regarding colostrum, use of pre-lacteal feeds, complementary food, weaning time and weaning food and cessation of breastfeeding.

The observer documented the sessions whether verbal or non-verbal aspects. The first session was audio taped after taking permission of the participants, other sessions were not because a difficulty was found as a result of the noise and unsuitable environment. Transcription was done by note-taker. By the end of each session, each participant took a brief Arabic booklet about breastfeeding and weaning that prepared by the researchers.

Data Analysis:

For quantitative data: Data were analyzed using SPSS version 16. Frequencies, percentages, mean and standard deviation were computed. Chi-squared test was used as the test of significance; $P < 0.05$ was considered significant.

For qualitative data: The FGDs were translated into English by the researchers. Coded material was compared and organized into themes that were then grouped into central categories. The information in each FGD was summarized and grouped according to these predefined information categories.

3. Results:

Table (1) shows the sociodemographic characteristics of the studied 43 mothers, 69.8% of the mothers were at the age 30 years or less. About half of the included mothers had secondary education, 39.5% having higher education and 4.7% of mothers had preparatory or no education. As regards mothers work, 55.8% were employers, 25.6% were teachers, 11.6% were unskilled workers and 7% were nurses. Those who work for more than 6 hours outside home represented 30.2%. Less than half of the studied mothers (48.8%), their family income was in the range from 300 to 450 Egyptian pounds per month.

As shown in Table (2), all the studied mothers knew that the breastfeeding is the best nutritional source for the baby. The majority of the mothers had good knowledge about the advantages of breastfeeding for the child and the mother. About 80% of the mothers knew that breast milk protects the child from diseases as well as strengths the relation between the mother and her baby. Nearly 70% of the participant mothers stated that breastfeeding protect mothers against cancer. 46.5% of the mothers aware that breast milk is a complete nutrition for the infant during the first 4 months. The majority (83.7%) of the participants knew that colostrum increase the immunity of the baby and 30.2% of the mothers reported that it is a first protection against infection.

As regards weaning, less than half of the mothers defined weaning correctly. On the other hand, good percentage of the respondents knew that weaning should be started by addition of juices (88.4%) and soft food as egg yolk (62.8%). More than half (53.5%) of the participants knew that 6 months is the suitable age for starting weaning. Unfortunately, 30.2% did not know the suitable age to start weaning. Nearly 42% of the mothers reported that baby must be weaned completely from breast milk at the age of two years and 39.5% said after one and half years (Table 3).

Table (4) presents the percentages of mothers regarding practice of breastfeeding, 67.4% initiated breastfeeding within the first 30 minutes after delivery. Only 9.3% started on the third day. Water, honey water and safe remedies were offered to the baby before lactation by 9.3%, 20.9% and 69.8% of the mothers, respectively. About one fifth of the mothers artificially fed their babies beside the breast milk. The important causes of artificial feeding were reported by 20.9%, 14% and 11% of mothers as follows low baby weight, mother work and breastfeeding weaken the mother, respectively. The majority of the mothers (93%) continued breastfeeding while their babies suffered from diarrhea.

Table (1): Sociodemographic characteristics of the studied mothers, Assiut MCH, 2010

Variable	No. (n= 43)	%
Mothers age:		
30 years	30	69.8
More than 30 years	13	30.2
Mean ± SD	27.6 ± 4.8	
Mothers education:		
Illiterate/ read& Wright	2	4.7
Preparatory	2	4.7
Secondary	22	51.2
Higher education	17	39.5
Mothers job:		
Employer	24	55.8
teacher	11	25.6
nurse	3	7.0
Unskilled worker	5	11.6
Work hours:		
6	30	69.8
More than 6	13	30.2
Husbands job:		
Employer	32	74.4
Businessman	4	9.3
Skilled worker	3	7.0
Unskilled worker	4	9.3
Family income: LE/month		
250-	9	20.9
300-	21	48.8
450-	8	18.6
600 or more	5	11.6

Table (2): Mothers knowledge about advantages of the breastfeeding, Assiut MCH, 2010

Variable	No. (n= 43)	%
The best nutrition for the baby:		
Breastfeeding	43	100.0
Artificial feeding	0	0.0
Advantages of breastfeeding for the child:		
Protect the child against disease	34	79.0
Complete nutrition in the first 4 months	20	46.5
Increase the intelligence of the child	18	41.9
Improve the child immunity	11	25.6
Help in early teeth eruption	11	25.6
Advantages of breastfeeding for the mother:		
Strengthen the relationship between baby and mother	34	79.1
Protect the mother from cancer	30	69.8
Prevent postpartum hemorrhage	16	37.2
Cheap	14	32.6
Breastfeeding is a natural contraceptive method	11	25.6
Safe mother time	2	4.7
Colostrum feeding:		
Increase the immunity	36	83.7
Protect the baby against diseases	13	30.2
Good nutrient for the baby	7	16.3
Easily digested	5	11.6
Increase the intelligence of the baby	3	7.0

Table (3): Knowledge of the studied mothers about weaning, Assiut MCH, 2010

Variable	No. (n= 43)	%
Definition of weaning:		
Stop of breastfeeding	21	48.8
Add other types of food beside breast milk	20	46.5
Do not know	2	4.7
Types of food used for starting weaning:		
Juices	38	88.4
Egg yolk	27	62.8
Mashed cereals	5	11.6
Any food	3	7.0
Suitable age of baby to starting weaning:		
Less than 4 months	1	2.3
4-6 months	6	14.0
After 6 months	23	53.5
Do not know	13	30.2
Complete weaning from breast milk:		
At one year	8	18.6
1.5 years	17	39.5
2 years	18	41.9

Table (4): Mother practice of breastfeeding, Assiut MCH, 2010

Variable	No. (n= 43)	%
Time of initiation of breastfeeding:		
In the first 30 minutes	29	67.4
In the first day	10	23.3
In the third day	4	9.3
Pre-lacteal feeds:		
Safe remedies	30	69.8
Honey water	9	20.9
Water	4	9.3
Giving artificial feeding;		
Yes	9	20.9
No	34	97.1
Causes of artificial feeding:		
Increase child weight	9	20.9
Work of the mother	6	14.0
Breastfeeding weaken the mother	5	11.6
Insufficient breast milk of the mother	3	7.0
Continuation of breastfeeding during diarrhea:		
Continue breastfeeding	40	93.0
Stop breastfeeding	3	7.0

FGD results:

Advantages of breastfeeding:

There was a general opinion among all the participants that breastfeeding is the best choice for baby feed. All of them reported more than one advantage of breastfeeding for the mother and her baby. All participants knew that the colostrum is very good, because it protects the baby against diseases. None of the participants reported having expressed and discarded the colostrum. They told that colostrum is good nutritive food which strength the child immunity.

The participants in general were less knowledgeable about exclusive feeding practices. Many of them believed that they followed exclusive breastfeeding, but in reality they gave water and other safe remedies. One participant said *"It is normal to give water especially in the summer"*. Few practiced exclusive breastfeeding only for the first 3 months. The main causes were the perception of insufficient milk, less satisfaction of the baby and return to the work. Mothers' relatives recommend to start giving the baby other food before six months to get used to eating food when the mother fell sick or may be died.

Half of the participants did not know the methods of suctioning and preserving of breast milk. The mothers believed that this practice is painful and requires certain hygienic measures. One of those stated that *"Even I know I don't do it because I afraid of"*.

Bottle feeding practices:

The majority of the women in the focus groups did not give anything to the baby before initiation of

breastfeeding. Unfortunately, some physicians prescribed safe remedies for baby as pre-lacteal feeds.

While asking mothers if they bottle fed their babies or not a small number of them reported that they gave bottle feeding to their babies for many reasons. The most reported reason is that there is no breast milk or insufficient milk in their breasts others stated "*To help child to gain weigh* ". One of the participants stated "*When I come back to work what the baby will feed*?".

Weaning time and complementary food

The majority of the mothers defined the weaning as the breastfeeding cessation. Nine mothers defined it as introduce assistant food with breastfeeding. Also the majority of mothers reported that the best time to start weaning when the baby aged 6 months and more. The main complementary food mentioned by all the interviewees was the easy digested food like mashed vegetables and fruits and mahlabia. Other complementary food mentioned was yoghurt, bean water, eggs, potatoes, and cow milk. Cerelac was mentioned by a few numbers of women.

Nutrition of lactating women

All participants told that the breast fed mother can eat the ordinary food of the home with an increase in the foods containing high calcium, high sugar like halvah named "halawa tahinia", treacle and desserts. Also they mentioned that fluids as arena named "helba", fruit juices and water play an important role in increasing the breast milk. Participants also gave special attention to the green leaves like Radish named "fegel", arugula named "Jarjir" and meat. Other mothers mentioned that breast fed mother should avoid spicy dishes as they may cause colic to the baby.

4. Discussions:

Results of this study indicated that mothers' knowledge concerning breastfeeding was in general satisfactory. Yet, they had less knowledge about weaning. In a study conducted on Indian mothers, the majority of the participants knew that breast milk is clean and sterile and promoted bonding between mothers and child. In addition, they were aware that colostrum protected the child from falling sick (Pant and Chothia, 1990) in comparison to 30.2% of mothers in our study knew that. These results are better than that reported by El- Kariri and Kannoa (2007) as 68.6% of mothers in Gaza strip knew more than three advantages of breastfeeding. Ekambaram and other researchers (2010) reported that the knowledge of the mothers in India was inadequate in areas of time of initiation of breastfeeding (92%), colustrum feeding (56%), duration of exclusive breastfeeding (38%), knowledge on expressed breast milk (51%) and continuation of breastfeeding while the baby is sick.

It is highly recommended that breastfeeding is to be initiated within the first thirty minutes after birth. However, many mothers in the Arabian countries start feeding their babies with pre-lacteal feeds until the mother lactates (Al-Shoshan, 2007).

In the present study, 67.4% of the mothers initiated breastfeeding within the first half an hour after birth. The rate of breastfeeding initiation within the first hour after labor was 78.4% in Gaza (El- Kariri and Kannoa, 2007) and 55.9% in Lebanon (Bata et al., 2006).

Many mothers in the Egypt believe that breast milk is secreted in the third day after labor and colostrum is not sufficient to be given as a feeding to the infant. This may explain the delay of initiation of breastfeeding of babies. Our results are previously observed by Youssef and their colleagues (1991) in their study about maternal approach to breastfeeding in Assiut.

In a study conducted on Saudi mothers whom been admitted for delivery at maternity hospitals in Riyadh. Pre-lactic feeding was practiced by 10.5% of mothers and 42% of mothers initiated breastfeeding within the first hour of delivery (Al-Shoshan, 2007).

In the present study, 20.9% of the mothers artificially fed their babies because they believed that bottle feeding increases the baby weight. This finding is lower than that reported by Al-Jassir et al. (2006) as 48.3% of lactating mothers in Saudi Arabia cited insufficient milk as a reason for introducing bottle feeding. While Al-Shoshan (2007) found that baby milk formula introduced at the first month by 9.7% of studied mothers in Saudi Arabia and 18.6% of them used it at the sixth month. The introduction of infant formula before one month and returning to the work postpartum were predictive of weaning before 3 months (Tarrant et al., 2010).

The majority of mothers in the present study reported that the best time to start weaning is when the infant aged 6 months. The main complementary food was easy digested food. This is different than that done in Gaza community as 6.3% women started weaning their children after 18 months. The most common practice (45%) was to wean children at two years of age. Egg, vegetable soup and fruit juice were common food given by mothers to their babies at the age 3 to 5 months (Kanoa et al., 2011).

In FGDs, the majority of women in our study had a good idea about the advantages of the breastfeeding but they had less knowledge about exclusive feeding practices. This finding is consistent with that reported by Fjeld et al. (2008) in Southern Zambia.

The present study revealed that working mothers rarely practiced exclusive breastfeeding. Work of the mother was the main obstacle for continuation of exclusive breastfeeding for 6 months. This is corresponded with previous research results (Kruger and Gericke, 2001; Kruger and Gericke, 2004).

Leong (2009) found that working women were more likely not to practice exclusive breastfeeding compared to non working women in Malaysia. Arts and his colleagues (2011) found that only 37% of infants younger than 6 months in Mozambique were exclusively breastfed. The practice of exclusive breastfeeding depends on various factors related to both mothers and their environment. Exclusive breastfeeding is not promoted in healthcare facilities because the health professionals do not encourage it (Moussa Abba et al., 2010).

Conclusion and recommendations:

The study showed good knowledge of studied mothers about the advantages of breastfeeding for the child and the mother but lack of adequate knowledge towards the practice and time of weaning. So the study recommended

implementation of health education program in primary health care settings to improve and support the breastfeeding practices among working mothers. Working outside the home was of most concern to lactating women in our study. Longer paid maternity leave at least for 6 months and reduction in working hours are recommended. Workplace accommodations could assist working mothers to continue breastfeeding after returning to their work. The present study also revealed deficient knowledge about exclusive breastfeeding practices. Interventions to improve exclusive breastfeeding should target family and community members and include training of health workers in counseling to resolve breastfeeding problems.

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Serum Visfatin is Specific Significant Predictor of Rheumatoid Arthritis Severity: A Comparative Study versus Interleukin-6 and Clinical Severity Scores

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Abstract: Objectives: To determine serum levels of visfatin in patients with rheumatoid arthritis (RA) of varying duration of disease and to correlate it with serum interleukin (IL)-6 and clinical and radiological severity scores. **Patients & Methods:** The study included 70 patients fulfilled either four of seven ACR criteria or having morning stiffness ≥60 minutes, symmetrical arthritis and small joint arthritis for at least 6 months and 20 cross matched age and gender volunteers (Control group). Patients' data including age, gender, weight, height and calculation of body mass index (BMI) were determined. All patients underwent clinical evaluation for disease activity assessed using a 28 joint disease activity score, (DAS-28), pain using visual analogue scale (VAS) and functional disability using the Swedish version of the Stanford health assessment questionnaire (HAQ) to calculate the Disability Index (DI). Postero-anterior radiographs of hands, wrists, and forefeet were taken and joint destruction was classified according to Larsen–Dale index. Blood samples were obtained from patients and controls for ELISA estimation of Rheumatoid factor (RF) and serum IL-6 and visfatin. **Results:** Mean DAS-28 score was 3.9±0.8; range: 1.4-6.8, mean VAS joint pain score was 60.2±5.2; range: 51-71 and mean DI was 12.3±5.1. Erosive lesions were identified in 43 patients (61.4%), while the remaining 27 patients (38.6%). Fifty-one patients (72.9%) were RF positive; 34 had joint erosions and 17 patients were free of erosion. Estimated serum levels of IL-6 and visfatin were significantly higher in patients compared to controls with significantly higher levels in patients had erosive lesions compared to those free of erosion. There was positive significant correlation between presence of radiological evidence of presence bone erosion and patients' age, clinical data and disease severity scores and serum levels of IL-6 and visfatin. Serum levels of IL-6 and visfatin were found to be specific predictors of radiological evidence of bone erosion. **Conclusion:** There was a positive significant correlation between serum visfatin levels and clinical and radiological severity of RA and could be considered as specific predictor for RA radiological severity.

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Keywords: Rheumatoid arthritis, interleukin-6, visfatin, prediction of radiological severity.

1. Introduction:

Rheumatoid arthritis is a chronic systemic autoimmune inflammatory disease in which the peripheral joints are the primary sites of inflammation, often leading to destruction of these joints. RA is characterized by symmetrical synovitis, progressive joint damage, pain, fatigue, and disability. The spectrum of RA ranges from benign remitting manifestations to rapidly progressive forms with increased mortality. About 10% of the patients show an intractable rapidly progressive course associated with severe extra-articular manifestations. Within the first three years, 70% of the patients develop radiological erosions of the joints and 31% deformities of the hands. Life expectancy is shortened by 3-18 years **(Kroot *et al.*, 2001, Maille *et al.*, 2004, Rat *et al.*, 2004)**.

Synovitis may explain most of the early symptoms and is also considered to contribute to the development of joint damage and disability. The correlation between inflammation and joint damage has been studied extensively, especially the relevance of inflammatory variables such as C reactive protein and erythrocyte sedimentation rate. Although there is a link between inflammation and the development of joint damage it is well established that damage may progress in spite of decreased inflammatory activity, and erosions may develop in patients who have few clinical signs of inflammation. Thus it has been suggested that pathological processes other than inflammation are involved in the destructive process **(van den Berg, 2001, Fonseca *et al.*, 2009, Plant *et al.*, 2000)**.

Although the exact cause of RA is still unknown, investigation of its pathogenesis has confirmed a role for various pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6. Accordingly, inhibition of these cytokines has become the new therapeutic strategy for RA **(Fonseca *et al.*, 2009, Lioté, 2005)**.

Pre-B cell colony-enhancing factor (PBEF), also known as visfatin, is a highly conserved, 52-kDa protein found in living species from bacteria to humans. It is a one of the recently discovered adipokine produced and secreted primarily by visceral white adipose tissue. PBEF or visfatin is also produced by endotoxin-stimulated neutrophils and inhibits neutrophil apoptosis through a mechanism mediated by caspase 3 and caspase 8 **(Matsui *et al.*, 2009)**.

PBEF exert three distinct activities of central importance to cellular energetics and innate immunity. Within the cell, PBEF functions as a nicotinamide phosphoribosyl transferase, the rate-limiting step in a salvage pathway of nicotinamide adenine dinucleotide (NAD) biosynthesis, so through regulation of cellular levels of NAD and so impact not only cellular energetics but also NAD-dependent enzymes such as sirtuins. Although it lacks a signal peptide, PBEF is released by a variety of cells, and elevated levels can be found in the systemic circulation of patients with a variety of inflammatory diseases. As an extracellular cytokine, PBEF can induce the cellular expression of inflammatory cytokines such as TNF-alpha, IL-1beta, and IL-6. Finally, PBEF has been shown to be an adipokine expressed by fat cells that exerts a number of insulin mimetic and antagonistic effects. PBEF expression is up-regulated in a variety of acute and chronic inflammatory diseases including sepsis, acute lung injury, rheumatoid arthritis, inflammatory bowel disease, and myocardial infarction and plays a key role in the persistence of inflammation through its capacity to inhibit neutrophil apoptosis **(Busso *et al.*, 2008, Luk *et al.*, 2008, Neumann *et al.*, 2007, Popa *et al.*, 2005, Sethi *et al.*, 2005)**.

The current prospective comparative study aimed to determine serum levels of visfatin in patients with RA of varying duration of disease and to correlate it with serum IL-6 levels and clinical and radiological severity scores.

2. Patients and Methods

The present study was conducted at Rheumatology and Rehabilitation Department in conjunction with Clinical Pathology Department since Sep 2009 till Sep 2010. After obtaining patients' fully informed written consent, all patients had rheumatoid arthritis (RA) attending the outpatient clinic for first time or for follow-up were enrolled in the study so as to collect 70 patients with varied duration of disease. Only patients who fulfilled either four of seven ACR criteria or having morning stiffness ≥60 minutes, symmetrical arthritis and small joint arthritis (metacarpo-/metatarso-phalangeal joints/wrists) for at least 6 months were included in the study. Acute phase reactions were measured by erythrocyte sedimentation rate (ESR; mm/h) and C-reactive protein (mg/l) using standard laboratory methods and performed at hospital laboratory. The study also included 20 cross matched age and gender volunteers free of any form of joint affection chosen from those attending hospital blood bank for blood donation after passing the preliminary laboratory investigations required for blood donation according to hospital protocol to serve as control group.

Patients' data including age, gender, weight, height and calculation of body mass index (BMI) according to the equation: BMI= weight (kg)/height (m^2) were determined and duration of disease were determined.

All patients underwent clinical evaluation of disease activity as assessed by the disease activity score, using a 28 joint score (DAS-28), as follows: ≤3.2: inactive, >3.2-≤5.1: moderate activity and >5.1: very active disease ***(*Prevoo *et al., 1995)***. Pain was assessed by a 0–100 mm horizontal visual analogue scale (VAS), with 0 indicates no pain and 100 indicates the worst intolerable pain and VAS score of 0-25 indicates mild pain, >25-50 indicates moderate pain, >50-75 indicates severe pain and >75 indicates intolerable pain **(Scott & Huskisson, 1976)**.

Functional disability was evaluated using the Swedish version of the Stanford health assessment questionnaire (HAQ) to calculate the Disability Index (DI). The eight categories assessed by DI are 1) dressing and grooming, 2) arising, 3) eating, 4) walking, 5) hygiene, 6) reach, 7) grip, and 8) common daily activities. The difficulty during each of these acts was assessed as follows: 0: without any difficulty, 1: with some difficulty, 2: with much difficulty and 3: unable to do, then the sum of the categories scores is calculated and divided by the number of categories. This gives a score in the 0 to 24 range **(Ekdahl *et al.*, 1988)**.

Postero-anterior radiographs of hands, wrists, and forefeet were taken at inclusion in the study and joint destruction was classified by comparison with standard reference films according to the Larsen–Dale index **(Larsen *et al.*, 1977)**. The joints assessed for this index are the wrists, where all metacarpo-phalangeal joints (=10), all proximal interphalangeal joints (=8), both first interphalangeal joints in the hands (=2), metatarso-phalangeal joints II–V (=8), and both first interphalangeal joints in the feet (=2). Thus 32 joints are scored in all. Each joint is graded 0–V, as follows: grade 0: no abnormality; grade I: slight abnormality with one or more of the following criteria: soft tissue swelling, juxta-articular osteoporosis, slight narrowing of the joint space; grade II–V: erosion and narrowing of the joint space of increasing severity as illustrated in the standard

reference radiographs referring to the grade of damage of bone and cartilage, respectively. The degree of erosive damage is the most decisive criterion in grading and the finding of at least one definite erosion on any of the hands or feet radiographs was sufficient to consider the patient as having erosive disease.

Whole blood sample (5 ml) were obtained from patients and controls under complete aseptic conditions and were collected in plain tube and allowed to clot and centrifuged at 5000 rpm for 10 minutes and serum was separated and kept at -80 C° for ELISA estimation of:

1. Rheumatoid factor IgM isotype was analyzed using the ELISA kit for RF IgM quantitation (Orgentec Diagnostika GmbH, Germany) according to the manufacturer's instructions. The titre of 20 IU/ml was regarded as positive, **(Kleveland *et al.*, 1988)**.
2. Serum IL-6 was measured with an ELISA kit from Pelikine™ Inc., Concord, USA. IL-6 values in fresh serum of healthy individuals are <20 pg/ml **(Gaines-Das & Poole, 1993)**.
3. Serum visfatin was measured by Visfatin C-terminal ELISA kit (Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA), **(Fukuhara *et al.*, 2005)**.

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using Wilcoxon's Ranked test for unrelated data and Chi-square test. Possible relationships were investigated using Pearson linear regression. Predictors for evaluation of radiological evidence of erosion were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) and Regression analysis (Stepwise Method). Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. *P* value <0.05 was considered statistically significant.

3. Results

The study included 70 patients; 47 females (67.1%) and 23 males (32.9%) with mean age of 52.9±6.7; range: 40-64 years. All patients had fulfilled the criteria of ACR with a mean duration of disease of 4.7±1.8; range: 1.6-10 years. Mean DAS-28 score for disease activity was 3.9±0.8; range: 1.4-6.8, mean VAS joint pain score was 60.2±5.2; range: 51-71 and mean DI was 12.3±5.1. Mean ESR level was 31.5±13.5; range: 9-60 mm/h and mean CRP level was 16.5±10.8; range: 6-72 mg/l. Details of patients' enrollment data are shown in table 1.

Erosive lesions were identified in 43 patients (61.4%) with a mean Larsen score of 33.2±8.7; range: 13-45 (Erosive group), while the remaining 27 patients (38.6%) with a mean Larson score of 7.3±1.3; range: 4-9 (Non-erosive group) with a mean total Larsen score of 23.1±14.3; range: 4-45, (Fig. 1).

Fifty-one patients (72.9%) were rheumatoid factor positive; 34 had joint erosions and 17 patients were free of erosion. Nineteen patients (27.1%) were rheumatoid factor negative; 9 had joint erosions and 10 patients were free of erosion. There was non-significant difference between erosive and non-erosive groups as regards frequency of rheumatoid factor positivity.

Estimated serum levels of IL-6 (Fig. 1) and visfatin, (Fig. 2) in studied patients were significantly higher, both as total and categorized according to radiological evidence for presence of erosion, compared to control group. Moreover estimated serum levels of IL-6 and visfatin were significantly higher in patients had radiological evidence of presence erosion compared to those free of erosion (Table 2).

There was positive significant correlation between presence of radiological evidence for presence bone erosion and patients' age, clinical data and disease severity scores and serum levels of IL-6 and visfatin, (Table 3). ROC curve analysis of correlated factors versus presence of radiological evidence of presence bone erosion showed that all of them could predict it specifically (Table 4, Fig. 3).

Age, duration of disease, DAS-28 score, VAS pain score, DI, serum IL-6 and visfatin were verified using Regression analysis (Stepwise method) excluding the non-significant and least significant factors as predictors of the presence of radiological evidence of bone erosion defined serum levels visfatin as specific predictor that was persistently significant in four regression analysis models, followed by serum IL-6 in three models, age in two models and DI in one model (Table 5), thus indicating that visfatin could be used as specific significant predictor for RA severity.

Table (1): Patients' enrollment data

Data			Findings	
			Number	mean±SD
Age (years)	Strata	40-50	22 (31.4%)	45.2±3.6 (40-49.8)
		>50-60	34 (48.6%)	54±2.7 (50-58.8)
		>60	14 (20%)	62.4±1 (60-64)
	Total		70 (100%)	52.9±6.7 (40-64)
Duration of disease (years)	Strata	≤5 years	43 (61.4%)	3.6±0.9 (1.6-5)
		>5 years	27 (38.6%)	6.5±1.1 (5.2-10)
	Total		70 (100%)	4.7±1.8 (1.6-10)
DAS-28 activity score	Inactive disease		14 (20%)	2.3±0.5 (1.4-3.1)
	Moderately active disease		47 (67.1%)	4±0.5 (3.2-5)
	Very active disease		9 (12.9%)	6±0.5 (5.3-6.8)
	Total score		70 (100%)	3.9±0.8 (1.4-6.8)
VAS pain score	Mild pain		11 (15.7%)	18.7±2.6 (15-23)
	Moderate pain		42 (60%)	34.8±6 (26-45)
	Severe pain		17 (24.3%)	60.2±5.2 (51-71)
	Total score		70 (100%)	38.4±15.3 (15-71)
Mean DI	<10		31 (44.3%)	7.5±1.4 (5-9)
	10-20		33 (47.1%)	15.1±2.9 (10-20)
	>20		6 (8.6%)	21.5±0.8 (21-23)
	Total		70 (100%)	12.3±5.1 (5-23)

Data are presented as numbers, ratio & mean±SD; percentages & ranges are in parenthesis

Table (2): Serum levels of IL-6 and visfatin estimated in studied patients categorized according to radiological presence of erosion compared versus control group

	Control (n=20)	Patients		
		Non-erosive (n=27)	Erosive (n=43)	Total (n=70)
IL-6 (ng/ml)	4.49±3.3 (1.5-11.6)	14.1±7.1* (4.8-23.4)	25.7±6.77*† (12.7-41.7)	21.2±9* (4.8-41.7)
Visfatin (ng/ml)	3.24±0.91 (2.1-5.4)	6.61±1.21* (4.7-9.87)	10.65±3.18*† (5.6-18.9)	9.1±3.26* (4.7-18.9)

Data are presented as mean±SD; ranges are in parenthesis *: significant difference versus control group
†: significant difference versus non-erosive group

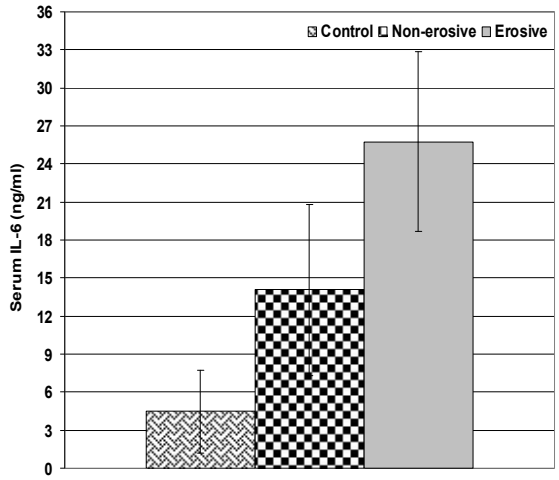

Fig. (1): Serum levels of IL-6 estimated in studied patients categorized according to radiological evidence of presence of erosion

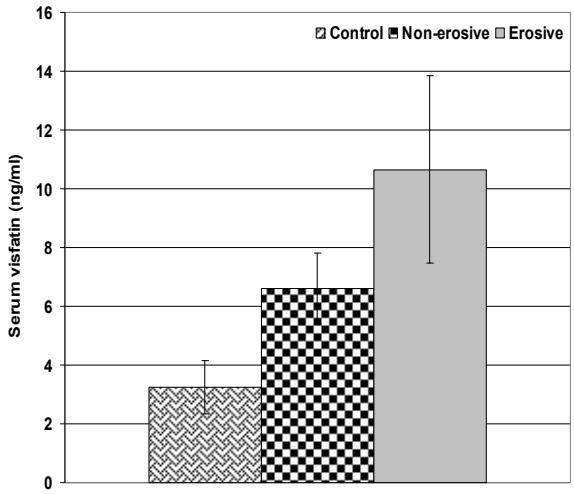

Fig. (2): Serum levels of visfatin estimated in studied patients categorized according to radiological evidence of presence of erosion

Table (3): Correlation coefficient between radiological presence of erosion versus age, clinical data and serum IL-6 and visfatin levels

	Age	Duration of disease	DAS-28 score	Pain VAS score	DI	Serum IL-6	Serum visfatin
"r"	0.373	0.365	0.486	0.504	0.413	0.390	0.607
p	=0.001	=0.002	<0.001	<0.001	<0.001	=0.001	<0.001

Table (4): ROC curve analysis of age, clinical data and serum IL-6 and visfatin levels as predictors for presence of radiological evidence of erosion as judged by area under curve

	Area under curve
Age	0.693
Duration of disease	0.717
DAS-28 score	0.752
Pain VAS score	0.789
ID	0.746
Serum IL-6	0.742
Serum visfatin	0.876

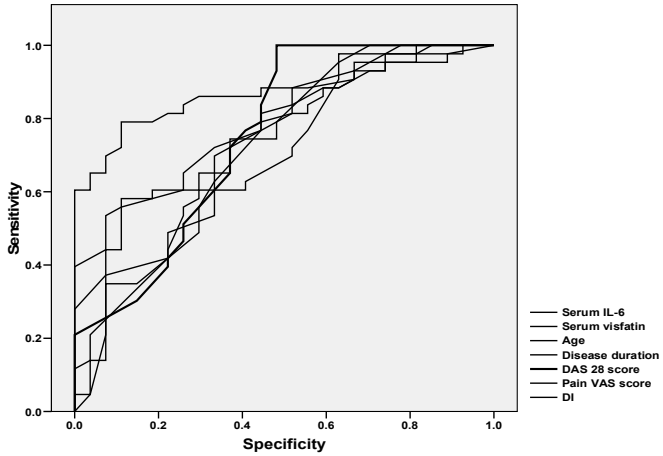

Fig. (3): ROC curve analysis of age, clinical data and laboratory findings as predictors of radiological presence of erosion

Table (5): Regression analysis models "Stepwise method" to identify the significant predictor for presence of radiological evidence of erosion

		ß	SE	t	p
Model 1	Age	0.235	0.006	2.809	=0.007*
	Duration of disease	0.128	0.145	1.475	>0.05
	DAS-28 score	0.104	0.284	1.081	>0.05
	VAS pain score	0.146	0.166	1.401	>0.05
	DI	0.185	0.008	2.096	=0.040*
	Serum IL-6	0.329	0.005	3.795	=0.004*
	Serum visfatin	0.485	0.013	5.814	<0.001*
Model 2	Age	0.231	0.006	2.695	=0.009*
	Serum IL-6	0.402	0.004	4.940	=0.001*
	Serum visfatin	0.531	0.013	6.204	<0.001*
Model 3	Serum IL-6	0.386	0.005	4.552	=0.002*
	Serum visfatin	0.604	0.013	7.117	<0.001*
Model 4	Serum visfatin	0.607	0.015	6.293	<0.001*

ß: standardized coefficient SE: Standard error *: significant parameter

4. Discussion

The current study aimed to enroll all patients with RA manifestation for more than 6 months; all enrolled patients were symptomatizing with a mean DAS-28 score for disease activity of 3.9±0.8, mean VAS joint pain score was 60.2±5.2 and mean DI of 12.3±5.1. These data could be attributed to that previously reported by **Gerber *et al.*(2003)** and **Scott *et al.* (2003)** who found the active joint count predicts subsequent performance and function for patients with recent onset, inflammatory synovitis more effectively than whether patients met ACR criteria for RA.

Disease severity assessment relied on evaluation of pain using VAS pain score, join affection score (DAS 28), disability index and laboratory evaluation of ESR and CRP levels. Such combination helped for patients' selection and goes in hand with **Klarenbeek *et al.* (2011)** who compared nine disease activity indices versus the American College of Rheumatology/European League against Rheumatism remission criteria in RA and tried to relate these indices to physical function and joint damage progression and found clinical DAS and simplified DAI were the most stringent definitions of remission, DAS28 and DAS28-CRP had the highest proportions of remission and concluded that all indices, higher levels of disease activity were associated with decreased physical functioning and more radiological damage progression.

Serum levels of IL-6 and visfatin were significantly higher in patients compared to controls and in those had radiological evidence of erosion compared to patients free of erosion. These findings go in hand with **Otero *et al.* (2006)** who investigated plasma levels of adipocytokines (leptin, adiponectin, visfatin and resistin) in patients with RA in comparison to levels estimated in healthy controls, and found patients with RA showed considerably higher plasma levels of leptin, adiponectin and visfatin than healthy controls, but no marked difference was observed in resistin levels between patients and controls. **Senolt *et al.* (2011)** found serum visfatin levels were significantly higher in patients with RA compared with healthy controls and significantly decreased following treatment with anti-B cell therapy.

The increased levels of both IL-6 and visfatin indicated pathogenic relation between both cytokines. In support of this assumption, there was a positive significant correlation between serum levels of both parameters on one side and between both and presence of radiological evidence of erosion.

Multiple studies tried to explore the relationship between IL-6 and visfatin and presence of RA and its severity; **Nowell *et al.* (2006)** experimentally found IL-6 trans-signaling regulated PBEF in a STAT-3-dependent manner, PBEF was regulated by the IL-6-related cytokine oncostatin M and that the involvement of PBEF in arthritis progression was confirmed in vivo, where induction of antigen-induced arthritis resulted in a 4-fold increase in the synovial expression of PBEF. On reverse, **Brentano *et al.* (2007)** found that in RA synovial fibroblasts, PBEF was up-regulated by Toll-like receptor ligands and PBEF itself activated the transcription factors NF-kB and activator protein 1 and induced IL-6, IL-8 and metalloproteinases 1 and 3 in RA synovial fibroblasts as well as IL-6 and TNF-α in monocytes.

Niederer *et al.* (2011) analyzed the expression of sirtuin 1 (SIRT1) which plays an important role in maintaining metabolic homeostasis in synovial tissues and cells of patients with RA and found SIRT1 was constitutively upregulated in synovial tissues and cells from patients with RA compared to osteoarthritis, silencing of SIRT1 promoted apoptosis in RA synovial fibroblasts, whereas SIRT1 over-expression protected cells from apoptosis and knockdown of SIRT1 resulted in a reduction of proinflammatory IL-6 and IL-8 in RA synovial fibroblasts.

Among clinical data including age, duration of disease, DAS-28 score, VAS pain score, DI and serum IL-6 and visfatin, Regression analysis as predictors of the presence of radiological evidence of bone erosion defined serum levels visfatin as specific predictor that was persistently significant in four regression analysis models.

In line with the specificity of visfatin for RA, **Nowell *et al.* (2006)** found that synovial fluid levels of PBEF were significantly higher in RA patients than in osteoarthritis patients. **Rho *et al.* (2009)** who found visfatin concentrations were associated with higher Larsen scores, and this association remained significant after adjustment for age, race, sex, disease duration, BMI, and inflammation. Thereafter, **Rho *et al.* (2010)** examined the relationship between adipocytokines and insulin resistance and coronary atherosclerosis among patients with RA and reported increased serum levels of examined adipocytokines and increased concentrations of leptin were associated with a higher insulin resistance index, even after adjustment for age, race, sex, BMI, traditional cardiovascular risk factors, and inflammation mediators, but concentrations of visfatin, adiponectin and resistin showed no association with insulin resistance.

Klein-Wieringa *et al.* (2011) found levels of IL-6, TNF-α, visfatin, and adiponectin were positively associated with radiographic progression over 4 years and this association was independent of

BMI and concluded that adipokines are predictors of radiographic progression in RA, possibly through distinct underlying biologic mechanisms. **Senolt *et al.* (2011)** reported that lack of change in the serum visfatin levels between baseline and week 16 following treatment with rituximab predicted worsening disease activity between weeks 16 and 24.

It could be concluded that there was a positive significant correlation between serum visfatin levels and rheumatoid arthritis severity as manifested clinically or radiologically and as serum IL-6 levels. Serum levels of visfatin could be considered as specific significant predictor for radiological severity and wider scale studies are advocated for evaluation of its utility as screening test for early cases.

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Efficacy of Prophylactic Fluconazole in Reducing Candidemia in High Risk NICU and PICU Patients

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Abstract: Background: Candida infection is a common cause of morbidity and mortality in neonatal intensive care unit (NICU) and pediatric intensive care unit(PICU) patients ,especially those with risk factors. **Objectives:** To determine the prevalence of Candida species in risky NICU and PICU patients and evaluate the efficacy of prophylactic Fluconazole in reducing Candida colonization and subsequent invasive candidemia in those patients. **Design:** Prospective, randomized, double blind placebo controlled clinical study. **Setting:** Tertiary level intensive care units at pediatric department. **Subjects:** 80 intensive care unit high risk group patient of neonatal and pediatric age. **Intervention:** children were randomly grouped during first three days to receive either Fluconazole or placebo till 28 days or less, if discharged or died earlier. Weekly surveillance cultures from oropharyngeal swabs, urine , stool and sputum (when available), samples were collected from all patients and cultured on sabaroud dextrose agar media . Blood culture on Bact/ALERT®3D culture system for Candida detection was done when candidemia was suspected. For positive cultures, isolates were identified by API 20c biochemical identification strips. Liver enzymes were monitored. **Results:** Baseline risk factors for Candida infection in Fluconazole and Placebo groups were similar. Candida colonization was reported in 35 patients (87.5%) in the placebo group which was significantly higher (P =0.0001) than that detected among patients in the Fluconazole treated group [10 patients (25%)] . Fluconazole treated group showed significantly lower colonization with Candida albicans (C. albicans) and higher colonization with non Candida albicans (non-C. albicans)versus placebo group. Invasive Candida infection was significantly higher (P =0.03) among placebo group than Fluconazole treated one .Invasive non-C. albicans infection was reported in 9/13 patients [6 patients (66.6%) in Placebo group and 3 patients (33.3%) in Fluconazole treated group]. No significant hepatotoxicity was noticed during Fluconazole therapy. **Conclusion:** Prophylactic Fluconazole in risky neonatal and pediatric patients in ICU is effective in reducing Candida colonization especially C. albicans but not invasive candidemia.

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Key words: Candida colonization; candidemia; antifungal prophylaxis; Fluconazole; risk factors for candidemia; ICU infection and nosocomial infection.

1. Introduction:

Bloodstream infections due to Candida have took considerable attention in several medical fields over the past few years due to their increasing incidence (57-61%) and attributed mortality rates(10-49%) [1,2], also the appearance of non-albicans species which displaying a different resistant susceptibility profile[3-5].

Candida species are responsible for around 80% of nosocomial fungal infections, and around 10-20% of all nosocomial bloodstream infections in intensive care units (ICUs)[6-8] especially patients with abdominal surgery, hematologic malignancies, and solid organ or bone marrow transplantation[9-11]. It is the third most common pathogens as cause of nosocomial bloodstream infections in premature infants[12] and the fourth commonest cause of blood stream infections in pediatric ICU patients[13].

The development of such infections is associated with increased overall morbidity, which lengthens the duration of ICU stay and increases the cost of hospitalization[14,15].

Many risk factors contributing to the development of fungal infections in ICU were identified. The main risk factors for candidemia include the prematurity[16,17], wide use of broad spectrum antibiotics therapy for a long time, long hospital stay especially with immunosuppressive drugs as in oncological diseases, immune-compromised patients, multiple organ failure (MOF), abdominal surgery, parenteral nutrition, hemodialysis, and the use of any invasive procedure like central venous catheter, and mechanical ventilation[18-20].

Prophylaxis with antifungal regimens has been proposed as an effective (and probably cost-effective) approach to prevent such infections in high-risk patients[21-23] but it remains controversial in most populations including surgical intensive care unit patients[24].In low candidemia rate populations the benefit of instituting preventative or prophylactic

strategies is weighed against the potential risks (eg, antifungal drug resistance or toxicity)[25]. So in order to reduce the morbidity and mortality contributed to this widespread type of fungal infection. Azoles and polyenes were used in a number of randomized controlled studies to assess their effectiveness as prophylactic regimens[26-28]. Azoles are associated with fewer adverse effects compared with polyenes and can be administered orally[29-31].

Our objectives were to determine the prevalence of Candida species in risky NICU and PICU patients at Zagazig University hospital which is a tertiary referral hospital, serving a large governorate in Egypt and to estimate the efficacy of using fluconazole as prophylactic therapy in those high risk patients.

2. Study Period, Patients, and Entry Criteria.

This study was a prospective, randomized, controlled double-blind study. Eighty consecutive risky patients from October 2009 to July 2011 were enrolled in this study. These patients were between neonatal age and 12 years of age and were admitted to the intensive care units of pediatric hospital, Zagazig University hospitals.

These patients included if they were admitted to NICU or PICU with risk factors for fungal infection including: prematurity, central line insertion, catheterization, mechanical ventilation, immune-compromised patient ,patients on immunosuppressive drugs neutropenia, patients with abdominal surgery, dialysis, or patients under total parenteral nutrition(T.P.N.).

They were randomized as soon as a preliminary report of sterile cultures and swabs were received, usually within 48–72 hrs.

Exclusion criteria: patients who were already on antifungal therapy, had positive Candida cultures, had known hypersensitivity to azole group of drugs, or those who had severe impairment of liver function at admission.

Informed written consent was obtained from the patient's parents. We recorded details of primary diagnosis, symptoms at the time of detection of candidemia, physical examination findings, and known risk factors for candidemia at the time of entry into the study. Randomization was done at the time of enrolment to receive either solution A or solution B (one of them being Fluconazole and other being placebo) by the shuffled sealed envelope method. So they were divided into two groups; group (I) include patients who received prophylactic fluconazole and group (II) include patients who received placebo one.

Baseline data on the demographic and clinical characteristics of the patients were collected and relevant clinical data was prospectively collected throughout the course of the study on a pre-designed Performa. Presence of one or more clinical signs consistent with fungal infection (e.g., temperature instability, increase in frequency of apnea, increase in oxygen requirement, etc.) was noted. Solution A or solution B was administered intravenously at dose of 3mL/kg/day as a single dose every 72 hours till day 14 and subsequently every 48 hours till day 28 of life in the neonatal period, while in patients older than 3 months the dose was doubled and given as a daily dose. Fluconazole preparation used for the study was colorless, in the strength of 1 mL = 2 mg. The placebo group received an equal volume of normal saline as it physically matched the Fluconazole solution. Candida surveillance cultures were collected on the day of randomization (day 1 to 3) and days 7, 14, 21, 28 and also as indicated by the treating physician. Candida workup for Candida colonization pattern obtained including weekly surveillance cultures from oropharyngeal swabs, urine, stool and sputum (when available), samples were collected in all patients and cultured on sabaroud dextrose agar media (Oxoid, Ltd.USA). Candida colonization defined as at least one positive surveillance culture[10]. The obtaining of surveillance cultures were discontinued before the determined four-week treatment period if systemic fungal infection documented, if the infant was discharged, died or transferred to another unit, or if significant hepatotoxicity was diagnosed based on biochemical monitoring. Decision of exclusion of any patient from the study and review of Candida culture pattern was done weekly.

If a baby developed invasive Candida infection determined clinically and by Candida growth in blood culture, solution A / B was stopped and Candida sepsis was treated with intravenous Amphotericin B.

Blood culture for candidemia on Bact/ALERT®3D culture system (biomerieux, Inc) cup to 4 ml blood was drawn under standard recommended antisepsis procedures and inoculated in pediatric blood culture bottles (Bact/Alert PFbottle) ,bottles were monitored for the presence of bacterial growth every 10 min. by the Bact/ALERT®3D culture system (automated continuously monitoring blood culture instrument). If the Bact/ALERT system recorded any positive bottle, direct gram stain and subculture on sabaroud,s dextrose agar media were done. Negative cultures were incubated till completion of the remainder period. All colonies appeared on the media were examined macroscopically, gram stain, C. albicans by germ tube test and finally by API 20c biochemical identification strips (biomerieux,Inc).

Complete blood counts, renal function test, and liver function tests were obtained at the start of therapy and repeated weekly for the duration of therapy.

Patients who did not have a follow-up blood culture were considered to have recovered as they were asymptomatic at discharge time from hospital and first follow-up visit. Further deterioration (not attributable to other cause) or positive blood culture (invasive candidemia) or urine culture for Candida after therapy was defined as prophylactic failure.

Statistical analysis:

Chi square χ^2 test ,fisher exact probability test were used when appropriate. P <0.05 was considered significant. Results were analyzed using SPSS software version 10.0.

3. Results

Data were collected, summarized, analyzed and presented in the following tables:

There was no difference in the demographic pattern, clinical characters or the risk factors for Candida colonization and candidemia between both fluconazole treated and the placebo groups (Tables 1 and 2).

Candida colonization occurred significantly more commonly in the placebo group as compared to the Fluconazole treated group (87.5% vs 25%, P = 0.0001) . The colonization rate by C. albicans was 60% with significant increase in placebo group (71.4%) in comparison to fluconazole treated group (20%) while "non C. albicans" colonization rate was 40% with significant increase among fluconazole treated group (80%) versus (28.6%) among placebo group (Tables 3,4).

The incidence of candidemia in the overall patients was (21.3%) among it (52.9%) were non C. albicans with profound increase (75%) among fluconazole treated group while increase of C. albicans (53.8%) among placebo group was detected (Table 5).

Regarding the clinical picture of candidemia among patients there was significant difference in the severity of the associated symptoms with candidemia between the two groups as regard lethargy and poor reflexes, mucocutaneous affection and hepato-splenomegaly (Table 6). Five patients died in the fluconazole treated group and seven in placebo group. Out of 12 patients who died, 6 patients had developed invasive fungal infection prior to death (2 in fluconazole treated group and 4 in placebo group). All those patients were started on Amphotericin B. Fluconazole was not found to be hepatotoxic with the dosage and the Duration used.

Table (1): Demographic and clinical characteristics of the patients in the studied groups

Characteristic		Group(I)		Group(II)		**P**
		N:40	(%)	N:40	(%)	
Sex (male/female)		27/13	(67.5/23.5)	22/18	(55/45)	0.25
Age	Preterm	8	(20)	10	(25)	0.59
	neonates	10	(25)	6	(15)	0.26
	Infancy	10	(25)	11	(27.5)	0.8
	preschool	8	(20)	7	(17.5)	0.77
	school age	4	(10)	6	(15)	0.5
Location	NICU	18	(45)	16	(40)	0.65
	PICU	22	(55)	24	(60)	0.65
Cause of admission						
-Prematurity		8	(20)	10	(25)	0.59
-Neurological disease		3	(7.5)	1	(2.5)	0.61
-Sepsis		5	(12.5)	5	(12.5)	1.00
-Post operative		7	(17.5)	3	(7.5)	0.17
-Oncolgical		4	(10)	1	(2.5)	0.35
-Hematological malignancies		2	(5)	3	(7.5)	1.0
-Pulmonary disease		4	(10)	6	(15)	0.49
-Dialysis		6	(15)	6	(15)	1.00
-Cardiac disease		1	(2.5)	5	(12.5)	0.09

Table (2): Risk Factors of Candida colonization and candidemia in the studied groups .

Risk Factor	Group(I) n:40		Group(II) n:40		P
	N	(%)	N	(%)	
-No. of antibiotics					
1-2	2	(5)	6	(15)	0.26
3-4	3	(7.5)	2	(5)	1.0
>4	4	(10)	1	(2.5)	0.35
-Mechanical ventilation	7	(17.5)	5	(12.5)	0.39
-C.V.catheter	6	(15)	5	(12.5)	1.0
-T.P.N.	6	(15)	7	(17.5)	0.76
-Surgery	5	(12.5)	6	(15)	1.0
-Corticosteroid therapy	6	(15)	5	(12.5)	0.1
-Neutropenia	3	(7.5)	5	(12.5)	0.7
-dialysis	4	(10)	3	(7.5)	1.0

Table (3): Surveillance cultures results and rate of colonization among both studied groups

Colonization rate	Group (I) n:40		Group (II) n:40		**Total** n:80	
	No	(%)	No	(%)	No	(%)
●No colonization	30	(75)	5	(12.5)	35	(43.7)
●Colonization rate	10	(25)	35	(87.5)	45	(56.3)
●Positive surveillance cultures:						
-One anatomic site of colonization	3	1+ve Oroph.* 1+ve Stool 1+ve urine	10	5+ve Oroph.* 1+ve Sputum 2+ve Stool 2+ve urine		
- Two anatomic sites of colonization	5	1+ve Oroph.*&sputum 1+ve Oroph.*&stool 2+ve Oroph.*&urine 1+ve Stool & urine	19	4+ve Oroph.*& sputum 6+ve Oroph.*& stool 2+ve Oroph.*& urine 7+ve Stool & urine		
- More than two anatomic sites of colonization	2	1+ve Oroph.*,stool & urine 1+ve Oroph*,stool & sputum	6	4+veOroph.*,stool & urine 2+veOroph.*,stool & sputum		

* Oroph.=oropharyngeal

Table (4): Different Candida species colonization among both groups

Species	Total n:80	Group(I) n:40		Group(II) n:40		P
	N (%)	N	(%)	N	(%)	
Total colonization rate .	45 (56.3)	10	(25)	35	(87.5)	0.0001*
●**C. albicans** species	27 (60)	2/10	(20)	25/35	(71.4)	0.008*
●**Non C. albicans** species	18 (40)	8/10	(80)	10/35	(28.6)	0.008*
-C.tropicalis		4	(40)	7	(20)	
-C.glabrata		2	(20)	2	(5.7)	
-C. krusi		1	(10)	0	-	
-C.parapsilosis		1	(10)	1	(2.9)	

*Comparison between group I and group II.

Table (5): Candida species candidemia among both groups

Species	Total n:80	Group(I) n:40		Group(II) n:40		P
	N (%)	N	(%)	N	(%)	
Total invasion rate	17 (21.3)	4	(10)	13	(32.5)	0.03*
●**C. albicans** species	8 (47.1)	1/4	(25)	7/13	(53.8)	0.6
●**Non C. albicans** species	9 (52.9)	3/4	(75)	6/13	(46.2)	0.6
-C.tropicalis		2	(66.7)	3	(42.9)	
-C.glabrata		1	(33.3)	2	(28.6)	
-C.parapsilosis		0	-	1	(14.3)	

*comparison between group I and group II

Table (6): Clinical picture of candidemia in both groups

Clinical picture	Group(I) N:4	Group(II) N:13	P
-Lethargy & poor reflexes	1	11	0.05*
-Hypothermia	2	5	1.0
-Fever	2	3	0.54
-Mucocutenous lesions	1	12	0.02*
-H.Smegaly	0	9	0.03*
-Food intolerance	3	8	1.0
-Death	2	4	0.58

4. Discussion

Candida species have become important and common causes of bloodstream infections in children, especially those hospitalized in PICUs, with an increasing incidence [32]. Although C. albicans remains the most frequently isolated species, there is a shift to non- C. albicans species, including C. parapsilosis, C. tropicalis, and C. glabrata, with an associated increase in mortality and antifungal resistance[33,34]. Morbidity and mortality remain high, underlining the importance of primary prevention of candidemia in PICU patients[32].

Patients with different risk factors for Candida colonization and candidemia were included in this study as patients with immunosuppressive therapy, immune-deficiencies, using broad-spectrum antibiotics, administrating of parenteral alimentation, dialysis, surgery were also included. These risk factors and more were mentioned in different reports[12,13,34-36].

In the present study, The total colonization rate was 45/80 (56.3%) with significantly (p=0.0001) less number of fungal colonization reported in Fluconazole treated group (25%) in comparison to the placebo group (87.5%) during the 28 days surveillance period . **Singhi and Deep [33] found** colonization by Candida in 69% patients by the end of two weeks stay in PICU. **Kicklighter et al [37] & Kaufman et al [38]** reported colonization rate 23% and 46%, respectively and after fluconazole treatment reduction to 4.9% and 15.1%, respectively was detected that was consistent with this study results as colonization in fluconazole treated group was significantly less than the placebo group .

The colonization rate by "non C. albicans" in the present study was (40%) with significant increase among fluconazole treated group (80%) versus (28.6%) among placebo group which is respectable to studies by **Baley et al [39]** and **Kicklighter et al [37]** in whom the colonization rate by "non C. albicans" was 39% and 47%, respectively .

Rodriguez-Nunez[40] mention that antifungal treatment seems to play an important role in the Candida species isolated, with C. parapsilosis and C. krusei being seen most commonly after fluconazole therapy, and C. glabrata after both amphotericin B and fluconazole.

The incidence of candidemia in the overall population is increasing (Candida is one of the leading causes of bloodstream infections in developed countries), and the rate of increase is greater in pediatric patients than in adults[34,41].

In the current study, The incidence of candidemia was (21.3%) among it (52.9%) were non C. albicans with profound increase (75%) among fluconazole treated group while increase of C. albicans (53.8%) among placebo group was detected.

Study by **Narang et al [11]** in the year 1998, from North India showed 22.8% incidence of invasive fungal infection in preterm neonates. Another study from the same institution showed that among the different Candida species, there is a shift from C. albicans to non C. albicans species as 56.5% fungal isolates from patients with fungal sepsis were non C. albicans (C. tropicalis in 21.7%, C. guillermondii in 13%, C. parapsillosis in 13% and C. krusei in 8.7%)[42]. In study by **Kaufman et al [38]** the incidence of invasive fungal infection was 20% in the placebo group and out of 10 fungal isolates 50% were non C. albicans. Interestingly C. krusei and C. glabrata are species with intrinsic resistance to Fluconazole[3,5,43] while C.tropicalis and C. parapsilosis tend to be less susceptible to Fluconazole than C. albicans[4,33].

Fluconazole is used in our ICUs since last four years, which could be the reason for high incidence of non- C. albicans species, which are less susceptible to Fluconazole. Similar timing of presentation of invasive fungal infection caused by those non- C. albicans species in the Fluconazole and placebo group highlights the fact that Fluconazole was not effective in preventing invasive fungal infection in the present study. Long-term repeated exposure of Candida species to a specific antifungal class may result in the gradual eradication of susceptible species like C. albicans and promote the proliferation of resistant species. There may be other significant factors apart from superficial colonization that contribute to fungal sepsis; as in **Kicklighter et al [37]** study, a reduction of fungal colonization by

prophylactic fluconazole did not bring down rate of invasive fungal infection.

The role of prior colonization seems to be very important for candidemia, and appears to be a necessary step before infection[44]. This was observed in this study as with increase colonization in placebo group (87.5%) versus Fluconazole treated group (25%), the candidemia increased as it became (32.5%) in placebo group versus (10%) in Fluconazole treated group. **Verduyn et al** [45] reported that colonization with Candida has been shown to precede candidemia and is regarded as an independent risk factor for systemic fungal infection. Also, in one group of pediatric patients with burns, the incidence of candidemia was remarkably increased from 0% when the fungus colonized one site, to 22.2% with two sites and 34.4% with three or more colonized sites[44].

Candida species are known to adhere to epithelial layers, endothelial cells, blood clots, plastic and acrylic producing a number of adhesive molecules that enhances their ability to persist, invade and disseminate. So, monitoring for colonization may help in predicting subsequent infection with identical strains in critically ill children being treated in PICU[33].

In the present study , mortality rate were (35.3%).which was on line with results obtained by **Filioti et al** [35] who mention that the mortality rate among infants can be as high as 43% to 54%. Several studies reported range (10% to 49%) mortality rate in children which is interestingly lower than the rate in adults, (31% to 78%) probably because of the difference in Candida species distribution between the two populations[1,2,33,34].

The question which is often raised is whether the morbidity and mortality in patients with invasive candidiasis is directly attributable to candidemia or should it be attributed to the critical nature of the underlying disease. **Wey et al** [46] found excess mortality attributable to candidemia apart from the underlying disease was 38%.

In conclusion, in view of the serious nature of candidemia with its attributable mortality, the early prophylaxis with fluconazole may be effective in limiting the Candida colonization and subsequent invasion (candidemia) which is a very strong reason for morbidity and mortality in NICU and PICU. Further studies should focus on validation of the risk factors that may contribute in candidemia in order to identify the population that could benefit most from antifungal prophylaxis and other preventive measures.

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Usefulness of Helicobacter Pylori Eradication for Platelet Recovery in Egyptian Idiopathic Thrombocytopenic Purpura Patients

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Abstract: Background: Recent studies have shown a relationship between Helicobacter pylori (H. pylori) and idiopathic thrombocytopenic purpura(ITP). **Objectives:** To clarify the relation between H. pylori and ITP, determine its prevalence in this disease and to evaluate the effect of its eradication on platelet recovery. **Subjects and methods:** 65 adult patients with ITP (platelet count < 100 x 10^3/µl) were investigated for the presence of H. pylori infection and its eradication by H pylori stool antigen (HpSA) enzyme immunoassay method (EIA) . H. pylori positive patients received standard triple therapy for seven days to eradicate infection. Platelet counts were monitored every 2 weeks and assessed 6 months after the end of H. pylori eradication therapy. Uninfected patients underwent immunosuppressive therapy and their platelet counts were followed up for the same duration. **Results:** 45/ 65 ITP patients, were H. pylori positive. They were significantly older and showed longer disease duration than H. pylori negative patients .There was significant increase in platelet count in both group after treatment and this increase was significantly higher in H. pylori positive group than negative one. Out of the 45 infected patients who received treatment, H. pylori was successfully eradicated in 39 patients. In 21 (53.8%) of these patients , significant good platelet response was detected when compared with unsuccessfully treated and H. pylori negative patients. **Conclusion:** Eradication of H. pylori infection led to good platelet response in ITP patients. Therefore, search for this infection must be attempted in ITP patients at diagnosis which will allow a good non immunosuppressive option for some of them.

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Key words: H. pylori, eradication therapy, platelet count, immune thrombocytopenic purpura.

1. Introduction:

Idiopathic thrombocytopenic purpura (ITP), also known as primary immune thrombocytopenic purpura is an acquired disease of both children and adults. It is defined as isolated thrombocytopenia with no clinically apparent associated conditions or causes of thrombocytopenia. So, its diagnosis relies on the exclusion[(1)]. The term idiopathic was coined because in the majority of cases the underlying cause was unknown. Recently, the list of etiologies has been steadily increasing, so, the term "idiopathic" is becoming obsolete, increasingly replaced by "immune" thrombocytopenic purpura[(2)]. Helicobacter pylori (H. pylori) is a gram-negative microaerophilic bacterium that colonizes the stomachs of over half the human population. It is the predominant agent of active chronic gastritis, gastric and duodenal ulcers. Also, it is a cofactor in the development of both adenocarcinoma and mucosal associated lymphoid tissue lymphoma[(3)]. Several studies have investigated the relationship between H. pylori and extra-gastroduodenal disorders. It is reported that it has been implicated in various autoimmune disorders[(4)]. H. pylori infection is driven by urease, flagella, and adhesions. Virulence factors such as CagA and VacA play roles in colonization and infection. Other virulence factors are H. pylori neutrophil-activating protein (HP-NAP) and cell-wall lipopolysaccharide (LPS)[(5-7)]. The role of H. pylori in the development of ITP is not yet known. Many hypotheses have been proposed to address the mechanisms by which H. pylori causes ITP. Platelet-associated immunoglobulin G, CagA, LPS etc., have all been reported to play a role in platelet apoptosis[(8,9)]. Since partial or even complete remission of thrombocytopenia has been recorded in some ITP patients after eradication of H. pylori it has been suggested that H. pylori may contribute in the pathogenesis of this disease[(10)]. Most studies of H. pylori and ITP are from Japan, Spain and Italy[(11)] To date the therapeutic option of H. pylori which is simpler and safer than immune-suppressives and splenectomy hasn't been carefully investigated in Egyptian ITP patients. This study was designed to clarify the relation between H. pylori and ITP, determine its prevalence in this disease and to evaluate the effect of its eradication on platelet recovery.

2. Study design:

Sixty five ITP patients (25 males and 40 females) were included and studied in Zagazig

University hospitals. ITP was diagnosed according to the American Society of Hematology (ASH) guidelines[1], based on thrombocytopenia (platelet count < 100 x 10^3/μl) with normal bone marrow or showing megakaryocytic hyperplasia. Secondary ITP caused by drugs, viral infection and collagen disease were excluded.

H. pylori infection was documented by detecting H. pylori antigens in stool specimens through Helicobacter pylori stool antigen (HpSA) enzyme immunoassay method (EIA)[12] and whenever possible, by histo-pathological examination using (Giemsa stain) for specimen obtained by an upper gastrointestinal endoscopy.

The stool sample from each patient was stored at 2-8°C for up to 24 hours or at -70°C if prolonged storage was required till the completion of a test batch. Thawing of the specimens was done by keeping them at room temperature for 1 hour. Premier Platinum HpSA plus kit (Meridian Diagnostic, Cincinati, Ohio. USA) was used for stool antigen detection as per manufacturer instructions. The test was performed in four steps:-

1)Specimen processing : A stool sample measuring 5-6 mm diameter was diluted in 200 μl of sample diluent and mixture was vortexed for 15 seconds. A total of 50 μl of the processed samples and equal volume of positive and negative controls were added to the appropriate micro-wells of the enzyme immune-assay (EIA) plate.
2)Sample-enzyme conjugation and incubation: A drop of enzyme conjugate was added to the wells and contents were firmly mixed for 30 seconds. The wells were sealed and incubated at 22-27°C for one hour. The contents of the wells were washed with buffer for five times.
3)Substrate incubation :Two drops of substrate were then added to each well and the plates were again incubated for 10 minutes at 22-27°C. A drop of stop solution was added to each well and mixed for 30 seconds.
4)The absorbance at 450 nm was immediately measured using a DaVinci (bioMérieux, France) microplate reader and were interpreted as positive if the optical density was more than 0.16 at wave length of 450 nm.

All infected patients gave a written consent ,immune-suppressives were stopped (if used by any) for one month and treated for 7 days with standard triple therapy (lansoprazole, 30 mg, clarithromycin 200 mg and amoxicillin 750 mg all twice daily)[13]. Eradication was confirmed by H. pylori stool antigen (HpSA) one month after completion of therapy. After completion of triple therapy infected patients were subdivided into successfully treated patients (eradicated infection) and unsuccessfully treated patients (uneradicated infection) based on repeated H. pylori detection tests.

Platelet counts were monitored every 2 weeks and assessed 6 months after the end of H. pylori eradication therapy. Uninfected patients underwent immunosuppressive therapy and their platelet counts were followed up for the same duration.

Rise of platelet count to normal value (150 – 450 x 10^3/μl) was considered as a complete response (CR), while increase of the count to less than 120 x 10^3/μl or 30 x 10^3/μl above the baseline count was considered partial response (PR)[14].

ITP patients whose platelet count didn't rise after H. pylori eradication or immunosuppressive therapy underwent splenectomy.

Statistical analysis:

Statistical analysis was done using the SPSS version 10.0. Data are represented as Mean±SD. Unpaired student t-test, fisher exact probability test and chi-square test, were used when appropriate .P<0.05 considered to be statistically significant in all tests.

3. Results:

Data were collected, summarized, analyzed and presented in the following tables:

Forty five (69.2%) patients were H. pylori positive (infected) (21 males and 24 females) with mean age 52 years (39-72 years), the remaining 20 patients (4 males and 16 females) were H. pylori negative (uninfected) with mean age 40.5 years (27-65 years), which is statistically significant different between the 2 groups (P<0.004). There was no statistically significant difference between both groups regarding the baseline platelet count (at the beginning of the study), mean values were 55.2 (11-92) x 10^3/μl and 56.7 (19-99) x 10^3/μl, respectively with P>0.05.

Disease duration was significantly shorter in H. pylori negative than H. pylori positive patients with mean values of 4.5 (4-10) and 8.2 (6-14) months, respectively. All previous data are shown in table (1).

Change in platelet count and comparison between its values for the H. pylori positive patients (before eradication therapy and after its completion) and for the H. pylori negative patients at the beginning of treatment and at the end of the same duration of treatment are represented by table (2) showing highly significant (P<0.0001) of both. Also, by the same table the difference between the mean values of platelets count for the two groups after completion of therapy is shown to be significant.

Infection in 39/45 (86.7%)of infected patients was successfully eradicated by completion of triple therapy, this subgroup is compared to those with

unsuccessfully eradicated infection [6/45 (13.3%)] and H.pylori negative patients regarding response of platelet count in table (3)which shows significant higher percentage (53.8%) of patients with good response among those with successfully eradicated infection than either those with unsuccessfully eradicated infection(0%) and H. pylori negative patients(35%) .

Table (1): Characteristics of H. pylori +ve and H.pylori –ve groups

Item \ Group	H. pylori +ve group (N:45)	H. pylori -ve group (N:20)	Test of significance	P
Age Mean±SD (Range)	52±16 (39-72)	40.5±10.2 (27-65)	t=2.95	0.004*
Sex Male/ Female	21/24	4/16	χ^2=3.11	0.078
Platelets count X10^3/ul Mean±SD (Range)	55.2±12 (11-92)	56.7±19 (19-99)	t=0.385	0.7
Disease duration	8.2±2.1 (6-14)	4.5±1.6 (4-10)	t=7.015	0.0001*

Table(2): Comparison between platelet count before and after therapy among studied groups.

Platelets count \ Group	H. pylori +ve group (N:45)	H. pylori -ve group (N:20)	t-test	P
Before treatment Mean±SD X10^3/ul (Range)	55.2±12 (11-92)	56.7±19 (19-99)	0.385	0.7
After treatment Mean ±SD X10^3/ul (Range)	230±20.2 (150-270)	186.7±10.7 (159-205)	9.014	0.0001*
t-test	49.9	26.7		
P	0.0001*	0.0001*		

Table(3): Response to therapy regarding infection eradication and platelet recovery among the studied groups.

Platelets count response \ H. pylori eradication response	H. pylori +ve group (N:45)		H. pylori -ve group (N:20)	P
	Successful eradication (N:39)	Unsuccessful eradication (N: 6)		
Good response	21/39 (53.8%)	0/6 (0%)	7/20 (35%)	0.02*
Partial response	4/39 (10.3%)	1/6 (16.7%)	3/20 (15%)	0.6
No response	14/39 (35.9%)	5/6 (83.3%)	10/20 (50%)	0.09

4. Discussion

Helicobacter Pylori has been considered for years as the only etiological agent of gastritis, peptic ulcer, gastric cancer and mucosa associated lymphoid tissue lymphomas[15]. Also, it has been found to be associated with a number of autoimmune disorders[16].

Globally, the prevalence of H. pylori infection in developing countries is markedly higher than that in developed countries[17,18].

Idiopathic thrombocytopenic purpura (ITP) is the most common autoimmune mediated hematological disorder. Its etiology, pathogenesis and molecular receptor targets remain unclear[11]. There is growing evidence of an association between H pylori eradication and platelets recovery in patients with ITP[19].

Aiming to participate in clarification of the relation between H. pylori and ITP, this study was carried out on 65 ITP patients among whom the

frequency of H. pylori infection was 69.2% (45/65) compared to 92% in Korea[3], 71% in Span[20], 62.5% in Japan[21], 62.7% in Italy[22], 56.3% in Australia[11], 29% in France[23] and down to lower rate (22%) in USA[4]. This variation in frequency of H pylori infection among ITP patients in these comparable studies may reflect the variation in ages of these studied groups of patients and give an impression about the potential regional variation of the prevalence of H pylori infection.

Recently, Semple and colleagues[24] demonstrated that in the presence of antiplatelet antibodies, the LPS of Gram negative bacteria can significantly enhance Fc-dependent platelet phagocytosis. These results suggest that infectious agents in combination with antiplatelet antibodies could affect platelet destruction in vivo, which may be at least one explanation for why thrombocytopenia worsens in some patients with ITP during infections[8].

Regarding age, in this study, H. pylori–infected patients were found to be significantly older (mean age:52 years) than H. pylori–uninfected (40.5 years) ones, which is consistent with that of similar studies[25,26]. This is not unexpected, as the prevalence of H. pylori infection in the general population increases with increasing age[27,28].

In respect to the mean duration of the thrombocytopenia, it was significantly longer in H. pylori-positive than that of H pylori negative patients (8.2 months versus 4.5 months) which was in agreement with that reported in different studies[29,30]. In contrast, as regard to other characteristics, such as sex and platelet count at the baseline all series that were reviewed failed to detect significant differences which is the case of this study.

In this study, data lend further support to a relationship between H. pylori infection and ITP as the platelet response of 64.1% (CR and PR) was noted after the eradication of H. pylori infection, whereas the corresponding rate was reported to be 50% by Emilia et al [31], 63.2% by Kohda et al [21] and 72.72% by Gasbarrini et al [32]. Moreover, 46.2% of the present study responders achieved CR versus 33.3% and100% of eradicated patients in reports by Emilia et al [31] and Gasbarrini et al [32] respectively. In contrast other studies refuted a significant association between of H. pylori infection and ITP[11] for example, a prospective study in the USA found that only 1/14 ITP who responded to H. pylori eradication had a rise in platelet count[4].

Variety in the rate of platelet response to bacterium eradication may be related either to the variability of host immune state (including HLA allele pattern , cytokines and chemokines produced in gastric mucosa in response to H. pylori infection) or to the bacterium's high genetic diversity, ie, to the existence of different H. pylori strains with possibly different pathogenic potential[33].

The response of the platelet count was also observed in one patient of six H. pylori– infected ITP patients who had unsuccessfully eradicated the infection in the present study. It has been advanced that the increased platelet count in patients who failed the H. pylori eradication or in those who received proton pump inhibitor monotherapy could have been mediated through a reduction in the quantity of H. pylori and/ or a bacteriostatic effect of the regimen[34].

Interestingly, the response of the platelet count was observed significantly increased after eradication therapy in H. pylori positive patients (mean 230)than H. pylori negative patients treated with immunosuppressives (mean 186.7) (P= 0.0001) . This finding could be explained in several ways, including an immune-modulatory effect of macrolides that is separated from the bacteriostatic effect[35].

In conclusion, considering the low costs, the noninvasiveness of diagnostic method, and much less toxicity and hazards of eradication therapy compared to standard ITP therapy (steroids or splenectomy), the assessment of H.pylori infection and use of its eradication therapy should be attempted in ITP.

Further studies are recommended on larger group of patients to fully ascertain the role of H pylori in ITP and for longer duration of follow up to assess the rate of relapse among the recovered cases and identify factors that may assist in selecting ITP patients who are more likely to respond to therapy.

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Density and sex ratio of seven spotted ladybird (*Coccinella septempunctata*) in three altitudes of Khorramabad district

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Abstract: Frequencies of seven spotted ladybird (*C. septempunctata*) were sampled from three altitudes of; 1700, 1800 and 1900 meters above sea level in the Rieg-sefid region of the Khorramabad district of Iran (33º48'N, 48º57'E, 1638m) in 2009 and 2010 during summer and autumn seasons. There was a higher frequency of females than males at all three altitudes (1700, 1800 and 1900 m) in both years. The average ratio of females to males in 2009 at an altitude of 1700 meters above sea level was 1.53:1; at the altitude of 1800 m it was 1.54:1 and at the altitude of 1900 m it was 1.55:1. The average ratio of females to males in 2010 at an altitude of 1700 meters above sea level was 1.49:1, at an altitude of 1800 m it was 1.51:1 and at an altitude of 1900 m it was 1.54:1. According to this information, results demonstrated that the ratio of females to males in seven spotted ladybird species increased at higher altitude. It was also observed that populations of this ladybird in farms at high altitude decreased and increased according to season.

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Keywords: sex ratio, ladybird, Khorramabad, coccinella septempunctata

1. Introduction

The seven spotted ladybird (*C. septempunctata*) is one of the most important predators of aphids. At the adult stage and during its immature stage (larval) the ladybird feeds on aphids and damages insect populations (Nunez-Perez et al., 1992; Sarwar & Saqib, 2010). This ladybird is found throughout most regions of Iran and it has been reported in all types of ecosystems (Sadeghi, 1991; Montazeri and Mossadegh, 1995; Ghahari et al., 2004; Farahi and Sadeghi namghi, 2009; Ansari pour, 2010; Ansari pour & Shakarami, 2011). This ladybird is the most dominant species in farms, gardens, mountains, pastures and meadows in the Khorramabad district (Ansari pour, 2010). The ladybird species is one that migrates. It covers short distances in search of food and this relocation involves short flights in search of food and longer flights for resettlement that includes gathering in places (Sadeghi, 1991). The seven spotted ladybird overwinters in different habitats among the surrounding forests, windbreaker and single shrubs, it can also move to the hills and mountains to overwinter (Honek, 1989; Hodek et al., 1993; Hodek & Honek, 1996, Honek *et al.,* 2007). Overwintering is spent as a collective rather than singly and this may increase its survival rate. Ecologists believe that life as a collective may contribute to a decreased risk of attack from predators and parasites (Sillen-Tullberg & Leimar, 1988; Turchin & Kareiva 1989; Mooring & Hartl, 1992). Adult ladybirds hibernate en masse, sometimes in small groups but more often a group consists of several thousand insects (Honek *et al.,* 2007). Population patterns of this ladybird species in different parts of the world show that insects migrate to high altitude areas for the overwinter period of hibernation (Bodenhimer, 1943; Savoiskaya, 1960; Sadeghi, 1991; Ceryngier, 2000). The population samples taken herewith showed that with increasing altitude, the ratio of female to male seven spotted ladybird's increases (Sadeghi, 1991; Ceryngier, 2000).

There is a considerable amount of research in the field on frequencies and gender ratios of the seven spotted ladybird (*C. septempunctata*) in altitude (Sadeghi, 1991; Ceryngier, 2000), but specific research has not yet been done on the species in the Khorramabad district. The seven spotted ladybird is the most important biological control agent in the Khorramabad district. Graduate studies can deepen an understanding of the biology of this ladybird and serve to increase its beneficial use in biological control programs to reduce or replace the use of agricultural pesticides. In this regard this research aims to provide a foundation for other researchers in this field.

2. Material and method

Sampling of the Seven spotted ladybird (*C. septempunctata*) was taken at three heights (33°48'N, 48°57'E, 1700m), (33°48'N, 48°57'E, 1800m), (33°48'N, 48°57'E, 1900m) from alfalfa fields from autumn to summer in 2009 and from autumn to summer in 2010 in the Rieg-sefid region of the Khorramabad district of Iran. The overwintering ladybird samples were collected from astragalus plants at different heights by hand; plants were shaken and the insects were kept in glasses containing 70% ethanol alcohol for counting and identification of numbers of male and female ladybirds in the laboratory. Exact numbers of males and females were counted and recorded in the laboratory, this work was done for four months in each year and the information obtained was recorded at each sampling at different altitudes of the Khorramabad district and sex ratios were determined thereafter.

3. Result and discussion

Results of sampling the seven spotted ladybird at three altitudes 1700, 1800 and 1900 m were as follows:

Sampling was done in the summers of 2009 and 2010 and demonstrated that populations of this species of ladybird from early summer to early autumn reduced at higher altitude. These findings are consistent with those of (Ceryngier, 2000). Research shows that in the first month of autumn this population decreases and this trend continues until winter approaches and then the ladybird population at high altitude grows. It can be said the population reaches its maximum in late fall and at the start of winter because these insects overwinter in highland locations.

There was a gradual increase of the ladybird population in the high region of Rieg-sefid of Khorramabad district from 2009/10/17 until 2009/12/1 and the population decreased in the same period in the alfalfa field (Fig. 1) demonstrating that this ladybird migrates to high ground for the period of overwinter hibernation; this has been validated by other research (Bodenhimer, 1943; Savoiskaya, 1960; Ceryngier, 2000). This increase in the ladybird population from 2010/10/10 until 2010/12/2 and the concurrent decrease in the alfalfa field (Fig. 2) indicated that overwintering occurred at high altitude locations.

According to Fig. 3 ladybird populations can be found three altitudes above sea level (1700, 1800 and 1900 m), the frequency of females was more than that of males which has also been commented on by (Sadeghi, 1991; Ceryngier, 2000). That research showed that the average ratio of males to females was consistent at the following altitudes in 2009; at the height of 1700 meters above sea level it was 1.53; at the height of 1800 m it was 1.54:1 and at 1900 m it was 1.55:1, and records for 2010 at the three altitudes of 1700, 1800 & 1900 m were 1.49:1, 1.51:1 & 1.54:1, respectively. These figures indicate that with increasing altitude, the ratio of female to male in seven spotted ladybird increased and this result is consistent with other research. It can be concluded that with increasing altitude the number of ladybirds increases Sadeghi (1991).

Table1. Number of seven spot ladybird in three different heights and them sex ratio in 2009.

Date	1700 m				1800 m				1900 m			
	Total	Female	Male	F/M	Total	Female	Male	F/M	Total	Female	Male	F/M
2009/7/20	103	72	31	2.32	110	69	41	1.68	142	83	59	**1.41**
2009/8/1	95	53	42	1.26	99	59	40	2.23	117	77	40	**1.93**
2009/8/14	67	43	24	1.79	72	44	28	1.57	93	52	41	**1.27**
2009/8/25	42	23	19	1.21	52	27	25	1.08	71	45	26	**1.73**
2009/9/6	31	16	15	1.07	55	35	26	1.35	69	41	28	**1.46**
2009/9/12	25	13	12	1.08	36	21	15	1.4	53	31	22	**1.41**
2009/9/29	17	10	7	1.43	24	16	8	2	43	27	16	**1.69**
2009/10/9	13	8	5	1.6	21	12	9	1.33	30	18	12	**1.5**
2009/10/17	13	9	4	2.25	18	10	8	1.25	24	14	10	**1.4**
2009/10/24	15	9	6	1.5	16	10	6	1.67	19	12	7	**1.71**
2009/11/7	16	10	6	1.7	13	8	5	1.6	25	16	9	**1.78**
2009/11/22	33	19	14	1.36	45	27	18	1.5	70	41	29	**1.41**
2009/12/1	57	32	25	1.28	67	38	29	1.31	98	59	39	**1.51**

Table2. Number of seven spot ladybird in three different heights and them sex ratio in 2010.

Date	1700 m				1800 m				1900 m			
	Total	Female	Male	F/M	Total	Female	Male	F/M	Total	Female	Male	F/M
2010/7/18	111	75	36	2.08	121	81	40	2.02	137	85	52	1.63
2010/8/1	101	57	44	1.29	98	55	43	1.28	117	70	47	1.49
2010/8/15	78	47	31	1.51	81	50	31	1.61	96	53	43	1.23
2010/8/27	63	35	28	1.25	68	37	31	1.19	75	47	28	1.67
2010/9/5	49	27	22	1.22	53	31	22	1.41	59	38	21	1.81
2010/9/13	33	17	16	1.06	44	25	19	1.32	47	29	18	1.61
2010/9/28	24	14	10	1.4	33	20	13	1.54	35	19	16	1.88
2010/10/10	25	13	12	1.08	31	18	13	1.38	37	22	15	1.47
2010/10/15	29	17	12	1.42	38	20	18	1.11	45	25	20	1.25
2010/10/26	34	21	13	1.61	41	25	16	1.56	50	29	21	1.38
2010/11/6	44	27	17	1.59	52	35	17	2.05	61	36	25	1.44
2010/11/23	57	34	23	1.48	67	42	25	1.68	79	48	31	1.55
2010/12/2	75	47	28	1.68	93	56	39	1.51	107	65	42	1.55

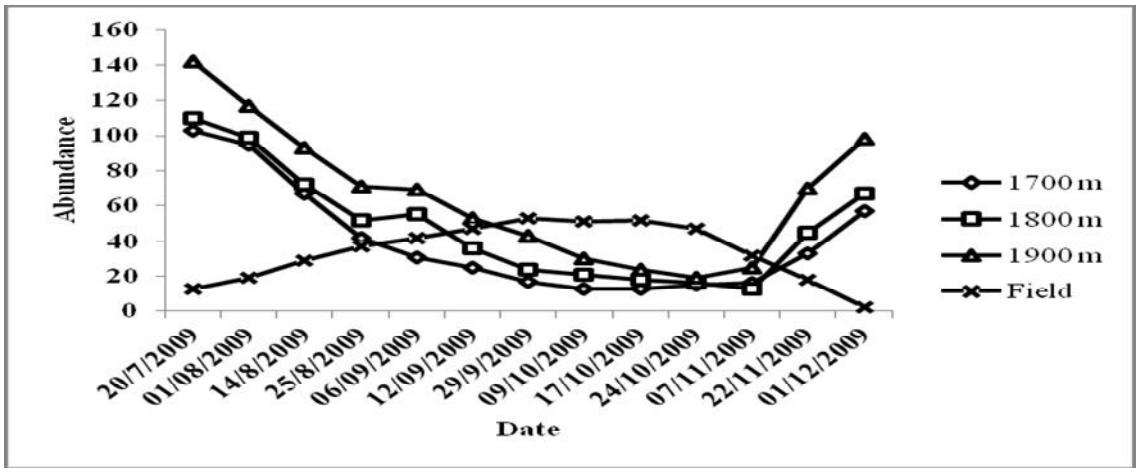

Fig1. Abundance of seven spotted ladybird (*C. septempunctata*) in three altitudes 1700, 1800 & 1900m and alfalfa field in this region from 20/7/2009 to1/12/2009

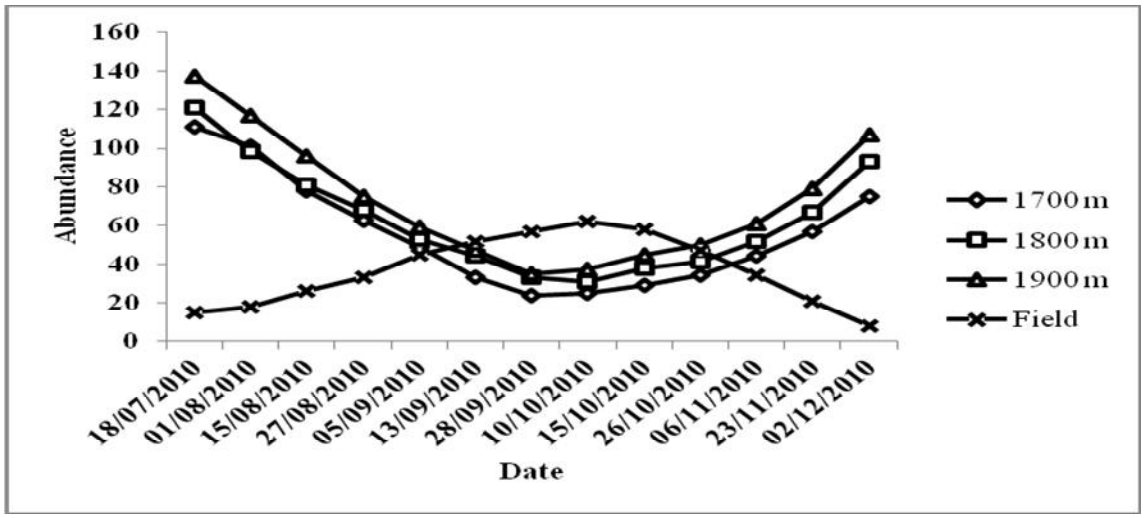

Fig2. Abundance of seven spotted ladybird (*C. septempunctata*) in three altitudes 1700, 1800 & 1900m and alfalfa field in this region from 18/7/2010 to2/12/2010

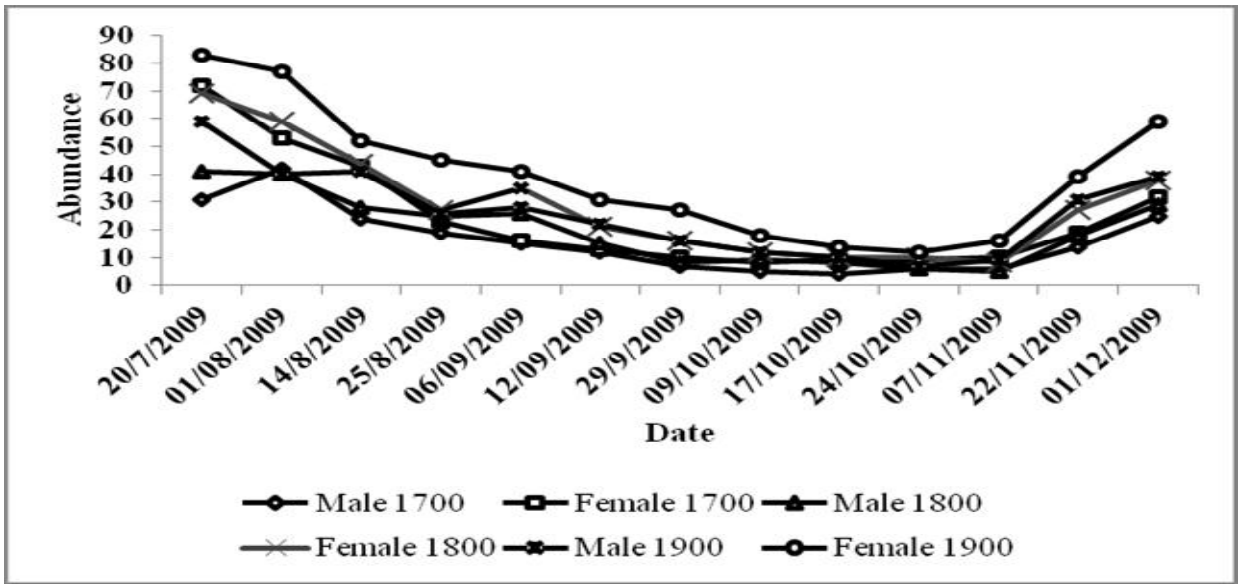

Fig3. Abundance of male and female seven spotted ladybird in three altitudes 1700, 1800 & 1900m and alfalfa field in this region from 20/7/2009 to1/12/2009

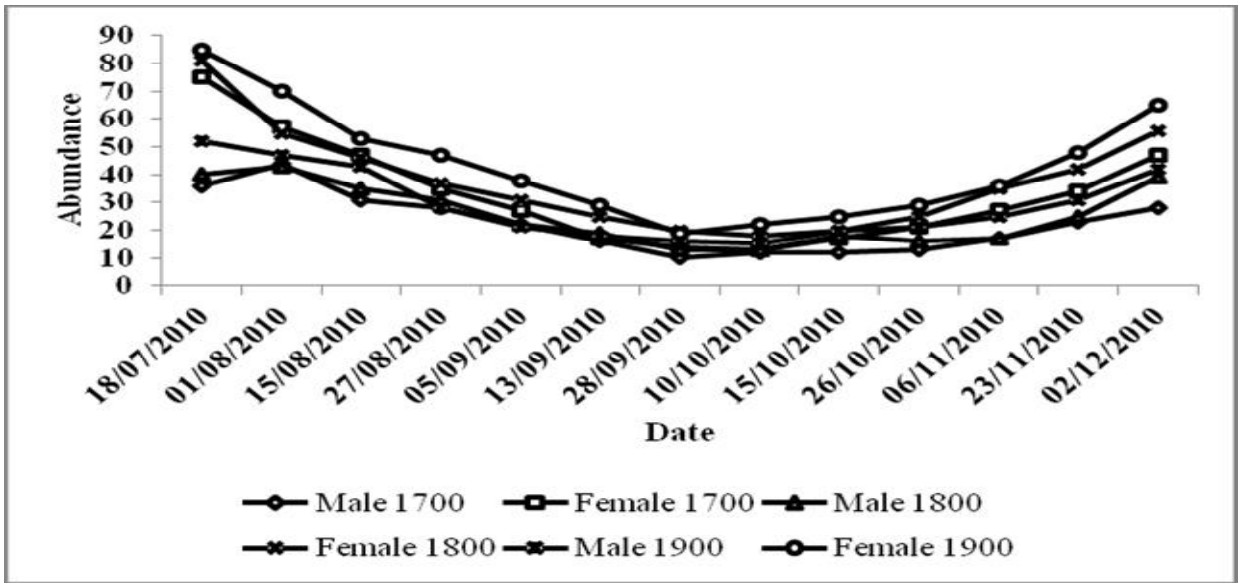

Fig4. Abundance of male and female seven spotted ladybird in three altitudes 1700, 1800 & 1900m and alfalfa field in this region from 18/7/2010 to2/12/2010

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Genetic characterization of *Pseudomonas aeruginosa* isolated from contact lenses and other sources by RAPD analysis

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Abstract:
Pseudomonas aeruginosa (24 isolates) collected from contact lens storage cases, contact lenses and contact lenses wearer in Saudi Arabia. PCR amplification of all isolates revealed one fragment with 230 bp that represented 16S rDNA gene. A total of 48 amplified DNA fragments (from 3500 to 90 bp) were observed using three RAPD primers; B-01, B-11 and B-14.Whereas, 42 fragments were polymorphic and the other 6 amplified fragments were commonly detected among all *Pa* isolates. The three primers showed a mean polymorphism of 87%, whereas, the polymorphic % B-01 primer was higher (89%) followed by primer B-11 and B-14 with 88 and 83%, respectively. The constructed UPGMA dendrogram showed two main clusters; the first included 10 isolates with a medium bootstrap 83%, the second included the remaining 14 isolates with two sub-clusters. The first sub-cluster divided to two branches; the first contained isolates 21 & 23 and the second was divided more to two sub-branches. The second sub-cluster was divided to isolate 2 that occupied unique branch and two other branches that divided again to several sub-branches. Isolate 8 revealed most high similarity with isolate 7(77%) followed by isolates 18 and 17 (74%). Isolates 3 & 1 and 22 & 15 showed similar percentage (72%). Isolates 7 and 3 displayed (70%). Conclusion: high DNA polymorphism occurrence of amplified fragments &Variable genetic similarities of *Pa* isolates (24) was noticed. The fluctuation of genetic similarity percentages of each of the isolates with others revealed the divergent genetic backgrounds of different serotypes.

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***Key words*:** *Pseudomonas aeruginosa* isolates, contact lens and contact lenses wearer, 16S rDNA gene, RAPD-PCR

1.INTRODUCTION:

Sight-threatening microbial keratitis associated with contact lens wear remains a serious concern for patients, eye-care practitioners, and the contact lens industry. Several decades of research and some major advances in lens and solution technology have not resulted in a decline in disease incidence (Fleiszig *et al*., 2010).

Contact lens wearer continues to be a significant risk factor for the development of acute sight-threatening corneal infections; microbial keratitis (Ibrahim *et al.* 2009; Edwards *et al.*, 2009).

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen implicated in sight-threatening occular infectious diseases such as keratitis (Willcox 2007; Green *et al.*, 2008a). For more than 20 years in this field, Pearlman *et al.*, (2008) have worked toward understanding why the corneas of contact lens wearers are more susceptible to infection. However, the widespread use of contact lenses is now recognized as an increasingly common risk factor for development of corneal infection in otherwise healthy eyes (Green *et al.* 2008b).

P. aeruginosa has remained the most common cause of contact lens-related keratitis, accounting for 60–70 % of culture-proven cases (Cheng *et al.*, 1999). *P. aeruginosa* is also one of the most commonly isolated organisms in non-contact lens-related ocular trauma events that lead to keratitis (Parmar *et al.*, 2006). *P. aeruginosa* keratitis is considerably more common in contact lens wearers compared with non-contact lens wearers, presumably because of the altered ocular environment. Biofilms produced by *P. aeruginosa* are thought to be the main cause of persistent ocular infections associated with contact lens wearer (Costerton *et al.*, 1999) through attachment to contact lens and contact lens storage case surfaces (McLaughlin-Borlace *et al.*, 1998). Bacterial contamination of lenses and storage cases has been reported even in association with good compliance with care and hygiene regimens. Biofilm-associated *P. aeruginosa* contamination is found in both contact lens cases and disinfectants with rates varying between 24 and 81% (Zegans *et al.*, 2002).

Phenotypic traits expressed in biofilms are partially responsible for the emerging resistance against antimicrobial therapy (del Pozo_and Patel

2007) of contact lens-related keratitis. In addition, emergence of multi-drug resistance in *P. aeruginosa* strains (Rossolini and Mantengoli 2005) becomes a major concern when antibiotics such as fluoroquinolones are used as monotherapeutic agents. Choy *et al.,* (2008) suggested that *P. aeruginosa* isolates from different infection origins may have different characteristics. Seventeen eyes (63%) lost more than one line of visual acuity with a resultant permanent medical downgrading in duty capability in nine cases. Increased awareness of the health risks of contact lens wear together with standardized treatment regimens based on improved pathogen detection using molecular diagnostics have improved outcomes (Musa *et al.,* 2010).

Typing of bacterial isolates has been used for decades for studying local outbreaks e.g. nosocomial outbreaks, as well as, for national and international surveillance when monitoring newly emerging (resistant) clones, e.g. for pathogens such as *P. aeruginosa* (Inglis *et al.,* 2010). During the last decades, genotyping techniques (DNA fingerprinting) such as RAPD analysis have largely replaced phenotypic techniques, such as serotyping, phage susceptibility typing and protein SDS-PAGE. With the development of faster, cheaper and more automated sequencing capacity, sequence-based typing, such as multilocus sequence typing (MLST)is gradually replacing these DNA fingerprinting techniques. Whereas, MLST is a reference approach for large scale surveillance and for population biology studies, there remains a need for rapid, less laborious and cheap approaches, such as RAPD, on a local scale. Although the interrun reproducibility of this approach is known to be limited, it still offers the possibility of studying within a single run the genotypic relatedness of a limited number of isolates that might possibly belong to a single outbreak e.g. in a hospital or hospital ward (Deschaght *et al.,* 2011). We aimed in this study to identify *Pseudomonas aeruginosa* strains isolated from soft contact lenses belong to healthy persons, patients with contact lens-associated red eyes (CLARE), asymptomatic wearer (CLSCaw) and patients with keratitis by 16S rDNA gene and studying the genetic similarity and variations of the isolates using PCR-RAPD analysis.

2.MATERIALS AND METHODS:

2.1. Materials:

2.1.1.Bacterial strains and growth condition

A collection of 24 *Pseudomonas aeruginosa* clinical isolates were obtained from contact lens storage cases, contact lenses and contact lenses wearer between November 2010 and December 2011 in Saudi Arabia as shown in Table (1). All strains were stored in tryptic soy broth (TSB) containing 30% glycerol at -80°C. Isolates were inoculated on chocolate agar plates and incubated overnight at 37°C.

All the strains were identified by 16S rDNA gene, colony´s morphology, Gram staining, mobility characteristic of polar flagellation, pigment production, fluorescence, and phenotypic analysis with API 20 NE identification kit (bioMérieux, Marcy l'Etoile, France). Cells were stored at 4°C for further experiments.

2.1.2.Primers:

A pair of primer was designed as forward(5′-CGACGATCCGTAACTGGTCT-3′) and reverse(5′-CCGGTGCTTATTCTGTTGGT-3′) with a final product size of 230 bp. RAPD analysis was performed using three 10-mer random primers (Operon Technologies, Alameda, Calif.), Table (2) showed their names and sequences

Table 1. Contact lens-related and non-contact-lens-related P. aeruginosa (Pa)

No. of *Pa* isolates	Sources	The case of the source
7	Soft contact lenses	Used by healthy persons
4	Soft contact lenses	Patients with contact lens-associated red eyes (CLARE)
1	Eyes of contact lenses wearers	Asymptomatic wearer (CLSCaw)
12	Eyes of contact lenses wearers	Patients with keratitis

Table 2. Names and sequences of RAPD primers used for PCR analysis

Name	Sequences
B-01	5′ TGC GCC CTT C ′3
B-11	5′ GTA GAC CCG T ′3
B-14	5′ GTA GAC CCG T ′3

2.2. Methods:

2.2.1.DNA extraction and PCR amplification of 16S rDNA gene

Genomic DNA was prepared from 18 h cultures in an exponential phase in Luria-Bertani medium. DNA extraction was performed according to the method of Ben Haj Khalifa *et al.,* (2010). Aliquots of 10 ml of bacterial culture were harvested by centrifugation at 12,000 rpm for 15 min at 4°C and washed once in sterile distilled water.

The pellets were resuspended in 400 µl of lysis buffer containing 2% glucose, 50 mM Tris-HCl (pH 8.0), 25 mM EDTA, 3 mg/ml lysozyme and 200 mg/ml RNase. The cell suspension was incubated for 1 h at 37°C.

PCR amplification was carried out in a DNA thermocycler (Biometra, Germany) for 30 cycles each. The PCR reaction was carried out in a final volume of 25 µl with 1X PCR buffer containing 10

mM Tris-HCl, 25 mM $MgCl_2$, 1 µl of template DNA, 0.2 mM deoxynucleoside triphosphate, 2 µM (each) primer and 0.5 U of *Taq* DNA polymerase (Promega). PCR conditions of 16S rDNA and RAPD amplification consisted of initial denaturation at 95°C for 2 min followed by 95°C for 1 min, annealing to primers at 54°C for 1 min (16S rDNA) and 50°C for 1 min (RAPD) and extension at 72°C for 1 min with a final extension step at 72°C for 5 min. PCR-amplified products were separated using agarose gel electrophoresis in 1% TBE buffer and stained with 0.2 µg/ml ethidium bromide. Amplified fragments were detected and photographed under UV light. In order to rapidly identify the 24 *Pseudomonas aeruginosa* clinical isolates, the 16S rDNA gene was amplified using accession JN377436 obtained from the NCBI GenBank.

2.2.2.Genetic analysis

RAPD fragments were scored as present (+) or absent. The data was used for similarity-based analysis using the program MVSP (version 3.1b) from www.kovcomp.com. RAPD analyses were analyzed using the Nei genetic similarity index (Nei and Li 1979) based on the equation: Similarity = 2Nab/(Na+ Nb)

3.RESULTS:

Results in (Table 1) illustrated the origin of the isolated twenty four strains; one of them was isolated from patients with endophthalmitis, and 4 from contact lenses belongs to a patient with contact lens-associated red eye (CLARE). Twelve strains from patients with keratitis strains from consecutive patients attending King Khaled Eye Hospital in Riadh, Saudi Arabia over a 12-month period. The remaining five strains were isolated from contact lens cases belonging to asymptomatic wearers (CLSCaw).

PCR amplification of 16S rDNA in *Pseudomonas aeruginosa* clinical isolates

PCR amplification of the 24 *P. aeruginosa* clinical isolates revealed one fragment with 230 bp that represented the 16S rDNA gene (Fig. 1).

Genetic characterization of *P. aeruginosa* (*Pa*) isolates by RAPD analysis

A total of 48 amplified DNA fragments ranging in size from 3500 to 90 bp were observed using the three random amplified polymorphic DNA (RAPD) primers; B-01, B-11 and B-14, whereas 42 fragments were polymorphic and the other 6 amplified fragments were commonly detected among the 24 *Pa* isolates (Table 3).

The three primers showed a meanpolymorphism of 87%, whereas the polymorphic percentage of primer B-01 was higher (89%) followed by primer B-11 and B-14 with 88 and 83%, respectively.

Primer B-01 resulted in 19 fragments, 17 of which were polymorphic with sizes ranging from 2000 to 100 bp; two fragments were common among the 24 isolates (Fig. 2 and Table 3). The total number of amplified fragments of the isolates varied considerably. For instance, in isolates (3 and 7) 14 fragments with different molecular sizes were amplified while 6 fragments were amplified in isolates (13, 14 and 21). The remaining isolates were intermediate. RAPD analysis revealed that the amplified PCR products of most of the 24 isolates vary in molecular size patterns even with the equal total fragments.

Primer B-11 revealed 17 fragments, 15 of which were polymorphic with sizes ranging from 3500 to 90 bp (Fig. 2 and Table 3). The total fragment numbers of the 24 isolates varied significantly in their amplified fragments: whereas isolate 11 revealed the highest with 13 fragments, followed by isolates 9 and 10 with 12, while six isolates revealed two.

Primer B-14 revealed 12 fragments, 10 of which were polymorphic with sizes ranging from 750 to 110 bp and two fragments with 250 and 190 bp were commonly detected among all isolates (Fig. 2 and Table 3).

The total number of fragments of the 24 isolates varied substantially: for example, isolates 3, 6, 9, 11 and 12 showed ten, while isolates 21 and 22 showed four. Although, several isolates revealed equal total fragments they noticeably difference in their molecular sizes. For instant, isolates (3, 6, 9, 11 and 12) with 10 fragments were also quite differences due to appearance and disappearance of some fragments. Similar observation was also detected in isolates (1, 2, 5, 8 and 10) with nine fragments, isolates (4, 7 and 20) with eight, isolates (15 and 17) with seven, isolates (13, 14 and 24) with six and isolates (21 and 22) with four.

Genetic similarity of *P. aeruginosa* isolates using UPGMA dendrogram

Genetic similarity between each two pairs of the 24 *P. aeruginosa* isolates was performed using the Nei similarity index on the basis of RAPD amplified fragments using the three random primers (Table 4).

Genetic similarity between the 24 *P. aeruginosa* isolates was calculated from the amplified fragment data using un-weighted pair group method with averages (UPGMA).

The constructed UPGMA dendrogram of the three primers showed two main clusters, whereas the first includes 10 isolates with a medium bootstrap 83% and the second includes the remaining 14 isolates with two sub-clusters.

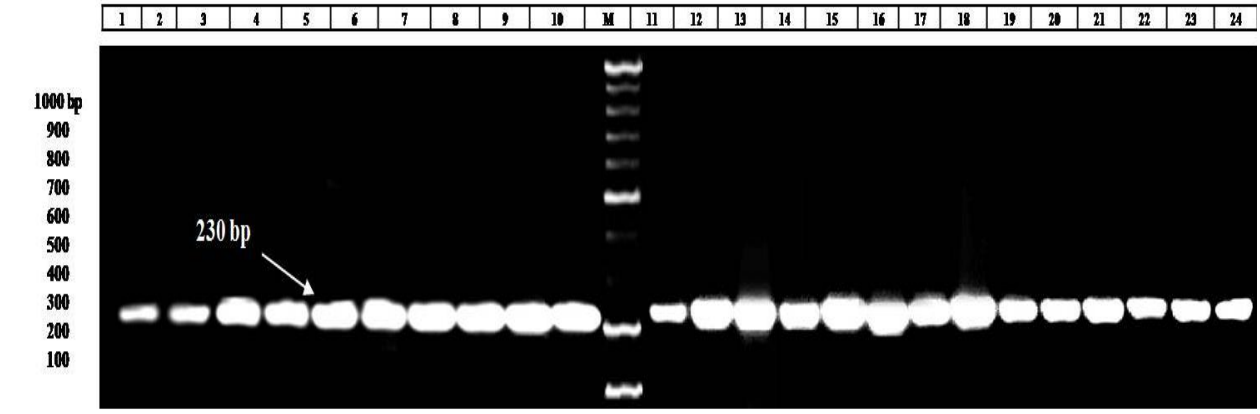

Fig. 1. PCR amplified products of 16S rDNA gene of the 24 *P. aeruginosa* isolates using the designed primers with expected size 230 bp.

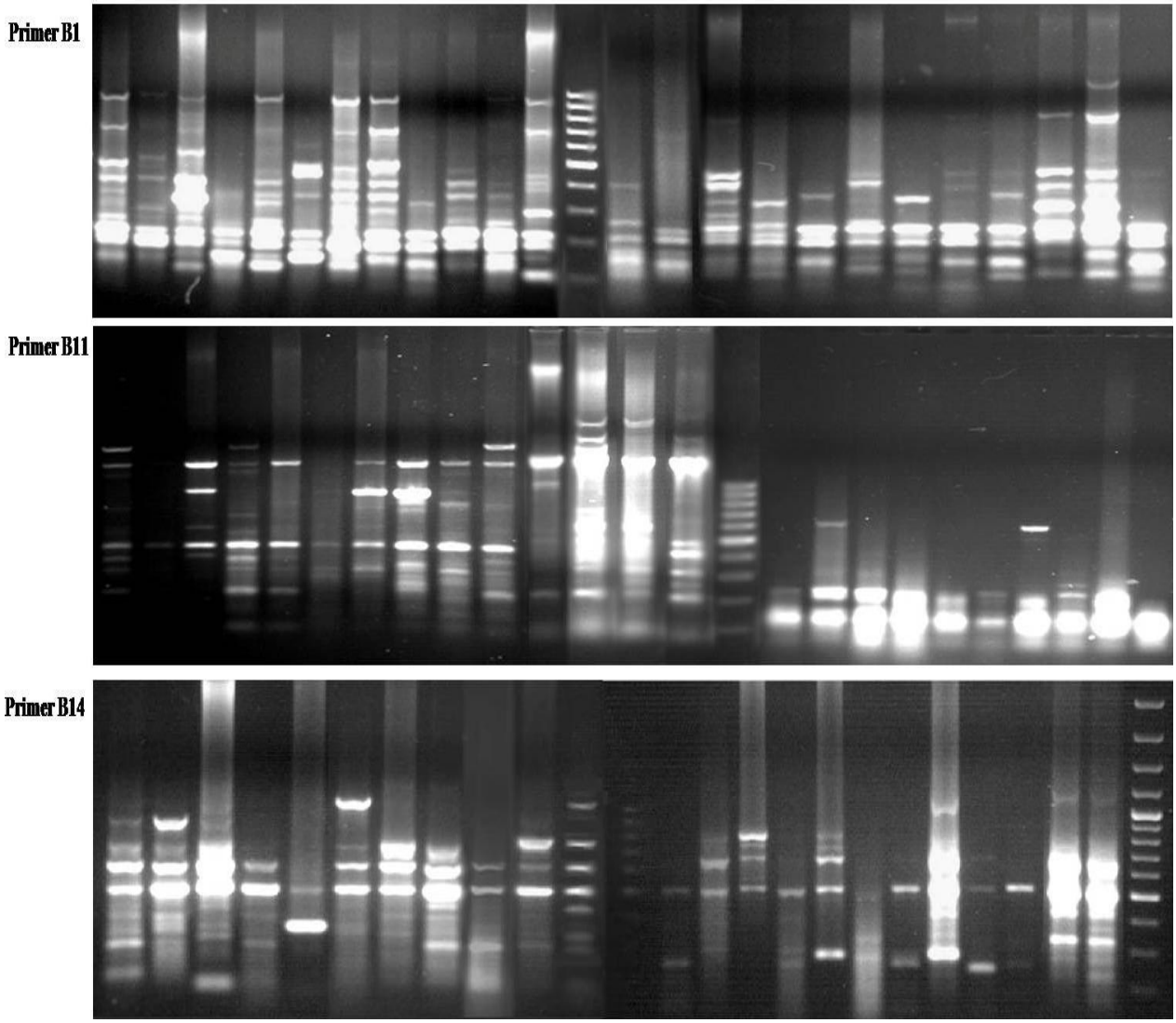

Fig. 2. RAPD amplified products of the 24 *P. aeruginosa* isolates using three random primers; B1, B11 and B1

Table 3. ***RAPD analysis of variable (polymorphic) fragments of the 24 P. aeruginosa isolates using three random primers; B1, B11 and B14***

Primer name	No	Ms (bp)	P%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	1	2000		+	+	+		+		+	+			+	+											+	
	2	1600			+	+				+																	
	3	1500				+																					
	4	1000		+	+	+		+		+	+			+	+			+							+	+	
	5	900				+	+	+	+	+	+			+													
	6	750			+	+			+																		
	7	690		+	+	+			+	+	+		+	+	+		+	+	+	+	+				+	+	+
	8	600	89	+	+	+		+		+	+		+	+	+			+	+			+	+	+	+	+	
B1	9	550		+																	+						
	10	500		+		+	+	+	+	+	+	+	+	+				+	+	+	+		+	+	+	+	+
	11	440			+	+				+	+	+	+	+	+			+	+	+	+	+	+	+	+		
	12	390		+	+	+	+	+		+	+		+	+				+			+	+	+		+	+	
	14	260																+	+	+							+
	16	180		+	+	+	+	+	+	+	+			+	+	+	+	+	+			+	+		+	+	
	17	160						+		+		+		+		+	+	+	+	+	+	+	+				+
	18	130					+			+		+			+	+	+	+	+	+	+	+	+	+	+	+	+
	19	100										+			+	+				+		+	+				+
	T=19			10	11	14	7	10	7	14	11	7	7	12	10	6	6	12	10	9	9	9	10	6	10	10	8
	1	3500		+	+								+	+	+												
	2	3000		+	+	+	+	+	+	+	+	+	+	+	+			+							+		
	3	2000										+		+	+								+				
	4	1500			+				+				+	+		+							+				
	5	1300			+	+				+	+	+															
B11	6	1000								+	+	+	+														
	7	820	88				+	+						+													
	8	750					+	+	+					+	+												
	9	560		+		+	+	+	+	+	+	+	+	+	+			+							+		
	11	450		+		+		+			+	+	+		+												
	12	350					+	+	+	+	+	+	+	+	+			+									
	13	290					+	+	+	+	+	+	+	+	+			+									
	14	270					+		+		+	+	+	+		+											
	15	220			+						+					+			+				+				
	16	150			+		+	+				+	+	+	+	+			+			+					
	T=17			6	8	6	10	10	9	8	11	12	12	13	11	6	2	6	4	2	2	3	5	2	4	2	2
	1	750								+	+		+	+	+												
	2	650				+		+	+			+		+	+								+				
	3	550		+	+	+			+				+		+			+		+							
	4	410	83	+	+	+	+	+	+	+		+	+	+	+		+	+		+			+				
	5	340		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+
B14	7	230		+	+	+	+	+	+		+	+	+	+						+	+	+	+			+	
	8	210		+	+	+	+		+	+	+	+		+	+	+							+				
	10	160		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+	+	+	+	+
	11	130				+		+	+	+	+	+			+	+	+	+	+	+	+	+					+
	12	110		+		+	+	+	+	+	+	+	+	+		+								+		+	+
	T=12			9	9	10	8	9	10	8	9	10	9	10	10	6	6	7	5	7	5	5	8	4	4	5	6
Total=48			*87	25	28	30	25	29	26	30	31	29	28	35	31	18	14	25	19	18	16	17	23	12	18	17	16

Table 4. ***Genetic similarity percentages of the 24 isolates based on RAPD products of three random primers.***

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
2	61																						
3	**72**	66																					
4	52	40	49																				
5	59	43	64	69																			
6	50	42	60	65	57																		
7	56	51	**70**	56	62	50																	
8	60	51	**70**	56	62	54	**77**																
9	43	37	53	61	54	54	55	60															
10	61	51	53	56	54	54	55	64	60														
11	60	55	60	69	**70**	62	63	67	55	68													
12	56	51	56	47	58	50	59	55	60	55	67												
13	33	38	**32**	47	37	41	39	39	47	34	36	39											
14	39	36	38	39	39	38	45	**32**	36	**31**	**30**	41	57										
15	56	47	57	52	59	50	**70**	56	47	56	51	60	38	56									
16	38	42	40	38	41	**32**	47	43	38	38	36	43	58	65	63								
17	36	**32**	38	**31**	**31**	39	37	33	41	36	**28**	37	44	55	56	64							
18	41	33	40	37	36	36	42	38	38	38	**32**	**31**	40	58	52	59	**74**						
19	36	41	38	40	44	30	41	37	41	36	34	41	57	55	50	64	62	65					
20	42	47	44	42	42	37	43	43	47	39	47	43	58	44	47	58	50	52	63				
21	37	**29**	36	37	**32**	**27**	39	34	38	38	**31**	**30**	41	44	42	55	45	47	45	48			
22	59	48	55	48	47	38	58	53	39	48	49	49	37	52	**72**	61	46	55	52	54	58		
23	62	45	52	45	48	34	50	50	**32**	41	46	37	39	48	50	50	42	50	48	50	61	67	
24	38	**27**	36	38	38	37	44	35	43	34	**29**	35	62	**71**	48	62	68	63	52	48	59	50	52

+= present of fragment, T=total fragments, P%= polymorphism %, *Average polymorphism %, Ms= DNA ladder with 100 bp

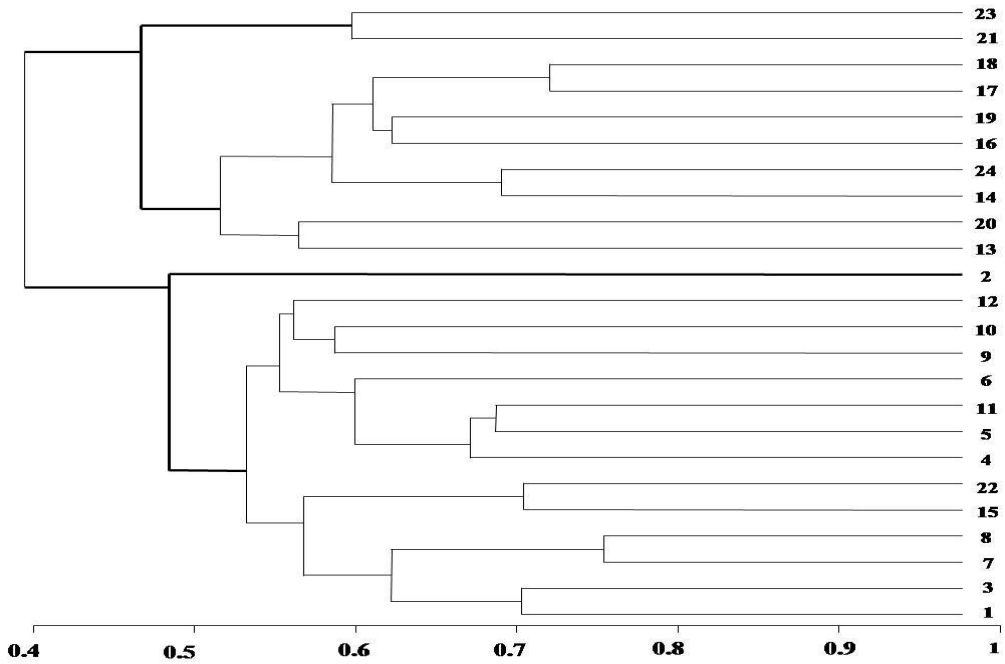

Fig 3. ***Dendrogram represented the genetic relationships among the 24 isolates using UPGMA cluster analysis of Nei's genetic similarity coefficients generated from three RAPD primers.***

Some pairs displayed 70% such as isolates 7 and 3, isolates 8 and 3, isolates 11 and 5, isolates 15 and 7.Consequently, the 24 *P. aeruginosa* clinical isolates revealed high DNA polymorphism using the three RAPD primers; either in the occurrence of amplified fragments or in the variable genetic

similarities of each isolate with the others. Despite the fact that they should display the most narrow and low variation due to the genomic structure of the same 24 *P. aeruginosa* isolates and the structure of the 10 mer-RAPD primers. Eventually, the fluctuation of genetic similarity values each of the 24 isolates with others using the three primers evidently revealed the divergent genetic backgrounds of such isolates with their high DNA polymorphism patterns.

4. DISCUSSION

The polymer type used to construct the contact lens may influence subsequent bacterial adhesion events. Contact lenses made from nonionic polymers with high water content may carry higher risks of bacterial contamination (Yen *et al*., 2003).

RAPD analysis recently emerged as a reliable means of typing several bacterial species, including *P. aeruginosa* (Kersulyte *et al.,* (1995); Mahenthiralingam *et al.,* (1996) and Mereghetti *et al.*, (1998). Moreover, Giordano *et al.,* (2001) characterized *P. aeruginosa* strains by three phenotyping methods: biotyping, antibiotyping and serotyping. Its aims were to evaluate their typing capacity in relation to various isolate profiles. The use of RAPD-PCR makes it possible to identify non-serotyped strains, and shows the necessity of this molecular typing technique for typing *P. aeruginosa* strains from patients with CF.

The obtained results were agreed with Hafiane and Ravaoarinoro (2011) who developed RAPD analysis for the routine typing of *P. aeruginosa* strains isolated from the sputum of cystic fibrosis patients (CFP) that are frequently difficult to type by conventional typing methods. They also showed that RAPD typing characterized 30 distinct genotypes and two small clusters of strains were observed among isolates with each primer. Strains belonging to one cluster were present in two (6%) of the 35 strains. Strains belonging to the other cluster were present in three (8%) of the 35 strains. The occurrence of these clusters indicates that cross-infection may occur. Their results indicated that only the RAPD method can establish a clonal relation whereas the other methods may only reflect phenotypical differences, and thus are inadequate to type these strains.

RAPD typing of bacteria is important for monitoring newly emerging pathogens and for examining local outbreaks. Accordingly, Deschaght *et al.,* (2011) evaluated the RAPD technique in combination with melting curve analysis (McRAPD) of the amplified DNA fragments to genotype isolates from five Gram-negative species, i.e. *Achromobacter xylosoxidans*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. RAPD typing of the 88 *P. aeruginosa* blood isolates gave 54 different RAPD fingerprints, 46 of which contained only one strain. The dominant RAPD type amongst the remaining 8 fingerprints contained 14 strains. There was little correlation between the presumed focus of the bacteremia and RAPD grouping although 5 of the 14 strains in the dominant RAPD type were thought to have a respiratory focus. The 75 CF isolates were likewise separated into 41 different RAPD types with 29 containing a single strain and the most dominant containing 12 (Wareham and Curtis, 2007).

The epidemiology of *P. aeruginosa* infections and colonization was studied prospectively on a 12-bed medical intensive care unit. *P. aeruginosa* strains isolated from patients and water samples were analyzed by RAPD-PCR typing. During the 6-month period, 60 of 143 (42%) water samples contained *P. aeruginosa* at various levels ranging from 1 to >100 colony-forming units per 100 ml sample. Genotypically, water samples contained 8 different colonotypes. Nine patients had infections due to *P. aeruginosa* and 7 patients were colonized. Isolates from patients showed a similar distribution of genotypes as did tap water isolates and strains of identical genotype as patient strains had been isolated previously from tap water outlets in 8 out of 16 (50%) infection or colonization episodes (Trautmann *et al.,* 2006).

The study of Campbell *et al.,* (2000) employed 600 samples to evaluate RAPD typing and found that it had a high degree of reproducibility with 98.5% yielding the same banding patterns for each triplicate. Epidemiologically related isolates, such as multiple isolates from individual patients and those from each of five sibling pairs, were appropriately "read" as identical by RAPD. Isolates that were epidemiologically unrelated, such as those from the environmental and the IATS type strains, each had a unique RAPD pattern. This method was highly reproducible and was able to differentiate apparently unrelated strains. RAPD typing is a robust, simple, and highly reproducible method that should be useful in clarifying the epidemiology of *P. aeruginosa* in patients with CF.

RAPD typing is best suited to situations in which a large number of isolates are to be evaluated. Results from RAPD analysis can be used in conjunction with the more cumbersome PFGE method to resolve and confirm the difference or identity among smaller numbers of isolates when an ambiguous result is obtained with RAPD.

Genetic fingerprinting of 30 *Pseudomonas aeruginosa* (*Pa*) isolates from three types of nosocomial infection cases from two Osun State Teaching Hospitals was compared using RAPD-PCR markers (Akanji *et al.,* 2011). Ten out of 50 operon primers tested showed polymorphism with reproducible results among the isolates and produced 131 bands of which 74 were polymorphic with sizes ranging between 200 and 3000 bp. Cluster analysis using the 74 polymorphic markers

classified the 30 *P. aeruginosa* isolates into two (*PgA* and *PgB*) genetic groups. Ben Haj Khalifa *et al.*, (2010) characterized 96 clinical isolates of *P. aeruginosa* recovered in a Tunisian teaching hospital during a 16-month period using RAPD analysis. Genotyping showed 83 RAPD types and the isolates showing the same serotype could show different genotypes.

Conclusion and perspectives

The identification and discrimination of 24 individual *Pseudomonas aeruginosa* isolated from contact lens wearers, non-contact lens wearer revealed that they are novel strains with different serovars and each isolate could be characterized by unique RAPD pattern.

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Physiological Studies on the *Aedes eagypti* larvae Culicidae, Diptera

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Abstract: The total and dry body weight treated larvae of *Aedes aegypti* was significantly decreased at different time intervals under investigation (6, 12, 24, and 48 hrs) post-treatment with *LC30* of *Bt* subsp. *Kurstaki* HD-1. The body water content was decreased significantly at 6 hrs post-treatment, while it was highly significant at the three other treatments. Also hemolymph volume was significantly decreased. A significant increase in hemolymph density was observed only at 12 hrs post treatment. The main of the total hemocyte count in the treated larvae at all inspected time was highly significant increased, also there was a marked variation in the hemocytes percentage of untreated and treated larvae of *Aedes eagypti* at time intervals.
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Key word: hemolymph, hemocytes, *Aedes aegypti,*hemolymph density,blood count,

1. Introduction

Aedes eagypti is one of the most serious and abundant medical insect pest in many countries in the world. *Aedes eagypti* Microbial pest control agents are considered as one of the new methods for the control of such insect pest, which have low impact on the peoples associated with the pest in environment. The production and application of Bt has been developed quickly . *Bt* toxins contain crystal proteins. Researchers have been working to develop environmentally responsible method for pest control based on microbiological control strategies. *Bacillus-thuringiensis (B.t)* is a gram-positive bacterium that can produce parasporal crystal protein during sporulation. These crystal proteins often show insecticidal activity against particular insect species and thus, *B.t.* is widely used in microbiological-based insect control. To date, the Cry genes used in transgenic plants have primarily been CryIA-type, CryZA and CryIF**; Schmidt *et al.,* 2009; Tabashnik *et al* .,2009; Grahan *et al*.,2010**). The increasing potential for resistance to Cry toxins to develop in insect pests has been demonstrated in previous laboratory research (**Tabashnik *et al.,* 1994; *et al.,*; Wu *et al.,* 2009**) and tolerance to the Cry toxins by insect pests has been observed in the field (**Li *et al*.,2004; Li *et al*., 2007**).To delay further insect resistance, it is important to discover and evaluate new Cry proteins with different mechanisms of action from those currently applied to pest control.Cry1Aa, CryIAbl, CryAc, Cry2A and Cry 2B. These *Bt* toxins are effective against dipterous pests. A generally accepted mode of action for Cry toxins describes the sequential steps of protoxin, activation, specific binding and cell toxicity). Both the required activation and more importantly binding steps confer remarkable pest specificity to Cry proteins (**Piott and Ellar 2007**). Ingested insecticidal crystal proteins are activated to a toxic form by proteinases from the digestive insect gut fluids. This paper describes the toxicity of *Bt* subsp. *Kurstaki* HD-1.against a serious medical disease vector. Thus the present investigation is preformed to encourage the using of *Bt* subsp. *Kurstaki* HD-1 as alternative method to chemical insecticide. This work is planned to investigate the following ; the effect of LC_{30} of the tested bacterium on some physiological parameters such as body water content, total and dry body weight as well as hemolymph volume, hemolymph density and also to determine its effect on the total and differential hemocyte counts of 2nd instar larvae of *Aedes eagypti*.

2. Materials and Methods

Test insects:-

Sources of colony:-

Adults susceptible strain of *A.aegypti* used in present study were obtained from a well established colony originated from Biology Department Faculty of Science for girls, King Abdul Aziz University.

Rearing Technique:-

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27 ± 2°C and 60 ±5% R.H). Each egg mass was placed in a clean Petridish (10cm diameter), previously washed with 10% formalin solution to avoid any contamination according to a constant technique .

2. Source of the bacterial pathogen.

Bt strain YBT – 226 was identified in *Aedesa egypti* screen and is the property of E.I Dupont de Nemours, *Bt* subsp. *Kurstaki* HD-1 was obtained from H.D. Burges, Institute for Horticultural Research , Little Hampton , UK . The conditions for

growth and sporulation on CCY medium were as described for *B. megaterium* KM . Purification of protein inclusions were purified from spore /crystal mixtures by centrifugation through discontinuous sucrose gradients (Thomas **and Ellar, 1983**). Protein yield was determined by the method of **Lowry *et al.* (1951)**.

3. Experimental larvae:

All the following investigated tests were accomplished on newly moulted 2nd instar larvae of *Aedesa egypti* treated with sublethal concentration (LC_{30}) of the bio-insecticide *Bt* subsp. *Kurstaki* HD-1 the tested larvae were obtained from the stock colony maintained in the laboratory of Biology Department-Faculty of Science for Girls, King Abdul Aziz University. Just after moulting and starved for about 12hrs then allowed to feed on treated larval media with LC_{30} of the bacterial pathogen. All the experiment were carried out under lab conditions (25 ± 2°C and 60 ± 5% R.H).

4. Physiological studies

4.1 Determination of total body weight and body water contents of the 2nd instar larvae.

Body water content was determined by the method of (Shapiro (1967). Measurements were adopted on ten larvae per each time interval (6, 12, 24, and 48hrs) after treatment. The body water content was calculated as the difference between fresh (total) body weight and body weight after drying for one day at 80°C in an oven.

4.2 Estimation of some physical parameters of larval hemolymph.

a. Hemolymph density

The density of hemolymph was determined for normal and treated larvae after the different time intervals, it was expressed as mg/ µ l. Ten measurements were used for each time interval.

b. Hemolymph volumes

The blood density was determined as described above. The blood weight was determined as the difference between filter paper weighted before and after 10 larvae was squeezed on this filter paper. The following equation was adopted to evaluate the blood volume:

The blood volume is expresses as µl/ Larva.

$$\text{Blood volume} = \frac{\text{Blood weight}}{\text{Blood denisty}}$$

4.3 Cellular immune response

4.3.1 Differential hemolymph counts (DHC_S)

Differential hemolymph counts were determined according to the technique of **Lim and Li (1981)**. The smears were examined under oil immersion (× 1000) and 100 cells from random fields were differentiated on each slide to determine the percentage of each type. Cell – shape, diameter, nuclear, cytoplasmic ratio and cytoplasmic inclusions were used for classification of the hemocytes using the classification a scheme of Li et al., (2007) types calculated by the formula:

$$\% = \frac{\text{Number of each hemocyte type}}{\text{Total number of hemocytes counted}} \times 100$$

$$\text{Number of WBCs\mm3} = \frac{x}{64} \times \frac{1}{\text{Vol. of each small square}} \times \frac{1}{\text{Dillution}}$$

$$\text{volume of each small square} = \frac{1}{4} \times \frac{1}{4} \times \frac{1}{10} = \frac{1}{160}$$

$$= \frac{x}{64} \times 160 \times 20 \text{WBCs\mm3}$$

determine 30% and 50% mortalities and slope value of the tested material.

3. Results

1. Effect of *Bt* subsp. *Kurstaki* HD-1. at LC_{30} on the total body, dry body weights and on the body water content of 2nd larval instar.

The total body weight of the treated larvae was significantly decreased at different time intervals under investigation (6, 12, 24, and 48 hrs) post-treatment. The same trends were also observed in the case of measuring the dry body weight, (Table 1). It is clear from presented results that the body water content was decreased significantly ($P< 0.05$) at 6 hrs post treatment, were as highly significant decrease was recorded at the other three time intervals post-treatment. The decrease in the total body weight (fresh weight) after larval treatment with microbial agent appeared to be mainly due to the decrease in the dry body weight and secondary due to the decrease in the body water content.

Table (1): Body water content of 2nd instar larvae of A.aegypti determined at different time intervals post-Treatment with LC_{30} of *Bt* subsp. *Kurstaki* HD-1Corresponding author

Hours post treatment	Body water content (mg)	
	Control mean± S.E.	Treated mean± S.E.
6	7.11 ± 0.241	6.07 ± 0.217 *
12	12.88 ± 0.555	9.32 ± 0.374**
24	18.68 ± 0.853	4.59 ± 0.401**
48	29.02 ± 1.431	9.17 ± 0.651**

n 10 larvae per test .
* Significant ($P< 0.05$) .
** Highly significant ($P< 0.01$) .

2. Effect of microbial agent (*Bt* subsp. *Kurstaki* HD-1) at LC_{30} on the hemolymph volume and density of the treated larvae:

Results in table (2) indicate that the hemolymph volume was significantly decreased at 12, 24 and 48 hrs post – treatment. These values were 2.23 ± 0.41, 1.56 ± 1.94 and 4.68 ± 0.245 µL as compared to 2.71± 0.111, 7.38 ± 0.331 and 13.44 ± 0.197 µL, respectively. A significant increase in the hemolymph density was observed only at 12 hrs post treatment. On the other hand, there is no significant differences in the values of hemolymph density in the untreated larvae as well as in treated larvae at 6, 24, 48 hrs post- treatments (Table 3).

3. Effect of *Bt* subsp. *Kurstaki* HD-1 at concentration of LC_{30} on the total hemocyte counts (THC_s) and Differential hemocytes (DHC_s) of 2nd larval instar of *A. aegypti*.

a. Total hemocyte (THC_s)

The blood cells, or hemocytes of insects form part of defense mechanism against insecticides, bacteria and other foreign bodies, they are mesodermal in origin and analogous to the leucocytes of the vertebrates. In the present study, the blood cells of the 2nd instar larvae of ***A. aegypti*** were classified into five types:- prohemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids . The mean THC_s at different time of treated and untreated 2nd instar larvae intervals was obtained in Table (4), it is clear from the present results that the mean of THC_s (cells / mm^3) in the treated larvae at all inspected times (6, 12, 24, and 48 hrs) was highly significant increased for example, the mean of THC_5 during the period was 33690 ± 395, 38000 ± 495, 24010 ± 403 and 20210 ± 409 (cells / mm^3) respectively. The corresponding figures of the untreated larvae during these periods were 25685 ± 245, 26335 ±211, 16040 ± 284 and 18855 ± 230 cells/ mm^3 respectively.

b. Differential hemocytes (DHC_s)

Results in Table (5) indicated that the differentiated hemocytes were greatly affected as result of post–treatment with B.t. When the 2nd instar larvae treated with tested material at $LC_{30,}$ the prohemocytes became smaller than intreated larvae. The injury causes other effects on the cytoplasm, break down of the cell wall; clumped with each other and extruding of their cytoplasm were observed.

Some morphological changes were observed in those of treated larvae. There are numerous shapes such as podocytes, amaobocytes and spindle shape. Some forms of plasmatocytes containing no distinguishing inclusion bodies in their cytoplasm which separated from the nucleus and contained large vacuoles**.**

Table (2): Hemolymph volume (µL/ larva) of 2nd instar of *A. Aegypti* determined at different time intervals post-treatment with LC_{30} of *B.t*

Hours post - treatment	molymph volume (µL /larva)	
	Control mean± S.E.	Treated mean± S.E.
6	2.14 ± 0.159	1,79 ± 0.145
12	2.71 ± 0.111	2.32 ± 0.141*
24	7.38 ± 0.331	1.56 ± 0.194 **
48	13.44 ± 0.197	4.68 ± 0.245 **

n 3 replicates per test .
* Significant ($P< 0.05$)
** Highly significant ($P< 0.01$) .

Table (3): Hemolymph density (mg / µL) of 2nd instar of *A. aegypti* determined at different time intervals post- treatment with LC_{30} of *B.t*

Hours post - treatment	Hemolymph density (mg/µL)	
	Control mean± S.E.	Treated mean± S.E.
6	0.86 ± 0.01	0.89 ± 0.008
12	0.87 ± 0.007	0.89 ± 0.014*
24	0.88 ± 0.007	0.88 ± 0.090
48	0.88 ± 0.005	0.88 ± 0.007

n 3 replicates per test .
* Significant ($P< 0.05$) .

Table (4): Total hemocyte counts (THC_s) (cells/ mm^3) of 2nd instar of *A. aegypti* determined at different time Intervals post -treatment with LC_{30} of *B.t*.

Hours post - treatment	Hemocyte counts (THC_s) (cells/ mm^3)	
	Control mean± S.E.	Treated mean± S.E.
6	25685 ± 245	33690 ± 395 **
12	26335 ± 211	38000 ± 495 **
24	16040 ± 284	24010 ± 403 **
48	18855 ± 230	20210 ± 409 **

n 10 replicates per test .
* Significant ($P< 0.05$)

Table(5): Differential hemocyte counts (DHCs) of 2nd instar of *A. aegypti* determined at different time intervals Post-treatment with LC_{30} of *B.t.*

ours post treatment	Percentage of hemocyte type (Mean ± SE)									
	Prohemocytes		Plasmatocytes		Granulocytes		Spherulocytes		Oenocytoids	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
6	10.50 ±0.05	7.70 ±0.21**	41.40±0.33	48.70±0.43**	24.70±0.35	18.30±0.20**	19.30±0.23	23.40±0.32**	4.10 ±0.13	1.90±0.11**
12	4.90 ±0.14	2.20±0.09**	42.40±0.16	49.80±0.45**	29.70±0.35	18.50±0.42**	20.20±0.31	27.20±0.62**	2.80±0.09	2.30±0.12*
24	1.60±0.08	3.64±0.15**	55.20±0.12	56.46±0.29**	24.50±0.29	20.60±0.27**	15.80±0.32	16.50±0.43	2.90 ±0.18	2.80±0.15
48	1.01±0.03	1.43±0.09**	52.89±0.31	58.70±0.65**	28.60±0.29	19.10±0.49**	15.40±0.24	18.70±0.57**	2.10±0.07	2.07±0.20

* Significant ($P< 0.05$) . ** Highly significant ($P< 0.01$) .

The effect of tested material on spherulocytes cells was small, they are often have an irregular shape the membrane is broken the nucleus moved to become laterally located.While, the injuries on granulocytes as a side effect of treatment were degeneration in cell wall with vacuolized cytoplasm, some cells were divided into two cells and other are aggregated together. Extrusion of nucleus from the cytoplasm is also observed in infected cells.Cytopathological changes were clearly observed on oenocytoids, indicated that these cells become irregular in shape with extruded cytoplasm and divided nucleus. Few cells appeared small with two nuclei and vacuoles in their cytoplasm.

4. Discussion

The larvicidal activity of *Bt* is due to toxins found in parasporal inclusions that are produced at time of sporulation (delta- exotoxins), they comprise a diverse group of proteinaceous toxins comparison of the amino acid sequences of the individual toxing resulted in the development of a new nomenclature and enabling correlations to be made between the sequence, larvicidal activity and evolutionary origin of toxin (**Crickmore, 2000**). Toxins with highest larvicidal activity (Cry4A, Cry4B, CryllA and Cyt 1A) are found in *Bti*. In addition to the 4 toxins listed above (**Perez, *et al.*, 2005**) also include CryloAa and Cyt2Ba. However, (Paul**, *et al.*, 2005**) recently reported high levels of resistance to *Bti* in isolated field population. So, the mechanism responsible for resistance Lead to develop modified methods for protein purification (**Tabashnik *et al.*, 2009**).

The total body weight, dry body weight and body water content of the treated larvae were highly significant decrease at different time intervals as compared to control (untreated larvae), and this decrease appeared mainly due to the decrease in the dry body mass, as reflected from the increase in water content percent, wet body weight and also due to the decrease in the body water content. This enables us to say feeding of *A.aegypti* larvae on diet treated with LC_{30} *B.t.i.* decreased the larval dry body weight. results' who found that in the Indian meal moth *lodia interpunctella* larvae, the B.T. induced gradual decrease in the fresh, dry body weights and body water content at three time intervals (6, 12, 24, and 48hrs). **Hegazi *et al.*(1998)** came to the same conclusion on the greater wax moth, *Galleria mellonella* larvae after injection with *Bacillus circus.*

The hemolymph volume was significantly decreased at 12, 24, and 48 hrs post treatment. The estimated significant decrease of the blood volume in the larvae may be attributed to water lose from blood and tissues as a result of bacterial infection. The present results are in accordance with that demonstrated by **Hegazi *et al.*, 1999; Crickmore, 2000 and Guo *et al.*, 2011.** The hemolymph density of untreated larvae showed non-significant difference in this parameter was observed at 12hrs post treatment, and this increase may be due to the increase in the total hemocyte counts and the increase of blood volume as well as the increase of bacterial metabolites. These results are in conformity with Guo[l] **e*t al.*, 2011** on *Plodia interpunctella* larvae with staphylococcus (**Hegazi *et al.*, 1998**) on *Galleria mellonella* larvae injected with *B. circus* (**Schmidt *et al.*, 2009, Saengwiman *et al.*, 20011 and Ritchie *et al.*, 2011**).

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Calculate & Analyze of Growth in Vicia FabaL. Plant

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Abstract— Tested using a modified split plot design in randomized complete block with the main treatments bean varieties and sub-levels of nitrogen fertilizer treatments were performed. The results show that the variance in total dry matter accumulation of bean varieties Treatment plant and the different levels of nitrogen fertilizer has been so significant at 1% of the total plant dry matter accumulation increased with increasing nitrogen. This is due to increase in line with the increased photosynthesis and leaf area index and dry matter accumulation in plants. Sigmoid curve diagram of a bean leaf area index figures showed that growth in primary school, which had up to 50 days after the Kennedy Planting of the LAI value is only 0.7 is taken to slow the spread of leaf area index for the legume family, the high levels of nitrogen fertilizer (80 kg/ ha) the first period to reduce the level of leaves, and secondly in the treatment of lower levels of nitrogen treatments were applied to the leaf area index was higher. Chart for RGR at different levels of nitrogen reduction process and that their maximum RGR in the early stages of development with 0.15 g per day is reached. NAR average value equal to 6.7 grams per square meter per day is 48 days after planting in the NAR to the 7.4 grams per square meter per day increased and then showed its decreasing trend.
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Keywords: Growth analysis, horse bean, fertilizer

1. INTRODUCTION

Analysis of plant growth parameters of bean

There are two types of plant growth analysis, attitude, attitude, classical and functional approach to separating these two approaches, for use in analyzing the growth took place in 1960.This title was first used by Keaston other words, the scientists used a functional approach to the dynamics with respect to the terms of the return that contains a view of the fitted curve and the other not. In the classical view, the events of the relatively small number of samples but with a large volume (high number of measurements) will follow the basic concepts in the books, Evans and Keaston and Venus, in view of the statistical function to fit curves of various samples, but low volume (less the number of measurements) will result in a lack of time and space, both views can be merged (the number of large samples and large size), but both of Perspective directions of growth parameters and determine the impact of the paths are different treatments(14, 11).

In this experiment, the growth paths of these parameters measured with the LAI, TDW and LDW-fit curve of growth factors is a function of attitude to the effects of treatments in different periods and different levels of nitrogen fertilization on components *RGR, CGR, NAR, LAR, SLA, LWR* will be analyzed by the graph.

2. MATERIAL AND METHOD

Research projects at the research farm, University of Ahwaz, with using a modified split plot design in randomized complete block with beans and treatment of minor figures, the main treatment was performed different levels of nitrogen fertilizer The main treatment plant, four varieties of beans (V) and secondary treatment, nitrogen levels (N) kg at Hectares (the source of urea) were used for analysis of shoot growth of plants harvested at the top (at the same level of 0.6 m) to the plant within 12 days and average five parameters and dry matter accumulation LAI and shoot dry matter accumulation was measured and growth components of LAR, CGR, RGR, NAR, LWR and SLA were calculated.

On all results, analysis of variance was performed followed by Duncan's test, the results were compared to the tables is presented charts with Harvard graph, Excel 2000 were analyzed with a computer program for agricultural growth SAS estimates were calculated.

3. RESULT

Relative growth rate (RGR)

Relative growth rate, the rate of overall growth rate of more sophisticated than a simple change of a variable rate of weight gain is when the plant is

based, in other words, the RGR in terms of growth rate in times of increasing size of the compared with the overall growth rate and the possibility of doing more to provide a fair, Blackman called the performance index. Contrasting the relative speed of decline in RGR in practical terms, the mean relative growth rate of the measurements performed at RGR is calculated and the trend is a declining function of the tissue due to loss of tissue dividing the amount of time.

Figure 1 with the following points of reflection can be investigated:

A. Chart for RGR at different levels of nitrogen reduction process and that their maximum RGR in the early stages of development with 0.15 g per day is reached.

B. The comparison shows that the levels of nitrogen treatments = 100 Kg N ha higher RGR values of the two treatments and is achieved. The steep decline in RGR Less steep decline in RGR and is. Treatments showed a similar decline over time.

C. The first treatment that can be seen in Figure 2 RGR, but the maximum time (60 days after planting) treatments The reason is probably the time of injection of nitrogen in treatments Of treatment Which is exacerbated by the growth of meristem tissue and thus decrease the slope of RGR has been less.

Net assimilation rate (NAR)

Assimilate net assimilation rate or the net amount represents net photosynthesis per unit leaf area is at the beginning of the growth of all leaves receive light and shadow is running low in the low NAR in the highest amount of breathing their But with the growth of leaves and ghosting and lack of light penetration into the plant goes up first, and secondly the breath of net photosynthesis in leaves under the shadow of the NAR decreased after the increase will begin to decline, NAR, this trend is visible in Figure 3 that the first 36 days after implantation, the NAR has been sampled on average, equal to 6.7 grams per square meter per day is 48 days after planting the NAR to the 7.4 grams per square meter per day increased and then showed its decreasing trend.

Injection of N = 100 Ha at the planting treatments NAR and the amount of leaf area expansion of the two treatments and But with time and the injection of 1/2 in the other fertilizer treatments 84 days after planting in steep decline in NAR and treatment of this treatment is less (Injection of fertilizer after flowering), but 108 days after planting, slope treatment Is less likely because of increased leaf growth, which leaves it up to shadow each other as well as differences in biological nitrogen fixation has caused this difference is not much different treatments.

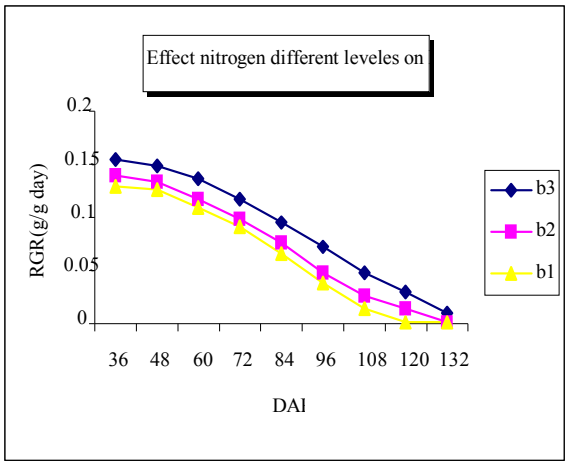

Fig. 1 Effect fertilizer on RGR

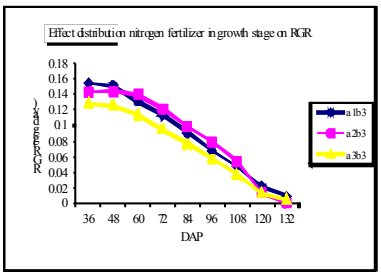

Fig. 2 Effect interaction fertilizer × varieties on RGR

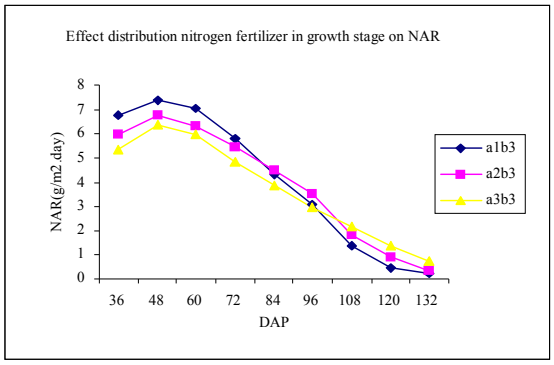

Fig. 3 Effect interaction fertilizer × varieties on NAR

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Solitary Wave Sol's for the Generalized Fifth Order KdV eqn

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Abstract: In this letter, different kinds of solutions including breather-type soliton and two soliton solutions are obtained for a generalized variable-coefficients fifth-order KdV equation by means of the bilinear method and extended homoclinic test approach. The proposed method can also be applied to solve other types of higher dimensional integrable and non-integrable systems.

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Keywords: Extended homoclinic test approach; bilinear form; generalized fifth-order KdV equation; Periodic solitary wave solutions.

1. Introduction:

The Korteweg-de Vries (KdV) equation has been derived in fields such as shallow water waves, stratified internal waves, ion-acoustic waves, plasma physics and lattice dynamics [1]. When high-order dispersion is considered, the fifth-order KdV (fKdV) equations have been seen in some physical contexts, usually investigated in the exponential asymptotic investigating a generalized variable-coefficient fKdV equation and numerical calculations [2, 3]. Several integrable fKdV, e. g.; the Lax's equation, the Sawada-Kotera (SK) equation and the Kaup-Kupershmidt equation, have been discussed, which have analytic solutions and infinite sets of conservation laws [4-6]. Besides, the higher-order KdV-modified KdV equations with higher-degree nonlinear terms describing gravity waves in the atmosphere have been the periodic and solitary wave solutions of which have been obtained in Li, (2008) [7].

Due to the inhomogeneities of media and non-uniformities of boundaries, the variable-coefficient nonlinear evolution waves, ion-acoustic waves, plasma physics and lattice dynamic equations can be used to describe the real physical backgrounds [8-11].

In this paper, with the aid of symbolic computation [9-11], our interest will be devoted

to investigating a generalized variable-coefficient fKdV equation such as the one given

$$u_t + a(t)uu_{xxx} + b(t)u_x u_{xx} + c(t)u^2 u_x + d(t)uu_x + e(t)u_{xxx} + l(t)u_{xxxxx} + m(t)u + n(t)u_x = 0, \quad (1)$$

where $u(x,t)$ is a function of space variable x and time variable t and $a(t)$, $b(t)$, $c(t)$, $d(t)$, $e(t)$, $l(t)$, $m(t)$ and $n(t)$ are analytic functions of t. If the parameters are specially chosen, a series of equations can be obtained, which are integrable [4-6] and can be used to describe such physical phenomena as the interaction between a water wave and a floating ice cover and the gravity capillary waves [2, 3].

In case of $d(t)=6$, $e(t)=1$, $l(t)=\xi^2$ and $a(t)=b(t)=c(t)=m(t)=n(t)=0$, Eq. (1) reduces to

$$u_t + 6uu_{xx} + u_{xxx} + \xi^2 u_{xxxxx} = 0, \quad (2)$$

which has been proposed for the interaction between a water wave and a coating ice cover in river channels and the gravity-capillary waves with the Bond number close to and slightly less than $1/3$, where $u(x,t)$ is the scaled depth, x and t are the scaled space and time coordinates, respectively and ξ is a small parameter [2].

For $a(t)=1$, $b(t)=2$, $d(t)=3$, $e(t)=-\theta$, $l(t)=(2/15)$, $c(t)=m(t)=n(t)=0$, Eq. (1) reduces to

$$u_t + 3uu_x + 2u_x u_{xx} + uu_{xxx} - \theta u_{xxx} + (2/15)u_{xxxxx} = 0, \quad (3)$$

which has been derived for the classical gravity-capillary water-wave problem, where θ is a scale parameter [3].

In the limiting case $a(t)=b(t)=15, c(t)=45$, $l(t)=1$ and $d(t)=e(t)=m(t)=n(t)=0$, Eq. (1) reduces to integrable SK equation of the form

$$u_t + 15uu_{xxx} + 15u_x u_{xx} + 45u^2 u_x + u_{xxxxx} = 0, \quad (4)$$

which has been investigated in [5, 12, 13].

When $d(t)=e(t)=m(t)=n(t)=0$, Eq. (1) reduces to integrable SK equation as

$$u_t+a(t)uu_{xxx}+b(t)u_xu_{xx}+c(t)u^2u_x+l(t)u_{xxxxx}=0, \quad (5)$$

some soliton solutions of which have been obtained in [14].

The integrable nonlinear evolution equations (NLEEs) possess several properties, e. g.; N-soliton solutions, Backlünd transformation, Lax's pair and infinite sets of conservation laws [1, 4-6, 9-11]. Since there are choices for the parameters, the variable-coefficient NLEEs can be considered as generalizations of the constant coefficient ones [9-11]. Under certain constraint conditions, the variable-coefficient models may be proved to be integrable and given explicit analytic solutions [15]. The corresponding constraint conditions on Eq. (1) in this paper, which are obtained by the Painlevé analysis [16] and conditions from the variable-coefficient models mapped to the completely integrable constant-coefficient counterparts [14] will be

$$a(t)=b(t)=\frac{15l(t)}{\rho}\exp[\int m(t)dt], \quad (6a)$$

$$c(t)=\frac{45l(t)}{\rho^2}\exp[2\int m(t)dt], \quad (6b)$$

$$d(t)=e(t)=0, \quad (6c)$$

where $\rho\neq 0$ is an arbitrary constant.

It is worth noting that there have been no discussions on Eq. (1) under conditions (6). Considering such insufficiency, we will apply the Hirota bilinear method [13, 17, 18] to investigate the integrability for Eq. (1) and the characteristic-line method [19] to discuss the effect of the variable coefficients in Eq. (1).

The structure of this paper will be organized as follows: In section 2, with symbolic computation, the bilinear form of Eq. (1) is obtained. In order to illustrate the proposed method, we consider a generalized variable-coefficients fifth-order KdV equation and new periodic wave solutions are obtained, which included periodic two solitary solutions, doubly periodic solitary solution. Finally, conclusion and discussion are given in section 3.

2. Soliton solutions of fifth-order KdV equation with variable coefficients

We make the dependent variable transformation

$$u(x,t)=2\rho\exp[-\int m(t)dt]\log[f(x,t)]_{xx}, \quad (7)$$

Where $f(x,t)$ is a real function of x and t. The bilinear equation of Eq. (1) turns out to have the following form

$$[D_xD_t+l(t)D_x^6+n(t)D_x^2]f(x,t).f(x,t)=0, \quad (8)$$

Where $D_x^m D_t^n$ is the Hirota bilinear derivative operator [18] defined by

$$D_x^mD_t^nf(x,t).g(x,t)=(\frac{\partial}{\partial x}-\frac{\partial}{\partial x'})^m(\frac{\partial}{\partial t}-\frac{\partial}{\partial t'})^n[f(x,t)g(x',t')]_{x'=x,t'=t}, \quad (9)$$

This definition is used to give

$$(D_x^6)f.f=2[f_{6x}f-6f_{5x}f_x+15f_{4x}f_{2x}-10f_{3x}f_{3x}] \quad (10a)$$

$$(D_xD_t)f.f=2[f_{xt}f-f_xf_t], \quad (10b)$$

$$(D_x^2)f.f=2[f_{xx}f-f_xf_x]. \quad (10c)$$

To solve the reduced Eq. (8) by means of the extended homoclinic test function [20-28], we suppose a solution of Eq. (8) as

$$f(x,t)=\exp(k_1x+c_1t)+p_1\cos(k_2x+c_2t)+q_1\exp(-k_1x-c_1t), \quad (11)$$

Where p_1, q_1, c_i, k_i, $(i=1,2)$ are parameters to be determined later.

Substituting Eq. (11) into (8) and equating all coefficients of $\exp[\pm(k_1x+c_1t)]$, $\cos(k_2x+c_2t)$, $\sin(k_2x+c_2t)$ to zero, one gets a set of algebraic equation for p_1, q_1, c_i, k_i, $(i=1,2)$. Solving this set of algebraic equations with the aid of Maple leads to many solutions, from which the following four solutions are chosen as:

'

The set of coefficients of solution (11) are given as:

$$k_1=k_1, \qquad k_2=k_2, \quad (12a)$$

$$c_1=-k_1[n(t)+16k_1^4l(t)], \ c_2=c_2, \quad (12b)$$

$$q_1=q_1, \qquad p_1=0. \quad (12c)$$

According to this set of coefficients, (11) leads to

$$f(x,t)=\exp\{k_1x-k_1[n(t)+16k_1^4l(t)]t\}+q_1\exp\{-k_1x+k_1[n(t)+16k_1^4l(t)]t\}. \quad (13)$$

Substituting this function into (7) gives a new periodic wave solution of (1) as follows:

$$u(x,t)=8\rho q_1k_1^2\exp[-\int m(t)dt]/\left\langle\exp\{k_1x-k_1[n(t)+16k_1^4l(t)]t\}+q_1\exp\{-k_1x+k_1[n(t)+16k_1^4l(t)]t\}\right\rangle^2. \quad (14)$$

Case (2):

For this case, the coefficients of the solution (11) are taken as:

$$k_1=k_1, \qquad k_2=k_2, \quad (15a)$$

$$c_1=-k_1[(k_1^4-10k_1^2k_2^2+5k_2^4)l(t)+n(t)], \quad (15b)$$

$$c_2=-k_2[(k_2^4-10k_1^2k_2^2+5k_1^4)l(t)+n(t)], \quad (15c)$$

$$q_1 = \frac{-p_1^2 k_2^2 (k_1^2 - 3k_2^2)}{4k_1^2 (3k_1^2 - k_2^2)},\ p_1 = p_1. \quad (15d)$$

These coefficients lead to a form of solution (11) as:

$$f(x,t) = \exp\{k_1 x - k_1[(k_1^4 - 10k_1^2 k_2^2 + 5k_2^4)l(t) + n(t)]t\} + p_1 \cos\{k_2 x - k_2[(k_2^4 - 10k_1^2 k_2^2 + 5k_1^4)l(t) + n(t)]t\} + [\frac{-p_1^2 k_2^2 (k_1^2 - 3k_2^2)}{4k_1^2 (3k_1^2 - k_2^2)}] * \exp\{-k_1 x + k_1[(k_1^4 - 10k_1^2 k_2^2 + 5k_2^4)l(t) + n(t)]t\}. \quad (16)$$

Substituting this solution form into (7) admits to a new periodic solitary wave solution of (1).

Case (3):

In this case, the coefficients of solution (11) are represented by

$$k_1 = ik_2, \qquad k_2 = k_2, \quad (17a)$$

$$c_1 = ik_2[16k_2^4 l(t) + n(t)],\ c_2 = -k_2[16k_2^4 l(t) + n(t)], \quad (17b)$$

$$p_1 = p_1, \qquad q_1 = \frac{p_1^2}{4}. \quad (17c)$$

These coefficients are used into (11) to give

$$f(x,t) = \exp\{ik_2 x + ik_2[16k_2^4 l(t) + n(t)]t\} + p_1 \cos\{k_2 x - k_2[16k_2^4 l(t) + n(t)]t\} + \frac{p_1^2}{4}\exp\{-ik_2 x - ik_2[16k_2^4 l(t) + n(t)]t\}. \quad (18)$$

Inserting this equation into (7) admits to a new periodic solitary wave solution of (1).

Case (4):

Finally, we can take a set of coefficients to solution (11) as follows:

$$c_1 = -\sqrt{3}k_2[16k_2^4 l(t) - n(t)],\ c_2 = -k_2[16k_2^4 l(t) + n(t)], \quad (19a)$$

$$k_1 = \sqrt{3}k_2, \qquad k_2 = k_2, \quad (19b)$$

$$p_1 = p_1, \qquad q_1 = 0. \quad (19c)$$

These coefficients lead to solution (11) of the form

$$f(x,t) = \exp\{\sqrt{3}k_2 x - \sqrt{3}k_2[16k_2^4 l(t) - n(t)]t\} + p_1 \cos\{k_2 x - k_2[16k_2^4 l(t) + n(t)]t\}. \quad (20)$$

Using this solution into (7) admits to a new periodic solitary wave solution of (1).

3. Conclusion

Using a bilinear form and the extended homoclinic test approach, we obtain breather-type soliton and two soliton solutions for the generalized variable-coefficients fifth-order KdV equation. The results show that the extended homoclinic test approach is very effective in finding exact solitary wave solutions for nonlinear evolution equation with variable coefficients.

It is worthwhile to mention that the proposed method is reliable and effective and can also be applied to solve other types of higher dimensional integrable and non-integrable systems of nonlinear evolution equations.

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Studying the factors relative to the being polygamy of the Bashtian Men

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Abstract: Marriage is the start point of the family formation among different cultures. One of the main criterions in this field is the number of wives. This survey is aimed to explore the factors affecting the polygamy. In this regard, using the quantitative performance, the economic and social factors relative to being polygamy of Bashtian men are examined. Data was gathered using some questionnaires. 200 questionnaires were distributed among the polygamous and monogamous men and then they were gathered and analyzed. According to the results, there is a meaningful relationship between polygamy and some factors including the power of men, the men desire for having more than one wife, the educational degree, the man job, the illness of first wife and dissatisfaction from the first wife's family.

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Key words: polygamy, marriage, culture

Introduction

Family is the central structure of a society which plays a main role in the social and personal life of each individual. One of the main issues relative to the family is to know the way of its formation and the factors affecting on. The start point of a family formation is choice of spouse which has influence on the performance of the family. There are various kinds of marriage: monogamy and polygamy. This survey is focused on polygamy.

By polygamy, we mean a kind of marriage through which a man can have several wives (Azdanloo, 2005). Polygamy is more current not only among the savage tribes, but also among the civilized countries. During Sasanian period, polygamy was a custom among the Arabs, Jewish and Iranians. Some authors believe that being polygamous of the tribes is rooted in the presence of the women in farmlands or herding. Polygamy is also current among the high – class men of the societies. This is because the men tend to have more children and to strengthen their social position (Arian pour, 1975). The behavior and mental health of such family members is under the influence of special relations (Mujahidi & Birshek, 2003). The happiness of married life is based the characteristics such as cordiality, generous disposition, self – sacrifice and intimacy. Some times, polygamy causes the women to injure each other and turns the family environment into a battlefield. Sooner or later, kindness of the family members changes into hostility. Therefore, through recognizing the factors which affect on the polygamy of the men, we can make some cultures by which the structure of the family can be strengthened. It is hypothesized that marriage is affected by various social, economic and cultural factors. Therefore, this survey aims to explore the social, cultural and economic factors relative to being polygamous of Bashtian men.

Review of literature

Polygamy is one of the main issues which have been rarely investigated because of the limitation of the traditional society environment. This survey is mainly aimed to explore the qualitative performance of the families.

Monireh Zare (1995) studied the effect of polygamy on the family system. According to the results, %74.7 of the people in her sample believed strongly in polygamy and 24.3 saying polygamy is because of the men's capriciousness. It is worth nothing that all of the men participated in the study believed polygamy means adhering to Islam, but most of the women believed that is because of the men's capriciousness. 1 out of 112 paradigm men was polygamy because he had no child. Although the polygamous men call themselves righteous and believe that being polygamy means adhering to Islam, the women believe that being polygamy of the men affects negatively on the family system.

She concluded that the family system is directly under the influence of some factors including the economic difficulties, number of the children, wide difference of the children's ages, bad educational conditions and difference of the women's ages (Zara& Bibikobadi, 1997).

Shahla Azazi (2000) has studied the effect of polygamy on the performance of the family. She believes that children of the wives of a man have dispute with each other because of the division of their father's heritage. There are many examples of fratricide in the history of both Iran and Europe. There is no heartfelt relation between the children because of the difference of their ages and the presence of stepmother or stepfather. Being absent from the house, the fathers can not have a good relation with their wives or daughters. She argues that the women are usually under the stress of being polygamous of the men (Azazi, 2000).

Aziz Allah Mojahid & Behrooz Brihak (1995) found that the mental health of the women having co – husband is less than the others'. Moreover, the children of the wives of the polygamy men have dispute with each other and sometimes this dispute makes the children criminal (Gharae: Moghadam, 2003).

Michael Tartlet (2005) has theorized that most of the African men have more than a single wife and the women are always pregnant. Africa is the poorest country of the world. Tartlet has asked, "Does the prohibition of being polygamous affect on the economic development of the Blacks?" he has used the polygamy model for answering this question and examines the effect of monogamy rule in this framework. He concludes that based on the monogamy rule, the number of pregnant women and the difference of husbands' and wives' ages will be decreased. Moreover, this rule increases the ratio of capital to the internal net income. When a polygamous man is authorized, the number of birth is high. Therefore, the marriage means investing capital. As a result, prohibition of remarriage causes the pregnancy to decrease by %40 and it also increases the country's saving and production by %70 and %170, respectively. He concludes that the government can improve the economic performance of the Africa through prohibiting polygamy of the men (Tertilt, 2005).

During his study about the Kuwaiti and Emirian patients, Chaney (1985 – 1988) found that the wives of polygamous men have more psychoses (Quoted from MOjahid & Birshek, 2001).

In a large sociological study in Dubai, Ghubash , Bebington & hamedi concluded that the divorced women ,Widows and the wives of polygamous men are mainly in danger of suffering from psychoses.

Oyefeso & Adegoke have theorized that the children of polygamous men are under the mental pressures including the struggles between the rival wives, the competitions between the mothers and insufficient cares. Further, the young boys of such men suffer more than the others from psychoses.

Owuamanam argued that the youths of polygamous men have low self – confidence compared to the others. Evaluating the educational progress of the children of monogamy and polygamy families, Cheri an found that the children of polygamy families have lesser educational progress compared to the children of monogamy families. Because the women compete with each other to attract the attention of their husband, the polygamy families may be disordered (Bites & plug, 1996). As a result, in most of the studyes about polygamous men, the various psychoses resulted from polygamy have been studied. Moreover, the studyers have not presented hypotheses in proportion to the factors affecting on being polygamy of Iranian men. Reviewing the evidences, this is no suitable study relative to such issues in Iran (Mojahid & Birshek, 2003).

Theoretical bases

Since the marriage and polygamy of men is the main relations established under the influence of some factors, sociologists have presented some view points about these factors. Polygamy of the men can be studied from anthropological and sociological perspectives.

-*viewpoints of anthropologists*:

Riviera, German anthropologist, believes that societies in which polygamy is customized follow various principles for the first and second marriages. One of these principles is to marry into the widow of the brother. He argues that being polygamy of the men follows various strategic objectives:

1- Demographic objective: to stable the tribe through having children
2- Economic objective: to increase the work forces through making money and making the parents secure
3- Political objectives: to establish the peace through increasing the family nets
4- Religious objective: to attract the Gods for supporting the women's tribes
5- Spiritual – social objective: to appropriate a top social position for the men.
6- Spiritual – material objectives: to satisfy the lust when a woman is pregnant (Rivier, 2005)

This study is aimed to test some of the main hypotheses including marring the widow of the brother, infertility of the woman and the social and economic significance of the males.

In "the cultural anthropology", Denial Bates & Fred plug theorized that only the old wealthy men can remarry. From the viewpoint of a man, the women are the main economic advantage. Not only can the women bear the boys who help their fathers

in political battles, but also they bear the girls who are lovely. Therefore, this is to say that women are the source of health, power and social position. It seems that polygamy is a custom among the political societies who impose the courageous ability of the men on the women (Bates & Plug 1996). Therefore, the viewpoint of Bates and Plug is used for studding the economic and social factors which affect on the polygamy.

Some authors argue that being polygamy is one of the characteristics of Patriarchy period.Chiefs of the tribes were endowed with a miraculous power and their properties showed their social and political position. Regarding this hypothesis, the women were the main part of the chiefs' properties. Morgan believes that the modern monogamy is the changed form of the old group marriage (Roohol Amine 1995).

- *viewpoints of sociologists*:

Based on the sociological viewpoint, the social exchanges of people leading to the social transformation are studied (Adibi & Ansari 2004). People have personal tendencies. These tendencies don't create joint objectives. It is hypothesized that people have selfish motivations. Therefore, motivation is either personal or private. It, so, refers to the personal desire for achieving goals. Authorities argue that emotional rewards compensate the financial loses. People are interested in satisfying their lust. Every person should be generous. Some authorities believe that when person can not satisfy each other, there is no social relationship between them.

Nevertheless, persons should pay a price to get profit. This price is related to the attempt of a person to satisfy others (Adibi & Ansari 2004).

One of the principles proportional to the exchange theory is social confirmation. This principle can be regarded as a creating factor. Due to exchange theory, there is a wide variety of rewards from which the social confirmation is of great importance (Adibi& Ansari 2004). Hymens argued that people act more better when they are encouraged (Ritzer 1998).

Because the Bashtians have tribal characteristics, they pay no attention to their personal tendencies. Tennis believes that the Bashtians have the characteristics of a spiritual society. He regards the following as the characteristics of a spiritual society:

1) *Organic intention*

In spiritual societies, persons seek serving each other. Organic intention is the main criterion of a spiritual society by which this society is separated from the superficial society. In superficial society, the human relations are under the influence of intention.

2) *Intimate relations*

People living in a spiritual society know each other's reaction. Therefore, they calm each other during bad conditions.

3) *Constant relation*

People who live in spiritual societies are acquainted with the past things of each other and this is because such societies are not so developed.

4) *Absolute relation of a few people*: Being small of spiritual society improves its quality. Therefore, the relation of people is improved and they have a good relation with each other (Sarokhani, 1994).

Tunis relates the following characteristics to the superficial society: intension, extensive, thoughtful and formal relation (Sarokhani, 1994). This is to say that the exchange theory is proportional to the superficial society. Therefore, this theory is not in proportion to the investigation of economic, social and cultural factors affecting on the choice of spouse.

Theoretical framework

Among the mentioned theories, the River's, Bit's & plug's viewpoints were chosen. Morgan has presented an evolutionary theory. He has regarded polygamy as one of the fivefold stages of marriage and argues that we have recently entered to the monogamy stage but evidences show that polygamy is prevalent in many modern societies. Therefore, it is not suitable to use the Morgan's theory in this study.

According to the exchange theory, people have personal aims and desires. All people may need something, but they have no joint goal. It is hypothesized that the unique and personal aims make people selfish. When persons are not satisfied from each other, there is no social interaction between them (skidmor, 1996). The exchange theory's adherents believe that the incomes are guaranteed by receiving reward. Expense refers to make effort in order to satisfy others. Profit means the difference between reward and expense. It is achieved when the reward is more than the expense (Skidmor, 1996). Due to exchange theory, persons seek the suitable opportunities for increasing their rewards. For example, a married person regards the followings as the resources of rewards:

- The pleasurable relation with the wife
- Explicit profits (assets)
- Peace of mind caused by the marriage

Riviera's theory of polygamy is a suitable theory, because it emphasizes on the marriage to the widow of the brother and the importance of bearing boys as the key factors affecting on polygamy phenomenon.

Bits & Plug have theorized polygamy phenomenon as an economic advantage which wealth, authority and social position of the men is based on. Therefore, in order to study the social and economic factors affecting on polygamy phenomenon, this theory is valuable.
William Gad argued that the structural changes are suitable for studying the economic, social and cultural factors which affect on the polygamy phenomenon.

Study hypotheses
H1: there is a relationship between the educational degree of men and polygamy phenomenon
H2: there is a relationship between the men's job and polygamy phenomenon
H3: there is a relationship between the illness of first woman and the polygamy phenomenon
H4: there is a relationship between compulsory marriage and polygamy phenomenon
H5: there is a relationship between imitation of men and polygamy phenomenon
H6: there is a relationship between the custom of marrying the widow of the brother and polygamy phenomenon
H7: there is a relationship between religious precepts and polygamy phenomenon
H8: there is a relationship between the men's desire and polygamy phenomenon
H9: there is a relationship between women's infertility and men's polygamy
H10: there is a relationship between women's bearing daughter and men's polygamy.
H11: there is a relationship between the men's dissatisfaction of the first women's family and polygamy
H12: there is a relationship between misunderstanding of the couple and the men's polygamy
H13: there is a relationship between the men's income and polygamy phenomenon
H14: there is a relationship between men's welfare possibilities and polygamy phenomenon

Study methodology
This is a descriptive survey in which data was gathered by use of a questionnaire. The questionnaire included some closed questions. The sample includes 200 monogamy and polygamy men of Basht region who were randomly selected with the help of statistics and registration administration. The variables used in the survey include: Educational degree. By educational degree, we mean a person's years of receiving education which is classified as the following:

1) Being illiterate
2) Elementary
3) Guidance
4) Secondary
5) High education

Family income: it is referred to the income of the family at the end of month.
Welfare possibilities: welfare possibilities refer to those possibilities includes personal house, personal car, television, personal library, Radio, Phone, Mobile, Computer and Internet.
Job kind: by job kind, we mean a set of activities by which a man makes money. There are two kinds of jobs:

Free job and governmental job
Infertility of the first wife: it means that the first wife is unable to bear a child.
Illness of the first wife: it refers to when the first wife has a disease under the influence of which she can not bear a child.
Disagreement of man with his first wife: lack of agreement between man and first wife in some affairs such as baby – sitting, proprieties, financial and living affairs.
Marrying compulsorily: when the man is obliged to marry a woman by his parents
The man dissatisfaction from the family of his first wife: when the man has a dispute with his father – in – law / mother- in- law
Imitation: when a man imitates another's manner and chooses another spouse
Marrying to the widow of the brother: when man misses his brother and is compelled to marry his brother's widow
Religious precepts related to polygamy: it refers to a set of rules by which the men's choice of spouse is limited.
By polygamy, we mean the union in marriage in which a man marries two women simultaneously.

Findings
Educational degree and polygamy
Educational degree of men is an independent variable. The relation between this variable and polygamy is studied by use of chi square test. Degree of freedom (df) and meaningfulness level (ml) of this variable have a meaningful relationship with the dependent variable. In results, hypothesis 1 is confirmed. As shown in table below, the polygamous men are always illiterate.
Table 1 shows the relationship between educational degree and polygamy

Job kind and polygamy

The kind of men's job is another independent variable examined. As shown in table 2, there is a direct relationship between the two variables. Therefore, hypothesis 2 is accepted because of the values of DF, ml and chi square. This is to say that those having free jobs believe in polygamy.

Table 2 shows the relationship between job kind and polygamy.

Illness of first wife and polygamy

Illness of first wife is another independent variable studied by use of chi square test. As illustrated in table 3, there is a meaningful relationship between the two variables because of the values of chi square, df and ml. Furthermore, most of men having an ill wife have a tendency to polygamy. On the other hand, most of polygamous men are those who have ill wives.

Table 3 shows the relationship between the illness of first wife and polygamy

Marrying compulsorily and polygamy

Being compulsory of first marriage is another independent variable examined by square test. As seen in table 4, there is a meaningful relationship between the two variables because of the meaningfulness level. Therefore, those who are married compulsorily tend to have another wife. When a man chooses his wife, he doesn't tend to remarry.

Table 4 shows the relationship between marrying compulsorily and polygamy

Imitation and polygamy

Imitation is another variable studied by chi square test. As shown in table 5, there is a meaningful relationship between the two variables. In result, hypothesis 5 is accepted .in other words, imitation is not regarded as the cause of being polygamy.

Table 5 shows the relationship between imitation and polygamy

Custom of marrying into the widow of brother and polygamy

Custom of marrying into the widow of brother and polygamy is another variable studied by use of square test. From table 6, with respect to the values of square test, DF and ml, there is no meaningful relationship between the two variables. Therefore, hypothesis 6 is not accepted. Results show that 3 out of 100 men marry into the widow of their brother.

Table 6 shows the marrying into the widow of brother and polygamy.

As illustrated in table 7, there is no meaningful relationship between the variables religious precepts and polygamy because of the value of meaningfulness level. Therefore, this variable is not accepted.

Table 7 6 shows the relationship between religious precepts and polygamy

Personal desire of man and polygamy

Desire of man is another independent variable studied by use of chi square test, df and ml. with respect to the results, this is to say that hypothesis 8 is confirmed. As seen, only %5 of monogamous men has a tendency to remarry. On the other hand, %23 of polygamous men believes in polygamy. Therefore, the polygamous men have more tendencies toward this phenomenon.

Table 8 shows the relationship between desire of man and polygamy.

Polygamy and degree of agreement between man and his first wife

The relation between polygamy and husband's and wife's agreement was studied by use of regression test and in the form of two – variable equation. This test was used because the independent variable has two categories. Therefore, such variables are studied by use of regression test. With respect to table 9, the Wald number is %4 and it shows that the independent variable criterion (B) is not meaningful. Also, with respect to the meaningfulness level, there is no meaningful relationship between the two variables.

Income of man and polygamy

The relation between income of man and polygamy was tested by use of regression test. As seen in table 9, the Wald number is .269 and it shows the meaninglessness of the independent variable criterion (B). The meaningfulness level shows that there is no relationship between the two variables.

Welfare possibilities and polygamy

In order to evaluate the relationship between the men's welfare and polygamy, this variable was examined by use of regression test. From table 9, this is to say that there is no meaningful relationship between the two variables.

Table 9 shows the relationship between possibilities and polygamy.

Multi – variable analysis

In order to investigate the relation between the independent variables of the study and the independent variable polygamy, the regression test was used. Tables 10 and 11 show the results. Table 10 includes the variables used in regression equation at stages. Variable 1 is infertility of the first wife. The Wald number is 43.584 showing the meaningfulness

of independent variable criterion (B). It shows that there is a meaningful relation between infertility of first wife and polygamy of men (%99). The second variable is bearing daughter (B=1.615). R=0.745 and it shows that there is a meaningful relationship between the two variables. The Wald number (20.199) shows the meaningfulness of the independent variable criterion (B).

The third variable is the men's dissatisfaction from the family of his first wife (B= 0.278). It is clear that there is a meaningful relationship between the two variables because R= 0.761. The Wald number is 5.188 and it shows the meaningfulness of independent variable criterion (B).

Regression equation:

Y= - 2.081 (infertility) +1.615(bearing daughter) + /278(dissatisfaction) +3.814

Table 10: choice of variable

-2 logs show the suitability of the model. The smaller this number, the more suitability is. the zero shows the complete suitability of the model. This criterion is 139.317, 109.712 and 104.156 for the variables infertility, bearing daughter and dissatisfaction, respectively. Therefore, it is a suitable criterion.

Model X^2: this criterion evaluates the effectiveness of variable of the equation. When x^2 is high, the variables are effective. As seen in table 11, the degree of chi square is increasing and it shows the effectiveness of the available variables.

Improvement: by this criterion, the change of 2 log likelihood is examined. With respect to table11, the chi square value is improved under the influence of second variable. Moreover, when the third variable decreases, the 2 log likelihood criterion is changed meaningfully.

Correct prediction: the value of dependent variable is compared to the predicted dependent variable by use of correct prediction criterion. Based on the table below, there is no meaningful difference between the predicted percentages (%81.9, %87, and % 86).

Table 11 shows the suitability of the model for used variables

Conclusion

This survey was aimed to investigate the factors related to being polygamy of Bashtian men. In past, polygamy was a custom among the people of Iran. Evidences show that some variables, including infertility of first wife, bearing daughter and dissatisfaction from the first wife's family, are the main factors affecting on the polygamy of men. In this regard, this is to say that marriage is under the influence of cultural and social behaviors of traditional societies.

Traditional society refers to the society in which it is very important to have boy. In such societies, men seek a way to solve their personal problems. They tend to have a powerful wife who can bear several boys. Sometimes, illness of first wife and bearing girl are the two factors affecting on polygamy. On the other hand, results show that there is no direct relationship between income of man and life possibilities. Polygamy has no direct relation with the economic condition. In Basht region, polygamy is a symbol, not a real need. It seems that their problem can be solved by polygamy.

At two – variable level, it was cleared that there is a meaningful relationship between polygamy and variables such as the man's desire, job kind and educational degree.

Table 1: relationship between educational degree and polygamy

Education	Number of wife		Total
	Monogamy	polygamy	
Illiterate	43	31	74
elementary	21	34	55
Guidance	8	15	23
Secondary	20	11	31
High education	8	7	15
Jurisdictional	0	2	2
Total	100	100	200

Table 2: relationship between job kind and polygamy

Job kind	Number of wife		total
	Monogamy	polygamy	
Free	66	81	147
Governmental	34	19	53
Total	100	100	200
	Df=1		Ml =%16

Table 3: relationship between the illness of first wife and polygamy

Illness of first wife	Number of wife Monogamy polygamy	Total number
No	96 69	165
Yes	4 31	35
total	100 100	200

Table 4: relationship between marrying compulsorily and polygamy

The man's role in choosing his wife	Number of wife Monogamy	polygamy	total
Extremely high	76	23	99
Relatively high	17	21	38
Partly	7	45	52
Relatively low	0	8	8
Extremely low	0	2	2
total	100	100	200
	Df= 1		Ml= 0.733

Table 5: relationship between imitation and polygamy

Imitation	Number of wife Monogamy polygamy	Total number
No	95 96	191
Yes	5 4	9
Total	100 100	200
	Df= 1	Ml=0.733

Table 6: marrying into the widow of brother and polygamy

Marrying into the widow of brother	Monogamy / polygamy	Total number
No	96.97	193
Yes	4.3	7
Total number	100.100	200
	Df=1	Ml =0.700

Table 7: relationship between religious precepts and polygamy

Religious percepts	Monogamy/polygamy	Total number
No	97 99	196
Yes	3 1	4
Total number	100 100	200
	Df=1	Ml=0.312

Table 8: the relationship between desire of man and polygamy

The man's desire to polygamy	Monogamy/polygamy	Total number
No	95 77	172
Yes	5 23	28
Total number	100 100	200
	Df=1	Ml=0.000

Table 9: relationship between possibilities and polygamy

variable	B	R	S.E	Wald	df	ml	Chance ratio
Agreement of husband and wife	-0.001	0.000	0.020	0.004	1	0.948	0.999
Income level	0.000	0.002	0.000	0.269	1	0.604	1
Welfare possibilities	%34	0.001	0.074	0.215	1	0.643	1.035

Table 10: choice of variable

variable	B	R	S.E	Wald	df	ml	Chance ratio
Infertility of first wife	-2.081	0.647	0.315	43.584	1	0.000	0.125
Giving birth daughter	1.615	0.745	0.359	20.199	1	0.000	5.027
Dissatisfaction from the first wife's family	0.278	0.761	0.122	5.188	1	0.023	1.321
Fixed number	3.814		0.916	17.342	1	0.000	45.354

Table 11: suitability of the model for used variables

Stage	Improvement Df	sig		Model Df	sig		-2 log likelihood	Correct prediction	variable
1	128.108	1	.000	128.108	1	.000	139.317	81.9	infertility
2	29.605	1	.000	157.713	2	.000	109.712	87	Daughter giving birth
3	5.556	1	.000	163.269	3	.000	104.156	86	Dissatisfaction

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An assessment of Dietary Intake Associated with the Coronary Heart Disease among Adults in Yerevan, Armenia

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Abstract: Epidemiologic studies have demonstrated the relationship between the dietary intake and coronary heart disease (CHD) in various countries. Extreme changes have occurred in lifestyles as well as dietary patterns in industrialized countries. Also, no study has been done to address the association between CHD and food consumption in these populations. This case-control study was conducted to assess the dietary intake in individuals with and without CHD during 2010 and 2011; we randomly selected 320 patients with CHD and 320 subjects without CHD (≥ 30 years old) from the hospitals, polyclinics and center of preventive cardiology in Yerevan. Dietary intakes with 135 food items over the previous 12 months were evaluated using a semi-quantitative food frequency questionnaire. We observed an inversely significant association between fruits, vegetables (not potatoes), whole grain, and plant food consumption and CHD. In a logistic regression, after adjusting for confounder risk factors, each 100 g increase in fruit or vegetables decreased 63% odds of CHD. The odds ratio for those with intake of sweet and dessert in the highest quartile was 2.64 (95% CI 1.65-4.21). 85% of cases and 81.3% of controls, consumed fish and seafood less than 200 g/wk (P>0.05), also, low intake of whole grain (below 100 g/d) was most common both in cases (95.9%) and controls (93.4%).This pioneering study indicates which fruit, vegetable intakes, whole grain and plant food independently associated with the CHD risk in the population under investigation.
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Introduction

Coronary heart disease (CHD) is one of the most common causes of morbidity and mortality in different communities (Hadaegh et al. 2009). Many risk factors for cardiovascular disease (CVD), including high blood cholesterol, hypertension, obesity, and diabetes are substantially influenced by dietary factors (*Liu et al., 2000).* Dietary intake and food habits are recognized to play key roles in the prevention and treatment of CHD (Lancaster et al., 2006). During the past decade, numerous key epidemiologic studies related to dietary intake and CVD have been published that suggested a strong association between CHD and dietary factors (Jakobsen et al., 2009; Holmberg et al., 2009; *Xu et al., 2006*). The recent and drastic changes that have occurred in industrialized countries have led to unhealthy dietary patterns (*Lairon et al., 2005*). Findings showed differences in dietary intake and risk of CHD and related health conditions among ethnic subgroups of Blacks living in the United States (Lancaster et al., 2006). Furthermore factors such as genetic predisposition as well as changing lifestyle including physical inactivity may also increase the coronary risk profile (Ghosh et al., 2003). As far as Armenia is concerned, there have been no studies investigating food pattern variables in explaining risk factors for CHD. This pioneering study was carried out to evaluate dietary intake in individuals with and without CHD in this country.

Materials and methods

Participants: This Observational Case-Control study was conducted during the period of March 2010 to February 2011 in the Yerevan State Medical University (YSMU) hospitals, polyclinics and in the Center of preventive cardiology. Patients aged ≥ 30 years as the case group (n=320) with established CHD were identified by cardiologist. The controls (n=320) consisted of individuals aged ≥ 30 years without CHD who attended for check-up in hospitals and polyclinics in Yerevan.

All participants were given a written informed consent prior to their participation in the study. The inclusion criteria were as follows: subjects, who attended YSMU hospitals and polyclinics and in the Center of preventive cardiology for check-up and age > 30 years. Patients were

excluded if they had any previous history of MI, admission for angiography, and previous history of any kind of heart surgery or angioplasty for CHD. Pregnant women and patient with history of systemic diseases according to the medical records were also excluded. The study protocol was approved by the Ethics Committee of the Yerevan State Medical University.

Data collection: In this study we assessed family history of diabetes, heart diseases, hypertension, socioeconomic status, lifestyle factors (including smoking habits, physical activity, alcohol drinking), and dietary intake for each subject. Next, anthropometric measures (height, weight, and hip and waist circumferences) were obtained. Waist and hip measures were assessed by using a soft tape measure, with waist measures taken at the midpoint between the costal margin and ileac crest and hip measures taken at the widest circumference. Finally systolic and diastolic blood pressure was measured twice with a standard mercury sphygmomanometer after the participants sat for 15 min; the mean of the two measurements was considered to be the participant's blood pressure at the time of health check-up.

Assessment of dietary intake: Information on the usual intakes of foods and dishes over the previous year was estimated using a semi-quantitative food frequency questionnaire (FFQ). Nutritionists and Public health specialists assisted to determine constructing a list of foods which ultimately consisted of approximately 135 foods and beverage items with a standard serving size that commonly consumed by Armenians. Before the FFQ was implemented in the study, it was adapted to Armenian conditions and was field-tested on 50 individuals.

Food items were classified into eleven categories: fruits (17 items), dried fruit (6 items), vegetables (23 items), meat, fish, poultry, egg and legumes (17 items), Dairy Products and Fats (18 items), miscellaneous (9 items), beverages (13 items), bread, cereal and potato (12 items), nuts (9 items), dishes and soups (8 items), and fast food (3 items). Subjects were asked to select their frequency of consumption and portion sizes of each food item in the past year on a daily (egg, bread), weekly (egg, rice or meat, vegetable, fruit), or monthly (egg, fish) basis by using household measures. If the subject did not consume that item, the "never" box was marked. For each subject, mean intake according to grams per day of each indicated categories were calculated.

Statistical analysis: A special database was developed to store and analyze the obtained data. The data collected through the questionnaire were entered into the database. Dietary variables are presented as means and standard deviations for normally distributed parameters. Comparison of numeric data was made using unpaired t-tests for normally distributed variables and the chi-square test for category parameters. To assess associations of CHD with food intake we calculated the lowest and highest quartiles of food groups. In logistic regression analysis, the odd ratio was computed according to the quartile value. All statistical analyses were done using the SPSS (Statistical Package for Social Sciences, Version 15). All p-values reported were based on two-sided tests, and the statistical significance was defined as $p < 0.05$ for all tests.

Results

Table 1 shows CHD risk factors of cases and controls. 50.6% of the patients with CHD were males and 49.4% were females. Also 44.1% of the controls were males and 55.9% were females. Cases had significantly higher prevalence of hypertension, MetS, current smoking, current alcohol consuming, and family history of CHD, while no statistically significant differences were found for obesity.

Table 2 shows the average consumption and standard error of each food group (g/d) for cases and Controls. In our study, the cases had significantly higher intakes of refined grain, sweets and dessert but lower intakes of fruits, vegetables (not potatoes), green leafy vegetables, fish, and seafood, nuts and plant food compared to the control group. No significant difference was observed in legumes, egg, whole grain and animal food intakes between individuals with and without CHD.

Distribution of case and control groups based on consumed food items is presented in Table 3. Among the food groups, the strongest associations were observed for fruits and vegetables. Percent of individuals who consumed fruits and vegetables less than 200 g/d was significantly higher in patient with CHD (case, 85% vs. control, 43.8%; P<0.000; case, 88.1% vs. control, 59.7%; P<0.0001 respectively). Reversely the percentage of cases who consumed processed meat ≥60 g/wk and egg ≥120 g/wk was significantly higher than that of the control group (cases, 33.1% vs. controls, 23.8%; P<0.05; cases, 27.8% vs. controls, 19.7%; P<0.05 respectively), while no statistically significant differences were found in distribution of cases and controls regarding the whole grain < 100 g/d, and fish < 200g/wk. P>0.05).

Participations defined by the CHD risk factors were calculated according to the mean of fruit and sweet & dessert intake (Table 4). In each category, the CHD group had a lower intake of fruit than controls. Also, other subgroups of cases had higher sweet and dessert consumptions except in current smokers.

Table 5 depicts the odds ratio and 95% confidence interval for CHD according to food items intake. Among the food groups included in logistic regression analysis that adjusted to calorie and gender, intake of sweet and dessert was observed to be risk factor for CHD. The odds ratio for those with intake of sweet and dessert in the highest quartile were 2.64 (95% CI 1.65-4.21), but fruit, vegetable, whole grain, and plant food (highest vs. lowest quartile) intake proved to be inversely significantly associated with CHD. More individuals in the CHD group were in the lowest quartile of them while, there were no statistically significant interactions for nuts and animal food consumption (highest vs. lowest quartile) with CHD.

Table 1: Characteristics of Patients with CHD and Controls

Variable	CHD group		Controls		P-value
	Males N (%)	Females N (%)	Males N (%)	Females N (%)	
Number	162(50.6)	158(49.4)	141(44.1)	179(55.9)	0.11
Current Smoking	108(66.7)	20(12.7)	66(46.8)	1(0.6)	0.000
Current Alcohol Consumption	124(70.9%)	51(32.3)	77(54.6)	29(16.2)	0.000
Hypertension	120(74.1)	130(82.3)	82(58.2)	124(69.3)	0.000
MetS	126(77.8)	129(81.6)	87(61.7)	135(75.4)	0.004
Family History of CHD	21(13)	24(15.2)	3(2.1)	7(3.9)	0.000
Obesity (BMI≥30)	65(40.1)	72(45.6)	57(40.4)	76(42.5)	0.40

Comparisons were based on the chi-squared test. P-value is for group differences after controlling for gender.

Table 2: Average Daily Food Consumption (g/d) in Case and Control groups

Food consumption	**Group**		**P value**
	Cases (n=320) Mean (SE)	Controls (n=320) Mean (SE)	
Fruits	129.1 (3.96)	223.4 (6.77)	P<0.0001
Vegetables (not potatoes)	140.1 (3.21)	215.8 (6.78)	P<0.0001
Green Leafy Vegetable	4.9 (0.23)	11.4 (1.05)	P<0.0001
Fish and Seafood	16.0(0.92)	19.7 (1.57)	P<0.05
Legumes	12.4 (0.80)	14.7 (0.99)	P>0.05
Egg	17.3 (1.08)	16.7 (1.12)	P>0.05
Whole Grain	25.3 (1.99)	30.0 (2.28)	P>0.05
Refined Grain*	181.3 (6.23)	157.1(4.36)	P<0.05
Nuts	14.2 (1.28)	22.8 (1.91)	P<0.0001
Sweets and Dessert **	34.8 (2.19)	24.7 (1.48)	P<0.0001
Animal Food	427.2(10.62)	441.2(10.74)	P>0.05
Plant Food	625.1(13.18)	791.3(16.64)	P<0.0001

* Refined grain included white wheat (lavash and matnakash), loaf, toast, rolls, macaroni, and rice.
**Sweets and dessert included candy, chocolate, sugar, jam, jelly, cake, cookies, and ice cream.

Table 3- Food Consumption in Case and Control groups

Food consumption	**Group**		**P value**
	Cases n=320 (%)	Controls n=320 (%)	
Fruit < 200 g/d	272 (85)	140 (43.8)	P<0.0001
Vegetable <200 g/d	282 (88.1)	191 (59.7)	P<0.0001
Total Fruit & Vegetable < 400 g/d	287 (89.7)	156 (48.8)	P<0.0001
Whole Grain < 100 g/d	307 (95.9)	299 (93.4)	P>0.05
Fish < 200g/wk	272 (85)	260 (81.3)	P>0.05
Processed Meat ≥60 g/wk	106 (33.1)	76 (23.8)	P<0.05
Egg ≥120 g/wk	89 (27.8)	63 (19.7)	P>0.05

Table 4- Mean and Standard Deviation (SD) of Fruit and Sweet and Dessert Intakes in Subgroups

Variables	**Fruit intake (g/day)**		**P**	**Sweet & dessert intake (g/day)**		**P**
	cases mean (SD)	controls mean (SD)		cases mean (SD)	controls mean (SD)	
Obese	141.5(78.7)	219.2(123.7)	P<0.0001	37(34.2)	21.2(21.7)	P<0.0001
Hypertension	120(64.7)	212.6(118.3)	P<0.0001	32(31.2)	20.5(20.4)	P<0.0001
MetS	129.7(71)	223.1(122)	P<0.0001	33.27(30.5)	21.51(21.61)	P<0.0001
Family History of CHD	131.9(95)	291(182.9)	P<0.0001	41.8(26.1)	24.2(17)	P<0.05
Current Smokers	120.1(66.2)	201.2(105.4)	P<0.0001	39.2(52.1)	37.1(39.7)	P>0.05
Current Alcohol Consumers	119.3(65.4)	216.6(132.5)	P<0.0001	31.8(29.7)	25.1(21)	P<0.05

Table 5 -Distribution of Cases and Controls in the Highest and Lowest Quartiles, the Odds Ratio (95% Confidence Intervals) According to Food Groups Intakes

Food groups intake	Cases N (%)		Controls N (%)		Odds ratio	95% CI
	1st quartile	4th quartile	1st quartile	4th quartile		
Fruit (g/day	116(84.7)	21(15.3)	44(24)	139(76)	0.06	0.03 – 0.10
Vegetable (g/d)	170(78.7)	46(21.3)	60(26.1)	170(73.9)	0.10	0.06 – 0.15
Sweet-Dessert(g/d)	63(39.6)	96(60.4)	97(60.2)	64(39.8)	2.64	1.65- 4.21
Whole grain (g/d)	91(56.2)	71(43.8)	69(43.7)	89(27.8)	0.58	0.36– 0.91
Nuts (g/d)	84(26.3)	64(43.2)	76(44.2)	96(55.8)	0.65	0.41 -1.03
Animal Food	80(55.2)	65(44.8)	80(45.7)	95(54.3)	0.83	0.49-1.41
Plant Food	90(67.7)	43(32.3)	34(22.5)	117(77.5)	0.11	0.06 – 0.21

Discussion

To our knowledge, this is the first epidemiologic study that evaluated the association between dietary intakes with CHD in Armenia. In this case-control study, as expected, in the t-test analysis, it was revealed that mean of fruit, vegetable, fish and sea food, and nuts intake was lower in the CHD group, whereas there was no significant difference for egg, legume, whole grain and animal food consumption in both groups.

We found that the mean daily intake of fruit and vegetable differed significantly between the two groups (case, 129.1 g/d vs. control, 223.4 g/d; case, 140.1 g/d vs. control, 215.8 g/d, respectively). The inverse association of fruit and vegetable intake with the CHD risk in some prospective cohort studies has been shown (*Fung* et al., 2008; www.plosone.org, 2012). One of the most important dietary recommendations in relation to potential health gains and the elimination of CHD to a large extent in the individuals aged below 70 years is "eat ≥400 g of vegetables and fruits per day" (Kromhout et al., 2002) while in the sample under study 89.7% of cases and 48.8% of controls consumed total fruit and vegetable less than 400 g/d (Table 3). Recently, the American Heart Association has recommended a diet that includes at least 4.5 servings of fruits and vegetables daily (www.heart.org, 2012). In the present study, in logistic regression analysis, the odds ratio of individual with total fruit and vegetable intake in the highest quartile compared to the lowest were 0.06 (95% CI 0.03– 0.1) and 0.1 (95% CI 0.06-0.15), respectively, after adjusting for sex and calorie (Table 5). Also, these were independently associated with CHD after they were adjusted for other risk factors including calorie, gender, smoking and exercising. For each 100 g increase in fruit or vegetable consumption, there was a 63% reduction in the odds ratio of CHD (data not shown). A meta-analysis of 9 cohort studies reported a weaker association, i.e. a 4% lower risk of the CHD incidence for each additional portion of fruit and vegetables (Dauchet et al., 2006). B.C.Zyriax et al, in Germany reported a 30% decline risk for every 100 g consumption of fruit and vegetable (Zyriax et al., 2005). In a case-control study among Indian population, persons consuming a median of 3.5 serving/wk green leafy vegetable had a 67% lower relative risk than did those consuming 0.5 servings/wk. (Rastogi et al., 2004).

A diet rich in fruits and vegetables due to a higher content of antioxidants, folate, and flavonoids has beneficial effects on markers of inflammation and oxidative stress which may inhibit the development of atherosclerosis and may result resulting in lower cardiovascular risks (Holt et al., 2009).

In this study, we also found that the mean of nuts intake particularly sunflower nuts, was significantly higher in controls than that of the case group (controls, 22.8 g/d vs. cases, 14.2 g/d;

P<0.0001) (Table 2). But based on the comparison of the lowest and highest quartiles removed the impact of nuts on CHD risk (Table5). Association of nuts consumption was reported with decreased CHD in earlier prospective studies (Dontas et al., 2007). In previous studies, it was found that consumption of at least 5 servings/wk of nuts or peanut butter was associated with lower LDL cholesterol, non-HDL cholesterol, and total cholesterol (Li et al.,2009). Nuts and peanuts contain many bioactive components which exert beneficial effects on these CHD risk factors (Ros, 2009; Kris-Etherton et al., 2008).

In our study (Table 1) no significant difference was observed in average legume and whole grain intakes between individuals with and without CHD (case, 12.4 g/d vs. control, 14.7 g/d; cases, 25.3 g/d vs. controls, 30.0 g/d; respectively). In addition, the percentage of individuals in our study, who reported consuming less than 100 g/day of whole grain, was 95.9% and 93.4% of CHD patients and controls respectively. These findings are in contrast with other studies that indicated legume (*Bazzano et al., 2001*) and whole grain (www.plosone.org, 2012) might have beneficial health influences to reduce the risk of CHD. It is remarkable to mention here that both legume and whole grain consumption was not considerable among the study population under study.

Although the cases had significantly lower intakes of total fish and seafood (16.0 g/d) compared to the controls (19.7 g/d), the low intake of fish (below 200 g/week) was most common among cases (85%) and controls (81.3%).

In a Meta-analysis of cohort studies, compared with those who never consumed fish or ate fish less than once per month, individuals with a higher intake of fish had lower CHD mortality. Each 20-g/d increase in fish intake was related to a 7% lower risk of CHD mortality (*P* for trend=0.03) (*Bazzano et al.,* 2004).

Given the objectives of our study, we conducted the analysis of plant and animal food consumption. In this study, controls had a significantly higher intake of plant food than the cases (791.3 g/d vs. 625.0 g/d; P<0.0001). This was mainly due to the difference in the intakes of fruit and vegetable, while in stratified analyses for CHD, significant association was not observed when the bottom and top quintiles of animal foods consumption were compared. In contrast, in a case control study, which was conducted in Indonesia there was no difference in plant food intake between the two groups. Average intake of plant food was 1,061 g/d for the case group and 1,028 g/d in the control group. Also, the odds ratio for the subjects who consumed animal foods in the highest quartile (above 210 g) to those in the lowest quartile (below 108 grams) was 4.8 (95% CI 2.25-10.30, *P<0.0001*) (Lipoeto et al., 2004), these differences may be related to food culture in this country. However, because of many other potential risk factors among diverse countries, dietary intake alone may not be the mere factor underlying lower CHD incidence.

Conclusion: Our finding revealed that fruit, vegetable, whole grain and plant food intakes independently were associated with CHD events. Thus these food groups could be predicted risk of CHD in this population. However, more studies are required to examine association dietary intake and CHD in Armenia.

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Symptoms of Obsessive Compulsive Disorder and Their Relation to Locus of Control in Armenian Participants

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Abstract: Obsessive compulsive symptom is a debilitating disorder marked by two distinct phenomena: recurrent, disturbing, intrusive thoughts (obsessions) and overt repetitive behaviors or mental acts (compulsions) that are performed to reduce distress caused by obsessions. Locus of control is concerned with the degree to which an individual perceives that she/he has control over a given event, or whether she/he perceives it lays outside his/her control. The study aimed to evaluate correlation of obsessive compulsive symptoms with locus of control. This research emphasized on cognitive and practical approach on OCD. For the current study 150Armenian participants were male40% and 60% were female. Participant'age ranged from 17 to 30in students and the mean age for the samples was 21.78(SD=3.52), and other samples were OCD patients with ranged age 25 to 58 and the mean age was 37.40(SD=9.61). Participants completed Armenian version questionnaire batteries including measure of Levenson locus of control, and Ybocs OCD scale. the multiple liner regression analysis with total OCD scores as the dependent variable had a significant association with powerful others scores. After including this variable, we found (total obsessive compulsive β= .42 F=25.7 T= 4.68 P.V <.01) so result showed robust association between powerful others and obsessive compulsive disorder, scores significantly predicted total OCD scores. Finally, powerful others were strong predictor then subscale obsessive and compulsive attendant severity OCD (obsessive: β= 32 F=11.8 T= 2.9 P.V <.05). Locus of control had only a main effect on obsessive compulsive disorder. That is, high levels of locus of control, indicating a powerful others and chance was associated with higher obsessive compulsive disorders, special obsessive thinking symptoms.
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Keywords: Locus of control; Obsessive-compulsive; Internal; powerful other; chance.

Introduction

Obsessive-compulsive symptoms is a rather disabling condition which is described by recurrent unwanted ideas, thoughts or impulses (obsessions), and repetitive, irresistible and often ritualized behavior (compulsions) to avoid anxiety or to neutralize the obsessions (American Psychiatric Association, 1994) Obsessive–compulsive disorder (OCD) is currently classified as an anxiety disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2000). Indeed, throughout most of the twentieth century, OCD has been regarded as an anxiety disorder or neurosis (Tynes et al., 1990).

OCD is a debilitating disorder marked by two specific phenomena: recurrent, disturbing, intrusive thoughts (obsessions) and overt repetitive behaviors or mental acts (compulsions) that are performed to decrease distress caused by obsessions. (Eisen and Rasmussen, 2002)

Although the specific content of obsessions and compulsions may vary from patient to patient, a common subject matter concerns uncertainty over responsibility and situation is external or powerful others for harm or mistakes. For example, thoughts such as ''I may have unknowingly hit a pedestrian with my automobile'' give rise to urges to check the road for injured people. The life span prevalence rate of OCD has been consistently estimated at 2–3%in the general adult population throughout the world (Angst, 1994; Karno, Golding, Sorenson, & Burnam, 1988), making it one of the more common psychiatric disorders.

Locus of control is concerned with the degree to which an individual perceives that she/he has control over a given event (internal), or whether she/he perceives it lays outside his/her control (external). The underlying presumption on the moderating effect of locus of control on the relationship between obsessive compulsive disorder is that individuals who define stressors as controllable will be more likely to attempt to cope with them through problem focused actions and there by promote existing health states. On the other hand, subjects with an external locus of control are more vulnerable to OC symptom.

The multidimensional Locus of Control construct has been around for about 30 years . (Wallston KA, et al., 1978) It has helped in the understanding of the role of beliefs in the context of health behaviors, health outcomes, and health care

(Luszczynska A, et al. 2005),. Normal locus of control comprises a person's beliefs regarding where control over his or her illness lies. A person's normal locus of control orientation is one of several factors that determine which health-related behaviors a person will perform.

These health-related behaviors, in turn, partially determine a person's health status (Wallston KA,et al. 1994) For example, Patients with spinal cord injury (SCI) who approved an internal locus of control experienced greater well-being and decreased pain feeling of SCI than those who approved an external locus of control. On the other hand, patients with SCI who accepted an external locus of control experienced more psychological distress, physical disability, lower self-esteem, and more helplessness and hopelessness than those who were not using an external locus of control (Chung MC et al2006),.In one study, compared with patients with low internal locus of control, patients with medium and high internal locus of control lived longer after lung transplant. (Burker EJ, et al, 2005). Some evidence suggest the observation that anxiety and powerful others health locus of control (PHLC) were significantly related to self-perception of precipitants such as stress and lack of sleep in epilepsy patients (Sperling et al 2006)that psychological adjustment and modification, adjustment may be one mechanism by which perceptions of control can affect extension of life or existence (Burker EJ, et al, 2005).

Method

For the current study 150Armenian participants were male40% and 60% were female. Participant'age ranged from 17 to 30in students and the mean age for the sample was 21.78(SD=3.52), and other samples were OCD patients with ranged age 25 to 58 and the mean age was 37.40(SD=9.61). Participants completed Armenian version questionnaire batteries including measure of Levenson locus of control, and Ybocs OCD scale In patient with a primary OCD according to DSM-IV criteria were recruited,. The other samples are students recruited at the university were selected for this cross-sectional study. The participants comprised 110 under graduate and post graduate students without record in concealing center and other groups are co morbid psychiatric disorder patients in hospital and center of counseling in clinical psychology in Yerevan sity, consecutively referred to a specialized OCD program of the 40 patients, OCD subjects have OCD in their life, and the other110 students without OCD symptoms.

Instruments

Yale-Brown Obsessive–Compulsive Scale: The Yale-Brown Obsessive–Compulsive Scale (Y-BOCS; W.K. Goodman et al., 1989; Goodman, Price, Rasmussen, & Mazure, 1989)is a widely-used semi structured, clinician-administered measure that assesses the severity of obsessions and compulsions. Ratings are based on information provided by the patient and surety, as well as clinical observations. The Y-BOCS is administered in two parts: first, clinicians utilize a symptom checklist to determine the types of obsessions and/or compulsions experienced by the patient. Next ,severity of these obsessions and compulsions are rated using a five point Likert scale ranging from 0 to 4, with higher scores indicating greater severity. The 10severity items, which assess distress, frequency, interference, resistance, and symptom control, yield three scores: an Obsessions Severity Score (range = 0–20), a compulsions Severity Score (range = 0–20) and a Total Score (range = 0–40). Six additional items examine features that can be used to aid with differential diagnosis and treatment (e.g., degree of insight, avoidance).

Levenson locus of control scale: Locus of control was measured with Levenson,I,P and C scales. Each scale includes eight items and is designed to measure the extent to which individuals believe that outcomes are due to their own actions, to powerful others or to chance. Participants asked to rate each statement on a 4point likert scale with 1=strongly disagree 4 strongly agree (petrosky,birkimer 1991). The Rotter(1966) I-E locus of control assesses an individual's attributions of control as being either internal(I) external(E) Levenson(Levenson1973,) modified I-E scale to distinguish attribution of control to other persons, powerful others(P) from such other external factors as fate or lock ,which she categorized as chance(C)Thus, her multidimensional instrument contains three separate I,P and C scales. In doing so,Levenson also attempted to reduce the biases in the Rotter . Levenson scale has Reliability and validity that had been identified by numerous researchers (Garcia, C., & Levenson, 1975).

Result

Regression statistics and item and reliability analysis calculated with SPSS version 19 ,Table 1 shows the mean severity of symptoms assessed by means and other indicator at the first time point of the examination under graduate students (B.S)showed a greater OCD symptoms than post graduate students (M.A) (BS :X=42 and MA : X=31.78). At the second time point patients of the obsessive compulsive disorder showed a greater total of OCD symptoms (patient=88). At the third time no differences

internality were found between patients, under graduate student and post graduate students (BS: X=33.10 MA: 33.11patients: X=32).

Scores on the two OCD subscales were very similar to scores obtained in the Study BS Scores with other student samples(M.A) in powerful others (BS:X=16.6, M.A=15.73) Scores on the powerful others in patients were found (M= 31.68, SD= 9.01); were also fairly consistent with data from previous student samples.

Table 2 presents descriptive information and the inter-correlations of the measures of the current study. As can be seen from Table 2, all measures had adequate internal consistency coefficients.

Separate hierarchical multiple regression analyses were performed in order to examine the impact of, locus of control and their interaction on the total OC scores, and on the two OC symptom clusters (obsessive thinking and compulsive behavior).

The first regression analysis in students was performed to examine whether the interaction of locus of control would predict total OC scores above and beyond the main effects of powerful others attitudes and locus of control. Table 2 presents the summary statistics for the multiple regression analysis with total OCD scores as the dependent variable had a significant association with powerful others scores. After including this variable, we found (total obsessive compulsive β= .42 F=25.7 T= 4.68 P.V <.01) so result showed robust association between powerful others and obsessive compulsive disorder, scores significantly predicted total OCD scores. Finally, powerful others were strong predictor then subscale obsessive and compulsive attendant severity OCD (obsessive: β= 32 F=11.8 T= 2.96 P <.05).

These probes revealed in table3 that subjects with powerful others attitudes, OCD symptom

severity was lower among those with internal locus of control as compared to those with chance locus of control (chance:β=.36 ,F= 24.5 ,T= 4.04 ,P.V <.01)(internal:β= .16 ,F= 24.5,T= 1.91,P.V <.059)However, for Participants with low internality attitudes, simple slope was not significant, indicating that their levels of OC symptoms were low regardless of their levels of locus of control. In other words, when OCD was high, the presence of powerful others and chance locus of control orientation increased the scores, whereas the occurrence of internality had a dampening effect by producing lower OCD scores. When the relationship scores were low, locus of control level did not influence the internal scores.

Factor analysis correlation Matrix coefficients were computed to examine the relationship between the OCD and the measures of symptom severity and cognition obsession and compulsion level of (P <.01). The results of this analysis, which are displayed in Table4, revealed a moderately strong and significant relationship between the OCD and the obsessing subscale of the Y-bocs. However, no other significant relationships with internal subscales were detected. In addition, the internality was not related to either the Y-BOCS total or subscale scores,(OCD with powerful others R=.663 but OCD with internal locus of control R=.060)A number of cognitive measures obsessive were significantly related to the chance(R=.50)

Table 1: General data on participants

demography in State under graduate students post graduate and patients,						
Variables	under graduate students		post graduate		patients	
	Mean	S D	Mean	S D	Mean	S D
OCD total	42.97	23.67	31.78	24.11	88.11	15.71
Internal	33.10	7.71	33.11	9.66	32.57	6.6
Powerful others	16.69	8.93	16.12	9.72	31.86	9.01
Chance	22.45	7.32	19.78	8.32	32.57	7.63
Obsessive	7.69	4.33	4.49	3.58	10.93	2.91
Compulsive	7.07	4.32	4.11	3.58	11.86	2.41
Severity	14.72	8.40	8.91	7.00	22.19	4.26

Table 2 Regression analyses on variables of locus of control and O C D in Students

Locus of control	internal				chance				Powerful others			
OCD	β	F	T	P-value	β	F	T	P.V	β	F	T	P-value
OCD total	.17	25.7	2.7	<.27	.42	25.7	3.845	<.01	.42	25.7	4.68	<.01
Obsession	.20	11.8	2.00	<.41	.25	11.8	2.07	<.05	.32	11.8	2.96	<.05
Compulsive	.16	9.33	1.6	<.97	.21	9.33	2.7	<.05	.32	9.33	2.0	<.05
Severity	.19	10.4	1.97	<.52	.22	10.4	2.12	<.05	.34	10.4	3.16	<.05

P<.01 and p<.05

Table 3: Regression analyses on variables of locus of control and O C D in patients

Locus of control	internal				chance				Powerful others			
OCD	β	F	T	p.v	β	F	T	P.V	β	F	T	P.V
OCD total	.19	19	1.57	<.014	.33	19	1.6	<.12	.61	19	3	<.05
Obsession	.071	.69	-.24	<.81	.007	.69	-.001	<.99	.40	.69	.16	<040
Compulsive	.16	7.4	1.6	<.11	.18.7	7.4	1.6	<.05	.30	7.4	2.77	<.05
Severity	.19	8.30	2.90	<.06	.19	8.30	1.8	<.07	.31	8.30	2.90	<.05

P<.01 and p<.05

Table 4: relationship among variables with factor analysis

Correlation Matrix

Correlation	1	2	3	4	5	6	7
OCD total	*						
internal	.069	*					
Powerful others	.665	-.244	*				
chance	.672	.115	.581	*			
severity obsessive	.720	.094	.475	.508	*		
severity compulsive	.715	.102	.488	.479	.884	*	
Severity OCD	.725	.108	.491	.492	.963	.967	*

P<.01

Discussion:

Recent studies on the importance of dysfunctional beliefs as well as attitude to internal locus of control or externality that characterize, suggest that the description of subtypes requires an examination of cognitive underpinnings that are potentially connected to the etiology and maintenance of symptoms. For example, effective treatment for obsessions without overt compulsions (e.g., Freeston et al., 2001)

The aims of this study were to investigate relationships between obsessive compulsive disorder and locus of control; and to examine the independent and relative contributions of obsessive severity and compulsive behavior .present study were found relationship between OCD and powerful others locus of control and this research were consistent with previous stud y that studied also highlights the importance of obsessive compulsive cognitions in the prediction at baseline of the symptoms of Mental Control, Contamination and Checking, but not of Obsessive impulses, independent of internal locus of control This is consistent with the results of many studies that have found that dysfunctional beliefs measured by scales can predict OC symptom (Julien, O'Connor, Aardema, &Todorov, 2006; Tolin, Woods, & Abramowitz, 2003). The study showed not only the capacity of dysfunctional beliefs to predict the development of obsessive and compulsive symptoms, but above all, dysfunctional beliefs are risk factors in the development of obsessions and compulsions following stressful events. Our study thus revealed stability in obsessive compulsive symptoms conceptualized as categories and a close relation between dysfunctional beliefs in general and symptomatology associated with impaired mental control, Contamination and Checking. (Novara, C et al., 2011).

Approach to locus of control is a cognitive in obsessive compulsive disorder and previous study was consistent with present study that found moderate associations between a range of obsessive beliefs and OCD severity, and certain obsessive beliefs continued to predict specific OCD .These

findings broadly replicate and extend findings of Abramowitz et al. (2009). And the other hand we favor a ‘‘cognitive’’ rather than a ‘‘neurological’’ understanding of obsessions (Moritz et al., 2007; Obsessive Compulsive Cognitions Working Group, 1997, 2001, 2003).

Present study showed Relationship between obsessive with powerful others locus of control were stronger than relation between compulsive and powerful others or chance and this result was indication that obsessive compulsive has cognitive approach and result of this research supported by result that found , metacognitive variables of need to control thoughts, beliefs about uncontrollability and danger, and fusion beliefs were also positively correlated with O C symptoms replicating previous findings (Myers S., Wells A., 2004).

This study has been suggested that perceived disruption of control and locus of control is important in maintaining anxiety and obsessive compulsive symptom. In particular, based on the review of the theoretical literature and empirical studies, it was argued that incorporation of the concepts might prove important to etiological theories of OCD. Given its phenomenology, it is perhaps surprising that such little attention has been given to the role of control cognitions in OCD. However, there is evidence that both anxiety and OC symptoms are associated with lowered levels of sense of control, and weaker evidence that OC symptoms are associated with elevated levels of desired control, both over thoughts and the environment. More importantly, a discrepancy between the concepts, where the desired level of control is not attained, may be an important factor in driving compulsive actions. This conceptualization may help to account for motivational aspects of the disorder (O'Kearney., 1998).

Between-group comparisons post graduate students reported lower OCD than under graduate students. Results were accepted because students in level of post graduate have a stable situation in developmental life.

Conclusion

Locus of control had only a main effect on obsessive compulsive disorder. That is, high levels of locus of control, indicating a powerful others and chance was associated with higher obsessive compulsive disorders, special obsessive thinking symptoms .According to this result, it seems that locus of control exerts an impact more on the thinking symptom than compulsive disorder

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Study of Serum Tumor Necrosis Factor Alpha and Interleukin 6 in Type 2 Diabetic Patients with Albuminuria

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Abstract:Background: Chronic kidney disease (CKD) is one of the major complications of type 2 diabetes and is the leading cause of end stage renal disease (ESRD). There are Growing evidences indicating that chronic low-grade inflammatory response is a recognized factor in the pathogenesis of development and progression of diabetic renal injury. The aim of this study was to analyze the relationship between inflammatory markers tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) with urinary albumin excretion (UAE) as a marker of renal injury. **Methods:** A total of 73 type 2 diabetic patients were divided into three groups according to urinary albumin excretion, normoalbuminuria, microalbuminuria and macroalbuminuria In addition, 10 apparently healthy subjects were included as control group. TNF-α, IL-6, Glycated hemoglobin (HbA1c) and creatinine were measured in all population. Urinary albumin excretion was measured by morning spot sample albumin / creatinine ratio (ACR). **Results:** Levels of TNF-α and IL-6 were found to differ significantly among studied groups. Both markers showed significant positive correlation with duration of diabetes, Albumin creatinine ratio and glycated hemoglobin and showed significant negative correlation with estimated glomerular filtration rate (eGFR), however TNF-α showed a better correlation with these variables when compared with IL-6. Stepwise regression analysis demonstrated that TNF-α is the independent predictors for ACR in total population with adjusted R^2 0.512 and P value less than 0.01. **Conclusion:** TNF-α and IL-6 are higher in type 2 diabetic patients with albuminuria and correlate well with the severity of albuminuria, however TNF-α was found to be a predictor of ACR suggesting the possible role of TNF-α in pathogenesis and progression of renal affection in type 2 diabetic patients.

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Keywords: Diabetic nephropathy; Albuminuria; tumor necrosis factor alpha and interleukin 6.

1. Introduction

The global diabetes burden is predicted to rise to 366 million by 2030 and would present itself as a major health challenge (1). Chronic kidney disease (CKD) is one of the major complications of type 2 diabetes and is the leading cause of end stage renal disease (ESRD) (2).The literatures are replete with studies about the involvement of innate immune system and low grade inflammation in pathogenesis of DM. and there is now clear evidence that inflammatory markers, acute-phase reactants and proinflammatory cytokines are strongly associated with the risk of developing type 2 diabetes (3-10). Based on this perspective several studies were conducted to address the role of this inflammatory cytokines in the development of diabetic complications. The exact mechanisms leading to the development and progression of renal damage in diabetes are not yet completely known. Growing evidence indicates that activation of innate immunity with the development of a chronic low-grade inflammatory response is a recognized factor in the pathogenesis of this disease (11). Cytokines are a group of pharmacologically active, low molecular weight polypeptides that possess autocrine, paracrine, and juxtacrine effects. These molecules cluster into several classes (i.e. interleukins, tumor necrosis factors, interferons, colony-stimulating factors, transforming growth factors and chemokines) (12). The cytokine TNF-α is a well-known member of the TNF superfamily consisting of 157 amino-acid peptide produced mainly by monocytes, macrophages, B and T lymphocytes. TNF signals through two distinct receptors TNFR1 and TNFR2 thereby controlling expression of cytokines, immune receptors, proteases, growth factors and cell cycle genes which in turn regulate inflammation, survival, apoptosis, cell migration, proliferation and differentiation (13). Human IL-6 composed of 184 amino acid and produced by various types of lymphoid and non-lymphoid cells, such as T cells, B cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangium cells, and several tumor cells (14). Different inflammatory molecules, including pro-inflammatory cytokines such as TNF-α and IL-6 play a critical role in the development of micro vascular diabetic complications, including nephropathy (15-16). The objective of the present study is to examine the relation between inflammatory cytokines TNF-α and IL-6 with urinary albumin excretion (UAE) in type 2 diabetic patients in early stages of diabetic kidney disease.

2. Patients and Methods

The protocol for this study followed the ethical standards and approved by the ethical committee of our institution and all subjects gave informed consent to participate in this study. This study was conducted on 73 type 2 diabetic patients, 29 males and 44 females. In addition, 10 apparently healthy, age and gender matched, subjects were involved in this study as a control group (5 males, 5 females).

Patients with the following criteria were excluded: age over 70 years, body mass index (BMI) above 30, current acute infection or any acute illness, systemic hypertension defined as blood pressure more than 140/90, history of ischemic heart disease, cerebrovascular stroke, malignancy, dyslipidemia, hepatic disorders, smokers, hematological diseases and patients with serum creatinine more than 1.5 mg/dl.

Patients were divided according to UAE which was measured by early morning spot urine sample for albumin creatinine ratio (ACR) into 3 groups, diabetic without microalbuminuria (ACR less than 30 mg/gm), diabetic with microalbuminuria (ACR between 30 – 300 mg/gm) and diabetic with macroalbuminuria (ACR more than 300 mg/gm).

All subjects underwent full history taking and clinical examination including measuring blood pressure weight and height. Mean arterial pressure (MAP) was calculated as {(2 x diastolic blood pressure (mmHg) + systolic blood pressure (mmHg)}/3. BMI Was calculated as weight (Kg)/ {Height (m)} ^{2} GFR was estimated using Modification of Diet in Renal Disease Abbreviated Equation (MDRD):

[GFR = 186 × (serum Cr)$^{-1.154}$× (age)$^{-.203}$× (0.742 if female) × (1.210 if African American)] (17).

Laboratory assessment

Blood samples were collected by sterile venipuncture and divided into 2 parts; the first part was collected on dipotassiumethelenediamine tetra-acetic acid (EDTA) tube for glycated hemoglobin (HbA1c). The second part was transferred into another plain vacutainer tube, left to clot, then centrifuged for 10 minutes at 4000 r.p.m. and the serum obtained for determination of TNF α and IL-6 was kept frozen at -20 ºC till analysis. Early morning 10- 20 ml of midstream urine was collected for measurement of urinary albumin and creatinine for measurement of albumin creatinine ration (ACR). ACR was calculated using the following equation: ACR = Albumin mg/ d l ÷ Creatinine g/ dl

HbA1c was measured using quantitative colorimetric measurement of glycohemoglobin as percent of total hemoglobin using kits supplied by STANBIO LABORATORY, USA.

Albumin in urine was estimated by Beckman's microalbumin test kit on Synchron CX9 autoanalyser.Beckman.Urine creatinine is measured by a modified rate Jaffe method.

Serum TNF-α was determined by enzyme linked immunosorbent assay method, using IDELISA^{TM} Human TNF-α ELISA kit. It utilizes a monoclonal antibody (capture antibody) specific for human TNF α coated on a 96-well plate. Standards and samples are added to the wells, and any human TNF α present binds to the immobilized antibody. The wells are washed and biotinylated polyclonal anti-human TNF-α antibody (detection antibody) is added. After a second wash, avidin-horseradish peroxidase (avidin-HRP) is added, producing an antibody-antigen-antibody sandwich. The wells are again washed and a substrate solution is added, which produces a blue color in direct proportion to the amount of human TNF-α present in the initial sample. The stop buffer is then added to terminate the reaction. This results in a color change from blue to yellow. The wells are then read at 450 nm

Serum IL-6 was determined by enzyme linked immunosorbent assay method using AviBion Human IL-6 ELISA kit, Ani Biotech Oy, Orgenium Laboratories Business Unit, FINLAND. The assay employs an antibody specific for human IL6 coated on a 96-well plate. Standards, samples and biotinylated anti-human IL6 are pipetted into the wells and the IL6 present in a sample is captured by the antibody. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed. Following this second wash step, TMB substrate solution is added to the wells resulting in color development proportional to the amount of IL6 bound. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm.

Statistical Evaluation

We used the statistical package of social signs (SPSS, version 16) to perform the analysis. Correlation between inflammatory markers (TNF-α and IL-6) with duration of diabetes, HbA1c, albuminuria and eGFR was performed by pearson correlation, one way ANOVA test or Kruskalwalis test was used as appropriate for comparison of quantitative variables among more than two independent groups. Multiple stepwise regression analysis was performed to determine the possible predictor for ACR among potential risk factors including inflammatory markers. P value ≤ 0.05 was considered significant.

3. Results

The cohort was divided according toACR into 3 groups. Group I; diabetics with normoalbuminuria, group II; diabetics with microalbuminuria and group III; diabetics with macroalbuminuria in addition to group IV; control group. Baseline characteristics and comparison of the studied groups are shown in table 1.

The groups are matched regarding age, sex, BMI and mean arterial blood pressure. Serum TNF-α and IL-6 differed significantly among the studied groups (Figure 1). Pearson's correlation coefficients (r) between serum TNF-α and Duration of diabetes mellitus, HbA1c & ACR showed a significant positive correlations with *P* value less than 0.001, while it showed a significant negative correlation with eGFR with *P* value less than 0.001.(Figure 2). IL-6 also showed significant correlations with the same parameters but with less r value (*P*< 0.01) (Figure 3). Interestingly both TNF-α and IL-6 correlated significantly positive with r value 0.521 and P value less than 0.001. Stepwise regression analysis demonstrated that TNF-α is an independent predictor for ACR in total population with adjusted R^2 0.512 and *P* value less than 0.01.

Table 1: Baseline characteristics and comparison of the studied groups.

	(Group I) Diabetics with normoalbuminuria ACR (mg/gm) 17.74 + 9.32 (n= 23)	(Group II) Diabetics with microalbuminuria ACR (mg/gm) 77.74 + 65.02 (n= 34)	(Group III) Diabetics with macroalbuminuria ACR (mg/gm) 468.69 + 57.40 (n=16)	(Group IV) Control group ACR (mg/gm) 4.40 + 4.01 (n= 10) Mean ± SD	*P* value
*Age (years)	55.96 ± 6.20	57.12 ± 7.65	61.31 ± 6.42	55.10 ± 6.72	> 0.05
**Sex (M/F)	7/16 (30.4/69.6%)	16/18 (47.1/52.9%)	6/10 (37.5/62.5%)	5/5 (50/50%)	> 0.05
*Duration of D M (years)	7.17 ± 2.95	14.85 ± 5.59	21.25 ± 6.01	-	< 0.001
*MAP (mmHg)	94.78 ± 4.09	94.36 ± 4.14	93.33 ± 3.16	91.50 ± 5.58	> 0.05
*BMI	27.35 ±1.76	28.29 ± 1.50	28.42 ± 1.36	27.63 ± 1.40	> 0.05
*HbA1c	7.33 ± 1.56	9.25 ± 1.52	11.80 ± 1.55	5.57 ± 0.42	< 0.001
*eGFR (ml/min/1.73m^2)	81.96 ± 26.41	68.32 ± 18.15	56.14 ± 12.92	83.56 ± 25.40	< 0.01
*TNF-α (pg/ml)	65.56 ± 69.39	173.24 ± 84.95	312.69 ± 75.56	13.50 ± 3.31	< 0.001
*IL-6 (pg/ml)	96.52 ± 151.29	214.91 ± 165.40	346.75 ± 191.70	5.20 ± 3.36	< 0.001

*: Mean ± Standard Deviation, **: Number and percentage, M/F: Male/Female
D M: Diabetes Mellitus, MAP: Mean arterial pressure, BMI: Body mass index, HbA1C: glycated hemoglobin, ACR: Albumin creatinine ratio, eGFR: Estimated glomerular filtration rate, TNF-α: Serum tumor necrosis factor alpha, IL-6: Interleukin 6.

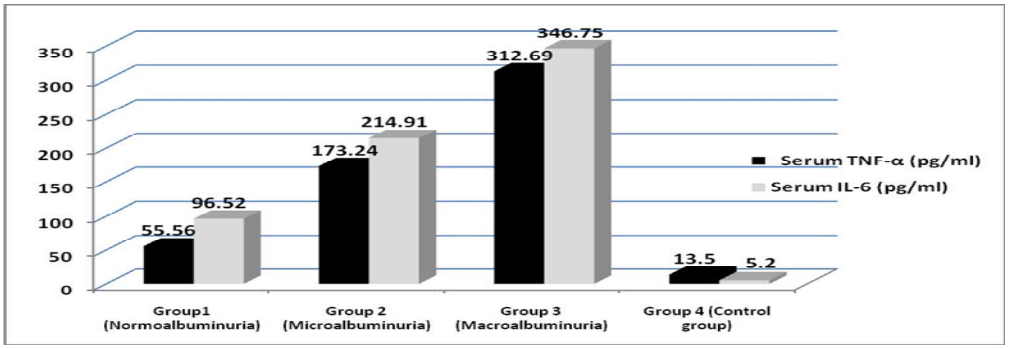
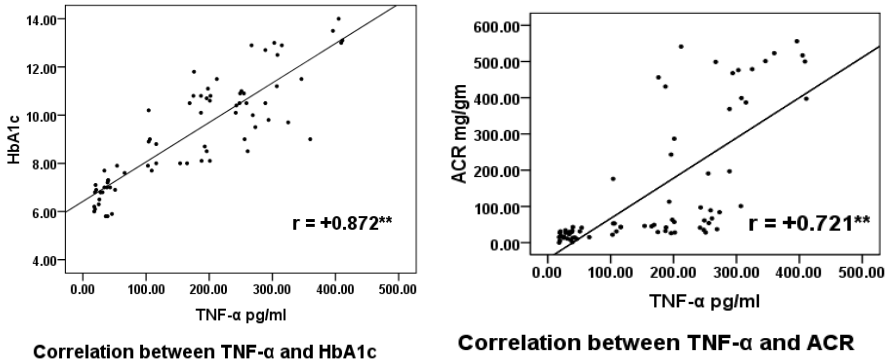
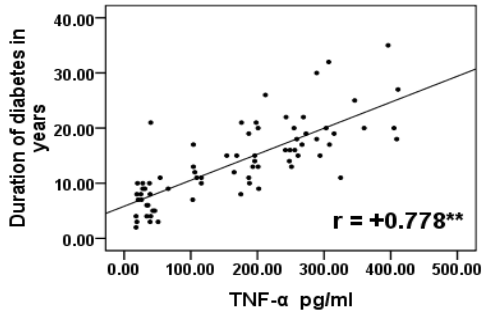
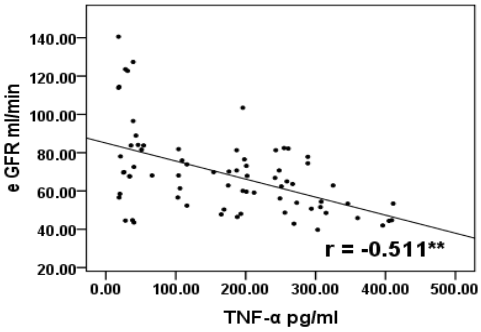


Figure 1: Comparison of TNF-α and IL-6 among the studied groups





correlation between TNF-α and duration of diabetes in years

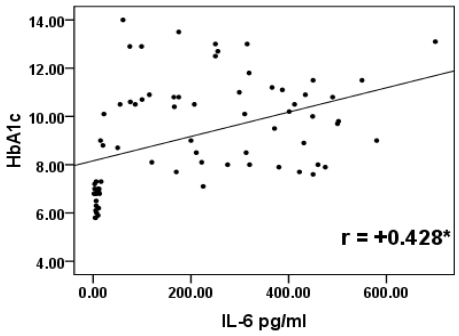


Correlation between TNF-α and e GFR

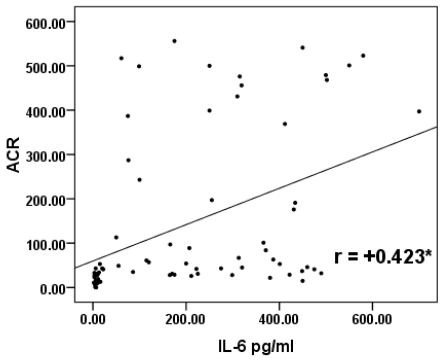
**: P value < 0.001

TNF-α: Serum tumor necrosis factor alpha,HbA1C: glycated hemoglobin, ACR: Albumin creatinine ratio e GFR: Estimated glomerular filtration rate

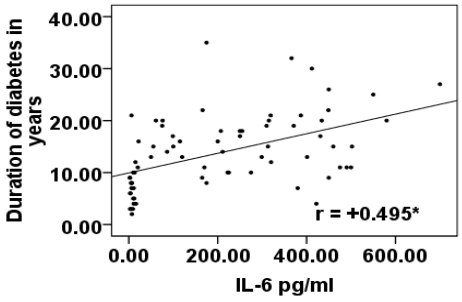
Figure 2: correlation between TNF-α with HbA1c, ACR, duration of diabetes in years and e GFR



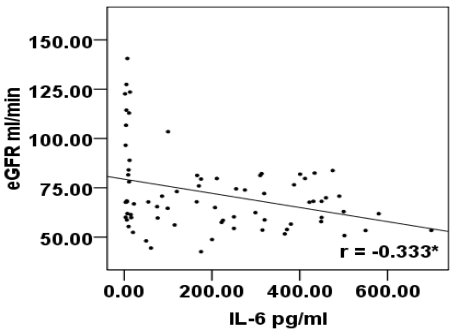
Correlation between IL-6 and HbA1c



Correlation between IL-6 and ACR



Correlation between IL-6 and duration of diabetes



Correlation between IL-6 and eGFR

*: P value < 0.01

IL-6:Serum Interleukin 6, HbA1C: glycated hemoglobin, ACR: Albumin creatinine ratio, eGFR: Estimated glomerular filtration rate,

Figure 3: correlation between IL-6 with HbA1c, ACR, duration of diabetes in years and eGFR

4. Discussion:

Diabetic nephropathy is one of the major microvascular complication of type 1 and type 2 diabetes mellitus and the leading cause of end stage renal disease. It was thought to be a result from interactions between hemodynamic and metabolic factors, however research during the past 10 years has provided insight into the etiology of diabetic nephropathy at the cellular and molecular level, and inflammation has emerged as being a key pathophysiological mechanism (18). Clinical investigations have implicated the roles of TNF-α and IL-6 in the development of diabetic nephropathy, suggesting that inhibition of those cytokines is promising remedy to ameliorate diabetic nephropathy.

In this present study we investigated the role of inflammatory cytokines TNF-α and IL-6 in relation to albuminuria in patients with type 2 diabetes. In our study we found that TNF-α and IL-6 differed significantly among studied groups. Furthermore there

were a significant correlation between each marker and HbA1c, albuminuria, eGFR & duration of diabetes. These results are consistent with other investigators who found higher levels of inflammatory cytokines among diabetic patients with albuminuria. Moriwaki et al. reported higher levels of TNF-α and IL-6 among diabetic patients without albuminuria compared to those with albuminuria and concluded that TNF-α and IL-6 may have some etiopathogenic roles in diabetic nephropathy (19). In a study included 95 diabetic patients conducted by Refaat H. et al. they found higher levels of serum and urine TNF-α in diabetic group with proteinuria and similar to our results found a good correlation between serum TNF-α and protein / creatinine ratio, HbA1c & duration of diabetes mellitus (20). Consistent with our results Maulana Azad studied IL-6 in 60 diabetic patients divided into three groups (normo, micro and macroalbuminuria) and found that IL-6 was higher in diabetic patients with macroalbuminuria compared to patient with microalbuminuria and normoalbuminuria and they also found a good correlation between IL-6 with HbA1c and urinary albumin excretion which was similar to our results but with a better r values (21). In our study stepwise regression analysis demonstrated that TNF-α was an independent predictor of urinary albumin excretion while IL-6 was not. In accordance with us Ng Dp et al. demonstrated that TNF-α system is likely to exert independent effects on albuminuria and renal function in type 2 diabetes while C reactive protein and IL-6 did not show that (22). Serum and urine TNF-α were found to be independently and significantly associated with ACR in diabetic patients (20, 23). Many investigators demonstrated a structural damage associated with inflammatory cytokines. Dalla Versa et al studied 74 type 2 diabetic patients regarding acute phase markers of inflammation including IL-6 in relation to structural kidney damage determined by mesangial fractional volume and glomerular basement membrane (GMB) width in a kidney biopsy and they found that IL-6 is significantly higher in group with increased GBM thickness and linear regression analysis demonstrated that IL-6 is one of the predictors of GBM thickness (24). Renal IL-6 expression has been related to mesangial proliferation, tubular atrophy and the intensity of interstitial infiltrates in diverse models of renal disease, suggesting a contributing role in the progression of renal disease (25). Experimental studies have demonstrated that urinary albumin excretion significantly correlates with renal cortical mRNA levels and urinary TNF-α excretion in animal models of diabetic nephropathy (26–27). Of more interest TNF-α inhibition by Infleximab reduced urinary albumin excretion in diabetic rat (28).

Conclusion

Serum TNF-α and IL-6 are elevated in diabetic patients with albuminuria and their levels are significantly higher with increasing levels of albuminuria. Both markers correlated well with ACR however TNF-α showed a better correlation than IL-6, moreover TNF-α found to be a predictor of ACR. This can suggest a possible role of TNF-α in pathogenesis and progression of renal injury in diabetic patients. Further studies are needed to confirm our finding and to study the possible role of TNF-α inhibitor in the prevention and treatment of diabetic nephropathy.

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3/2/2012

A Prospective Study Comparing Lidocaine 2% Jelly versus Retrobulbar Anesthesia in 23-G Suturless Vitrectomy for Macular-Based Disorders: Efficacy and Intraocular Pressure

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Abstract: Purpose: To compare Lidocaine 2% Jelly versus retrobulbar anesthesia on efficacy and IOP in 23-G suturless vitrectomy for macular-based disorders. **Materials and Methods**:A prospective clinical trial was conducted on 40 patients allocated into two equal groups; group 1 received topical lidocaine 2% Jelly and group 2 received retrobulbar anesthesia (6ml volume of bupivacaine 0.5% solution with 10 IU/ml hyaluronidase). Both groups received a standardized sedative consisting of midazolam, fentanyl and /or propofol intraoperatively. All patients underwent a 23 G-suturless vitrectomy for macular-based disorders. IOP was measured in both groups, immediately before and after anesthesia application, and at 5 and 10 minutes after application before start of surgery. Pain scores were assessed using a numerical visual analogue scale immediately after surgery. Patient comfort, physician assessment of intraoperative patient's compliance, need for supplemental anesthesia, volume of local anesthetic used and any complications were recorded. **Results**: There was a statistical significant variation in elevation in mean IOP in group 2 (retrobulbar group) compared to group 1 (lidocaine 2% Jelly) ($P< 0.01$). Mean IOP was elevated only in group 2 after injection and was reduced at all time-intervals. The two groups did not vary significantly in subjective pain score and surgeon's satisfaction scale. A statistical significant difference was noted regarding anesthetic supplement being more in group 1 (topical group) compared to group 2 (retrobulbar group).**Conclusion**: Topical Lidocaine 2% Jelly is as effective as retrobulbar anesthesia for pain control in patients undergoing 23G suturless vitrectomy for macular-based disorders. Lidocaine 2% Jelly is similar to retrobulbar anesthesia regarding patient's comfort and surgeon's satisfaction. Moreover, the Lidocaine 2% Jelly is found to have fewer effects on IOP prior to surgery. Lack of akinesia in this group (group 1) also did not prevent or hinder a successful surgical outcome.

[Ehab El Zakzouk; Sherif Emerah; Ayman Shouman; Mona Raafat, and Hala Bahy **A Prospective Study Comparing Lidocaine 2% Jelly versus Retrobulbar Anesthesia in 23-G Suturless Vitrectomy for Macular-Based Disorders: Efficacy and Intraocular Pressure**. Life Science Journal 2012; 9(1):883-887]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 129

Key words: Topical Anesthesia - Lidocaine Jelly - retrobulbar - suturless vitrectomy.

1. Introduction:

Topical anesthesia was first proposed by Fichman (**Feibel, 1985**) as an attractive alternative to the traditional method of injecting local anesthetic agents, resulting in faster visual recovery and high patient satisfaction[1]. The advantages of topical anesthesia include its ease of application, minimal to absent discomfort on administration, rapid onset of anesthesia and most important of all, elimination of the potential risks associated with other injections (retro, peri and subtenon) [2,3] .

Topical anesthesia has been successfully used by different authors for cataract surgery, trabeculectomy and phacotrabeculectomy surgeries [4-6] . **Yepez *et al.*** published a prospective study on 134 eyes operated with standard 20-gauge vitrectomy under topical anesthesia (4% Lidocaine drops)and preoperative or intraoperative sedation with various vitreoretinal diseases[7]. According to the authors, all patients experienced mild pain or discomfort during pars plana sclerotomies external bipolar cautery and conjunctival closure[4,7]. Now, with 23-G suturless vitrectomy technique becoming increasingly popular because of the decreased surgical trauma, faster wound healing and improved postoperative comfort, it was done under topical anesthesia with different authors [4,7-9].

In this study, we compared retrobulbar anesthesia to unpreserved lidocaine 2% Jelly in 23-G suturless vitrectomy, but specifically for macular based disorders regarding the efficacy and intraocular pressure changes.

2. Materials and Methods

Approval from the Ethical Committee in the Research Institute of Ophthalmology (RIO) and informed consent from the patients were obtained. Forty eligible patients scheduled for 23-G suturless vitrectomy for macular based disorders (namely idiopathic macular hole and epiretinal gliosis) at the RIO were enrolled in this study. Inclusion criteria were patients who were eligible to perform this procedure under local or topical anesthesia.

Exclusion criteria were patients with bleeding disorders, dementia or mental instability, deafness, movement disorders, hyperanxiety and inability to complete the visual analogue scale (VAS) of pain line (for example; confusion, communication barriers, visual impairment). No patients received sedatives before entering the operating theatre.

Patients were allocated into two groups; 20 patients in each group. Group 1 received lidocaine 2% Jelly and group 2 received retrobulbar anesthesia. On entrance to operating room (OR), patients were cannulated and a standardized mild intravenous sedation regimen was administered by one of the anesthetists of the study. Patients were instructed to be able to interpret the pain by the VAS scale. The sedation consisted of midazolam hydrochloride 1mg/ml, fentanyl citrate 0.05 mg/ml and propofol 10 mg/ml. This sedative mixture was used in all patients in the study. The dose of intravenous sedation was defined as low (a total intraoperative dose of midazolam < 3 mg, fentanyl < 85 µg and propofol < 70 mg) or high (a total intraoperative dose of midazolam > 3mg, fentanyl > 85 µg, propofol > 70 mg). The patients in both groups were monitored by an EGG, pulse oximetry and non-invasive blood pressure manometer. The patients in group 1 (Lidocaine 2% jelly; n = 20) received 0.2 ml of unpreserved lidocaine 2% jelly (xylocaine, Astra Zeneca, Mississauga, Canada) both in the superior and inferior conjunctival fornices 10 minutes before surgery. Additional lidocaine 2% jelly was inserted into both fornices at the start of surgery and supplemented if needed. The patients in group 2 (retrobulbar; n = 20) received local anesthetic mixture through the retrobulbar technique where a 27 G needle was introduced transcutaneously into the inferotemporal quadrant of the orbit at the junction of the lateral third and medial two-thirds of the inferior orbit rim and running tangentially to the globe. Passing initially close to the orbit floor and medially, the globe equator is passed, at this time the angle of direction was adjusted upwards and the needle advanced to enter within the muscle cone posterior to the globe. 6 ml of bupivacaine 0.5% with 10 IU/ml hyaluronidase was then injected after gentle aspiration was done[10].

Intraocular pressure (IOP) was measured in both groups, immediately before anesthesia application immediately after anesthesia application and at 5 and 10 minutes after application before start of surgery.

Patients were instructed to inform the surgeon with any pain or discomfort throughout the procedure.

All surgeries were performed by the three surgeons included in the study using the same technique for macular-based disorders namely macular hole and epiretinal gliosis.

Three 23-G- transconjunctival sclerotomy ports were created for infusion and illumination and introducing the vitrectomy probe (Alcon Laboratories Inc., Fort Worth, TX, USA). To create the 23-G-port, the conjunctiva was displaced by approximately 1-3 mm with 2 pressure plates.

A 23-G trocar cannula was first inserted through the conjunctiva and sclera, parallel and 3.5 mm posterior to the limbus and then at an angle of approximately 5° until it just passed the end of the bevel. At that point, the handle was slightly raised to an angle of approximately 30° and the cannula was then inserted into the hub.

The trocar was removed while the cannula was stabilized with a forceps. The same surgical technique was applied to introduce the illumination and the vitrectomy probe through the other two 23-G-ports. A complete vitrectomy was performed. Injection of a membrane blue dye (DORC, Inc, Holland) was performed to stain the ILM (internal limiting membrane) through the vitrectomy probe port via a cannula. Then, an ILM removal forceps was introduced through the same port to remove the ILM (macular rehexis). Air-fluid exchange, then injection of SF6 (sulfur hexaflouride) was done at the end of the operation.

During the procedure, the surgeons were in constant communication with the patients to assess their compliance and if additional anesthesia was needed. In cases of severe unbearable pain during the procedure, supplemental topical anesthesia was given in group 1, and a medial canthus injection was given in group 2. The total volume of anesthetic used was calculated.

If pain still persisted, additional sedative mixture was given I.V. and the total volume was calculated. Patient comfort and pain were evaluated immediately after surgery by an independent observer using the visual analogue score (VAS). The VAS scale was incorporated into the experimental design and pain score was illustrated on a 100 mm line with end values of “no pain” and “pain could not be worse” corresponding to the extremes of pain intensity.

Subsequently, the independent observer also collected surgeon’s responses to complete a five point satisfaction scale immediately after surgery rating the overall surgical experience. The surgeons were instructed to consider the patient’s comfort and the ease of the procedure (for example; eye movements, squeezing, any intraoperative complications regarding the surgical and/or the anesthetic technique). The final score was an estimate of all these findings. The scale used to assess the

surgical experience was as such: 0 = extremely poor, 1 = poor, 2 = fair, 3 = good and 4 = excellent. Further information included patient's demographic data.

Primary outcome measures were: patient comfort and physician assessment of intraoperative patient compliance. Secondary outcome measures were: intraocular pressure measurement, need for supplemental anesthesia, volume of local anesthetic used and any complications.

Statistical analysis:

The student's t-test was used to compare the group statistically. Numerical data were given as mean ± SD. P-values < 0.05 were considered statistically significant.

3. Results

Forty patients were enrolled in this study. 20 patients received topical anesthesia, 10 patients had epiretinal gliosis (50%) and 10 patients had idiopathic macular hole (50%) 20 patients received retrobulbar anesthesia, 10 patients had epiretinal gliosis (50%) and 10 patients had idiopathic macular hole (50%). There was no statistically significant difference between groups with respect to age, sex, weight and duration of surgery (Table 1)

Table (1): Patients' demographic data

	Group 1 (n = 20) (Topical lidocaine 2% jelly group)	Group 2 (n = 20) (retrobulbar group)	*P* value
Age (years)	45.3±11.4	46.8±12.1	0.51
Sex (M:F)	8:12	7:13	0.33
Weight (kg)	64.5±11.8	63.8±10.4	0.81
Duration of surgery (min)	34.5±13	36.1±12	0.47

Values are mean ± SD (Standard deviation).

There were no anesthesia related complications. 2 patients in the topical lidocaine group (group 1) had small retinal tears that were managed intra-operatively (one patient with epiretinal gliosis and one patient with idiopathic macular hole). Only one patient in the retrobulbar group (group 2) with epiretinal gliosis manifested with a retinal tear that was also managed intra-operatively.

Patients receiving topical lidocaine 2% jelly anesthesia were more likely to require additional anesthesia (n = 4/20) (p< 0.001) compared to patients receiving retrobulbar anesthesia (n = 0/20). A larger mean volume of topical anesthetic was required in the lidocaine 2% jelly group than the retrobulbar group (p < 0.001) (Table 2).

Table (2): Volume of total anesthetic solution & number of patients needing additional supplements in the 2 groups

	Group 1 (n = 20) (Topical lidocaine 2% jelly)	Group 2 (n = 20) (retrobulbar group)	*p* value
Volume of local anesthetic (ml)	0.83	0.37	< 0.001*
Additional anesthetic supplement (%)	4/20	0/20	< 0.001*

* p value statistically significant.

The total mean quantity of sedatives (midazolam, fentanyl and propofol) was rated low for both groups with no statistical significance. Both groups did not vary significantly regarding the pain score the lidocaine 2% jelly group (mean 17.4±11.1), the retrobulbar group (mean 16.1±14.3), p = 0.691 and the surgeon satisfaction scale; in lidocaine 2% jelly group (mean 3.2±0.4) and in the retrobulbar group (mean 2.9±0.5), p = 0.317 (Table 3).

Table (3): Comparable characteristics between lidocaine 2% jelly and retrobulbar groups

	Group 1 (n = 20) (Topical lidocaine 2% jelly group)	Group 2 (n = 20) (retrobulbar group)	*p* value
Mean quantity of midazolam (mg)	1.2	1.3	0.171
Mean quantity of fentanyl (μg)	41.2	45.7	0.473
Mean quantity of propofol (mg)	40.9	43.1	0.464
Mean intraoperative discomfort (VAS)	17.4±11.1	16.1±14.3	0.691
Mean surgeon satisfaction score	3.2±0.4	2.9±0.5	0.317

There was a statistical significant difference in IOP measurement immediately after anesthesia application being higher in retrobulbar group compared to lidocaine 2% jelly group (17.41±3.17, 13.12±3.13, respectively). But IOP levels were statistically insignificant in other time recordings

Table (4): Intraocular pressure measurements (mmHg) of both groups

	Group 1 (n = 20) (Topical lidocaine 2% jelly group)	Group 2 (n = 20) (retrobulbar group)	*p* value
IOP immediately before anesthesia application	13.07±2.7	13.19±2.13	0.31
IOP immediately after anesthesia application	13.12±3.13	17.41±3.17*	< 0.001*
5 minutes after anesthesia application	13.46±2.31	12.87±2.92	0.54
10 minutes after anesthesia application	13.31±2.2	12.91±2.83	0.73

*P-value statistically significant

4. Discussion

Topical anesthesia has been reported to be a safe and effective alternative to retro bulbar and peribulbar anesthesia. Conventional 20-G vitrectomies have been successfully performed under topical anesthesia with sedation[4]. Most of these reports have recorded grade 2 level of pain and discomfort during cauterization of scleral bed, during incision of sclerotomy, suturing of sclerotomy and conjunctiva. 25-gauge vitrectomies have been successfully done under topical anesthesia without sedation using anesthetic–soaked pledget at the site of sclerotomies[11]. The pledget delivery of anesthetic had the added advantage of prolonged delivery of the anesthetic to the areas where the sclerotomies are planned, thereby contributing to reduced pain and discomfort during the procedure[12].

Theocharis *et al.,* indicated that topical anesthesia could be considered an alternative to other anesthetic procedures in 25-G and 23-G vitrectomies. In our study, we used the 23-G vitrectomy system for macular based disorders mainly idiopathic macular hole and epiretinal gliosis. The 23-G technique relies on the trocar and cannula system for the sclerotomies. Conjunctival periotomy is not required and there is no contact of instruments with sclera or pars plana. Some studies suggested that 23- G vitrectomy was ideal for topical vitreoretinal surgeries in selected cases. Moreover, topical anesthesia has several advantages: early return of visual acuity without the potential complications of injection (for example; hemorrhage, chemosis, globe perforation, increased orbital pressure, ptosis, diplopia retinal detachment)[13-16]. Also the added advantage of topical anesthesia was that patients could be instructed to move the eye in the required direction intra-operatively whenever necessary as there was no akinesia[8]. Still yet, there are some disadvantages related to the local drops such as the need for administration of several doses prior to and during surgery, the short anesthetic effect and the potential for cummulative toxicity[17]. But, with lidocaine 2% jelly, there was the advantage of increased contact time with the ocular surface, providing prolonged release of lidocaine hence providing a sustained effect. Many published studies evaluated the clinical efficacy of lidocaine 2% jelly in ophthalmic surgery and suggested that it provided adequate anesthesia and patient comfort[17,18]. **Lai *et al.*** using the VAS for intraoperative pain assessment reported topical 2% lidocaine jelly without systemic sedation to be a safe and effective anesthetic method but in patients for phacotrabeculectomy[5].

Similar findings were published by **Assia *et al.*** using lidocaine 2% jelly as the sole anesthetic agent in cataract surgery and the VAS to grade the intraoperative pain[19]. But we had to correlate to other studies that compared the 2% lidocaine jelly to other local techniques in vitrectomy procedures.

Theocharis *et al.* concluded that lidocaine 2% jelly with or without per oral morphine and dixyrazine offered adequate analgesia to perform sutureless vitrectomy compared to peribulbar anesthesia, and the lack of akinesia did not prevent a successful surgical result[8]. These findings correlated with the results of this study, where there was no difference between the lidocaine jelly and retrobulbar group regarding the patient's comfort and surgeon's satisfaction. Despite the use of systemic sedatives that may affect patients' response and might cause anterograde amnesia, the patients in our study received low doses of these drugs, so there was no difference between both groups regarding the above mentioned parameters. Patients in both groups had favorable visual and surgical outcome with no anesthetic complications. Only 2 patients in the lidocaine 2% jelly group and one patient in the retrobulbar group manifested with intra-operative retinal tears and they were all successfully managed intraoperatively. Still yet, patients in the lidocaine 2% jelly group required a higher volume of local anesthetic and a higher need for additional anesthetic

supplement compared to the retrobulbar group with a significant statistical difference.

On the other hand, IOP was significantly higher in the retrobulbar group compared to the lidocaine 2% jelly group immediately after local anesthetic application. But IOP returned to baseline in the retrobulbar group in the rest of the time recordings. This was explained by the direct injection of local anesthetic inside the muscle cone.

The pain scale used (VAS) in our study, has been used previously and has been found to be valid and reliable[20,21]. The VAS has properties consistent with a linear scale, at least for patients with mild to moderate pain, and hence VAS score can be treated as ratio data, so a change in the VAS score could represent a relative change in the magnitude of pain sensation[20,21].

In conclusion, we found that topical lidocaine 2% jelly to be as effective as retrobulbar anesthesia for pain control in patients undergoing 23 G sutureless vitrectomy for selected cases of macular-based disorders (namely idiopathic macular hole and epiretinal gliosis). Lidocaine 2% jelly was found to be similar to retrobulbar anesthesia regarding patient's comfort and surgeon satisfaction. Moreover, it was found to have fewer effects on IOP prior to surgery compared to retrobulbar. In addition, it could be more advisable as it does not involve injections which may lead to complications seen with retrobulbar techniques such as hemorrhage, retinal tears or globe perforation. Finally, it was also found that the lack of akinesia in the lidocaine 2% jelly group did not prevent a successful, uneventful surgical outcome.

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3/2/12

Acupuncture versus Ultrasound-Guided Peribulbar Block in Pediatric Strabismus Correction: A Prospective Randomized Study

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Abstract: Objective: Postoperative emesis after strabismus surgery continues to be a problem, despite the use of anti-emetic measures. The purpose of this study was to identify an anesthetic technique associated with the lowest incidence of vomiting after squint surgery. **Materials and methods:** A prospective, randomized, double-blind study was conducted to evaluate the effect of Acupuncture at P6 versus ultrasound guided medial canthus peribulbar block with propofol infusion on emesis in 180 pediatric patients undergoing strabismus correction.**Results:** The incidence of emesis was significantly lower in the peribulbar (4/90, 4.4%) compared to the acupuncture group (17/90, 18.8%) ($p < 0.01$). **Conclusion:** Among the two techniques, peribulbar block with propofol-based anesthesia is the technique with the lowest incidence of postoperative emesis compared to the acupuncture technique.
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Key words: Anesthesia, Strabismus, peribulbar, Propofol, Emesis, acupuncture

1. Introduction:

Vomiting following strabismus surgery in children is a frequent finding with a reported incidence of approximately 40-50%[1]. Post-surgical vomiting can delay recovery and result in an unplanned hospital admission after outpatient surgery. As anesthetists continue to search for more cost effective approaches to improving patient outcome, attention has focused on acupuncture. It is a simple, inexpensive, and non-invasive method to prevent PONV (post operative nausea and vomiting).

The mechanism by which P 6 acupoint stimulation prevents PONV has not been established. Both the role and efficacy of P6 acupoint stimulation in the prevention of PONV are unclear. P6 acupoint stimulation significantly reduced the risk of PONV in some studies[1,2].

Ultrasound imaging provides information that cannot be otherwise obtained when light transmission into the eye is obstructed or when anatomy around the globe needs to be assessed during local anesthesia injection by using sound waves to produce diagnostic photos. This is done as the sound waves pass through tissues, bouncing off of tissue interfaces, back to the transducer. The ultrasound unit then converts the sound energy to electrical energy and a photo is then displayed in the monitor as selected by the anesthetist[3].

In this study, acupuncture technique versus ultrasound-guided peribulbar block were used in pediatric strabismus correction to assess the technique of choice regarding the lowest incidence of PONV.

2. Materials and Methods:

The study was approved by the Ethics committee of the Research Institute Of Ophthalmology. One hundred and eighty American Society of Anesthesiology (ASA) grade I and II children aged 4 to 15 years undergoing strabismus surgery were allocated in two groups in this study, group 1 receiving peribulbar anesthesia (n =90); group 2 receiving acupuncture (n = 90). Exclusion criteria included: children with ear problems, history of bleeding, diathesis, bronchial asthma, allergy to non-steroidal anti-inflammatory drugs (NSAID) and gastric and intestinal diseases. Informed consent was obtained from the parents. The postoperative data were gathered by an anesthetist blinded to the technique used and who was not present in the operation theatre at the time of surgery.

Children in the study were kept fasting for solid food for six hours preoperatively and clear fluid was allowed until four hours before surgery. An intravenous cannula was inserted in the preanesthetic area in presence of parents under EMLA cream. Both groups were premedicated with midazolam 0.15 mg/kg. Anesthesia was induced in all children by administration of 2-3mg/kg IV propofol, rocuronium 0.6 mg/kg to facilitate proseal laryngeal mask airway introduction[4].

Immediately after induction, all children received IM diclofenac sodium 1mg/kg^{-1} and dexamethasone 1mg/kg^{-1} [5]. A propofol infusion was started at a rate of 300/µg/kg/min for the initial 15 minutes, then decreased to 150/µg/kg/min for the remaining time of surgery and stopped 10 minutes before end of surgery. The children were

mechanically ventilated with 100% oxygen and were fully monitored (ECG, pulse oximeter, capnograph and non-invasive blood pressure). The fluid deficit was replaced with Ringer's Lactate solution after being calculated. Group 1 (n = 90) received peribulbar injection just before the start of surgery by the anesthetist using 0.5% Levobupivacaine. A 12 mm length, 27 gauge needle was inserted in the plica semilunaris, just lateral to the caruncle between it and the globe. The needle was then shifted slightly medially, displacing the caruncle medially away from the globe[6]. The needle was advanced in antero-posterior direction, with the globe directed slightly medially by the needle, until a click was perceived under ultrasound image (Alcon) and 4-6 ml of 0.5% Levobupivacaine was injected.

In group 2, the acupuncture group (n = 90), the P6 acupoint which lies between the tendons of the palmaris longus and flexor carpi radialis muscles, 4 cm proximal to the wrist crease, was identified and acupuncture needles were used under complete aseptic conditions[7].

Any signs of inadequate depth of anesthesia (for example: tachycardia, tachypnea, increased end tidal carbon dioxide) were noted. The rate of propofol infusion was increased by 25 μg/kg if signs of light anesthesia were observed.

At the end of the surgery, the stomach was decompressed using a nasal catheter through the proseal LMA (laryngeal mask airway). Residual neuromuscular block was reversed with neostigmine 50 μg/kg and glycopyrolate 10 μg/kg IV, and when respiration and reflexes were satisfactory the proseal LMA was removed. The children were then transferred to the recovery room. The level of wakefulness was assessed by the Alderete recovery score[2] immediately after surgery then after 15 minutes, 30 minutes, 60 minutes and 2 hours postoperatively or until the children were fully awake. Patients who experienced persistent moderate pain for more than 2 hours postoperatively were given proparacetamol 15mg/kg IV. Postoperative vomiting was assessed by a numerical rank score (Table 1). Patients with one or more episodes of vomiting were administered palonosetron[8,9].

The children were allowed to eat when they so desired. They were observed in the postanesthesia care unit (PACU) for 2 hours or until they were having only mild pain and no vomiting, then were transferred to the ward and kept there overnight.

Statistical analysis

Statistical analysis was performed using the Chi-square test, Fisher test and Kruskal-Wallis test. Probability value (p value) less than 0.05 was considered statistically significant.

Table (1): Emesis score

No vomiting	1
1 or 2 episodes of vomiting	2
More than 2 emetic episodes in 30 minutes	3

3. Results

The two groups of patients were comparable with respect to age, sex, weight and duration of surgery (Table 2). No bradycardia, hypotension or laryngospasm were observed during induction in any of the two groups. No involuntary movements, sweating or tachycardia suggestive of light anesthesia were observed at any time in any of the two groups. A large number of patients in group 1 and 2 (42.8% and 37.1% respectively) were able to respond to verbal commands, as well as to maintain their airway unassisted immediately after extubation. At 15 minutes postoperatively, 87 out of 90 patients (96.6%) in group 1 and 85 out of 90 patients (94.4%) in group 2 were fully awake. At 30 minutes postoperatively, all patients in the two groups were fully awake and had achieved maximum Aldrete recovery score. The intraoperative phase was smooth and uneventful except for some episodes of bradycardia induced by surgical manipulation of eye muscles. These episodes occurred more commonly in the acupuncture group (group 2); (12 out of 90 patients, 13.3%) compared to the peribulbar group (group 1); 2 out of 90 patients; 2.2%) indicating a significant statistical difference ($p < 0.05$).

The number of patients who required treatment for pain was comparable in the two groups in the first 24 hours postoperatively. However, the mean time to first analgesic requirement varied significantly among the two groups; the peribulbar group (group 1) was 6.1±1.8 hours and the acupuncture group (group 2) was 3.5±2.0 hours ($p = 0.001$). No patients in any of the two groups experienced severe pain at any time. Comparison of the incidence of postoperative emesis over the first 24 hours revealed a significant difference between the 2 groups ($P < 0.01$) 17 out of 90 patients (18.8%) in group 2 experienced vomiting during the first 24 hours postoperatively compared to 4 out of 90 patients (4.4%) in group 1. Severe persistent vomiting that required anti-emetic drugs was found in 2 patients out of the 4 in group 1 and 8 patients out of the 17 in group 2 (Table 3).

Table (2): Demographic data

	Peribulbar group (group 1, n = 90)	Acupuncture group (group 2, n = 90)
Age (years)	7.4±3.3	8.1±2.5
Sex (M:F)	43:47	42:48
Weight (Kg)	22.8±10.6	23.0±11.1
Duration of operation (min)	35.5±15.0	36.0±13.0

Values are mean ± Standard deviation (SD)

Table (3): Postoperative nausea and vomiting (PONV) in the 2 groups

Variables	Peribulbar group (group 1, n = 90)	Acupuncture group (group 2, n = 90)
PONV (n)		
During 0-2h	1 (1.1%)	2 (2.2%)
During 2-6h	2 (2.2%)	4 (4.4%)
During 6-24h	1 (1.1%)	3 (3.3%)
During 0-24h	4 (4.4%)	17 (18.8%)*
Number of children requiring anti-emetic treatment	2	8

* *p* value significant < 0.05 n = number of patients with emesis Score ≥ 2.

4. Discussion

PONV is among the most unpleasant experiences associated with squint surgery and one of the most common reasons for poor patient satisfaction rating in the postoperative period.

Macario *et al.* [10] quantified patients preferences for postoperative out come. PONV was among the ten most undesirable outcomes following surgery. Furthermore, the deleterious effect of PONV is not only limited to the patients, but has also profound, economic impact on the surgical unit. Multiple factors are responsible for postoperative vomiting after strabismus surgery in children[11]. The primary causes may be the use of inhalational agents and opiates in the perioperative period and the stimulation of the oculocardiac reflex during surgery. Ocular pain in the postoperative period and early mobilization of the patient also contribute to an increased incidence of postoperative emesis. Propofol-based TIVA is associated with lower incidence of postoperative emesis compared with conventional general anesthesia[12]. **Subramaniam** and his colleagues [13] also reported that the incidence of PONV was 81.39% in group GA (receiving general anesthesia) vs. 54.76% in the other group where patient received double injection peribulbar block and explained this high incidence in both groups by the long duration of vitreoretinal surgery and waned effect of the block.

Chhabra *et al.* [14], could not detect a significant difference in incidence of PONV on comparing peribulbar block as an adjuvant to GA to GA alone in ophthalmic surgery. This may be due to the antiemetic effect of propofol as they used total intravenous anesthesia (TIVA) as an anesthetic technique. Previous researches that investigated the double-injection peribulbar block in pediatrics, used volumes up to 10 ml of local anesthetic mixture[13]. Owing to the fact that the mechanism of increased IOP is related to the mechanical pressure effect from the volume injected, particularly in pediatrics with special anatomical configuration, a smaller volume (4-6 ml) was used in this study. This smaller volume was associated with transient IOP increase with no operative effect.

However incidence of postoperative emesis with opiates and propofol-based TIVA is still high (28%-60%)[11]. Along with propofol-based anesthesia, we used non-steroidal anti-inflammatory and regional anesthesia technique. To ensure adequate and sustained postoperative pain relief, we used adjunctive IM diclofenac sodium 1mg/kg in the two groups of patients, finding a higher incidence of postoperative vomiting in the acupuncture group. In many studies the use of fentanyl with TIVA was associated with low incidence of PONV after pediatric strabismus surgery[8,11] but in our study we used only propofol with peribulbar block which is an acceptable technique in adults[15] and has been safely used in children[16]. A few studies have mentioned the role of peribulbar block in reducing emesis after ophthalmic surgery in children[14] by inhibiting the oculocardiac reflex. We combined peribulbar block with propofol-based TIVA technique which decreased the incidence of OCR 2.9 %.

However, we did not find any significant correlation between the occurrence of OCR and postoperative emesis in any of the two groups. The peribulbar block, by providing good analgesia and akinesia in the postoperative period, may give the

child time to adapt to visual changes. This outcome, in addition to its opiate-sparing effects, contributed to the low incidence' of postoperative vomiting in this group. Also there was statistically significant difference between the postoperative vomiting incidence in the peribulbar and the acupuncture groups (4.4% to 18.8% respectively).

In conclusion, single medial canthus injection peribulbar anesthesia under ultrasound image using a short needle and a small volume is effective in pediatric eye surgery. It provided low incidence of OCR, less intraoperative narcotic requirements, hemodynamic stability, less PONV and improved postoperative analgesia. It also had the advantage of, being simple and safe, with the lowest incidence of complications compared to the acupuncture group.

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3/2/2012

Educational Language Teaching: A New Movement beyond Reflective/Critical Teaching

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Abstract: This paper aims to extend our understanding of what it means to be a language teacher of sufficient caliber by elucidating ways in which professionals in teacher education can help teachers proactively construct and promote their *Educational Identity*. Our descriptions of the historical outlooks to language teachers constitute the starting point for a wider discussion on the expansion of the language teacher's identity in being and becoming an *Educational Teacher*. Having made reference to a variety of ground-breaking approaches in English language teaching and learning, encapsulated in the notion of *Applied ELT*, this article highlights some of the future challenges that should be involved in language teacher training courses. However, the purpose of this paper is by no means to promise more than it can deliver; rather, the introduction of *Educational Language Teacher* should rest on the guarantee that what is being offered to teachers can and will support how well they deal with life issues in the classroom. Issues raised here should be able to (a) contribute to a better understanding of the future directions in language teacher education, (b) present a series of recommendations on actions that need to be taken and, consequently, (c) lead to improvements in the planning of teacher training programs.
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Keywords: Teacher education; reflective language teaching; educational identity; life issues; educational language teacher

1. Introduction

The English language, during the colonial era, was considered to be an instrument in the hands of colonizers for bringing to the fore their own culture and curbing other countries' cultural expressions through cultural invasion (Freire, 1985), exploitation (Said, 1993), and marginalization (Ha, 2004). Most simply put, a great part of linguistic imperialism comprised the deculturation of the speakers of other languages (Phillipson, 1992) whose unfortunate consequence, i.e. ESL/EFL learners' loss of identity (Norton, 1997; Ricento, 2005), could then be observed in most of the theories and practices in the field of ELT.

However, with the advent of Postmodernism, the validity of the mainstream Western scientific practices, along with their teaching methods which were prescribed by native theoreticians for a variety of *unknown* contexts, were put into serious question (Kuhn, 1962; Kumaravadivelu, 1994; Pennycook, 1989; Prabhu, 1990). Likewise, the native speakers were stripped of their sheer ownership of the English language and there was no longer any distinction between native and non-native speakers of English (Swales, 1993; Walker, 2001; Widdowson, 2003). Rather, due to the emergence of World Englishes (Kachru, 1982) with its notions of *inclusivity* and *pluricentricity*, the possession of the English language became de-nationalized and indigenized by other varieties of English around the world (Higgins, 2003; Smith, 1976; Widdowson, 1994).

Similarly, during the postmodern era of ELT, many scholars denounced the idolatry of the *native* language teacher (e.g., Rampton, 1990; Phillipson, 1992; Kramsch, 1997) and the idea of teachers' being classroom *consumers* (Kincheloe, 1993; Prabhu, 1990; Richards, 1990; Stern, 1991) but, instead, gave a high prominence to classroom *action research* (Altrichter, Posch, & Chamot, 1995; Curry, 1996; Schmuck, 2006; Wallace, 1998) and looked upon language teachers as *reflective* (Cutforth, 1999; Kumaravadivelu; 1999; Mena Marcos, Sanchez, & Tillema, 2011), *critical* (Giroux, 1988), *participatory* (Freire, 1972), *exploratory* (Allwright, 2003), and *transformative* (Kumaravadivelu, 2003) teachers.

Nonetheless, in this paper we argue that in an age of globalization and neo-colonization we must extend the language teachers' role beyond that of a reflective practitioner and transformative intellectual and give them a new identity. This study, therefore, mainly centers on introducing *Educational Language Teacher* as a new concept in language teacher

education. It examines the basic issues of where and under what conditions language teacher education works best, including not only qualifying language teachers to realize and promote their professional identity (Bauer & McAdams, 2004; Beijaard, Meijer, & Verloop, 2004; Varghese et al., 2005) and abandon their undue reliance of native identities (Abell, 2000), as well as to be reflective practitioners and transformative intellectuals in their teaching, but also developing teacher training courses in which teachers are empowered to become *educational teachers* in the sense that, henceforth, they should go beyond teaching language per se towards extending their knowledge of other disciplines so that they can help learners develop as 'whole-person' individuals.

2. Timeline: Roles of Language Teachers

Language teaching has undergone three stages, namely Pre-modern era, Modern era and Postmodern era (Pishghadam & Mirzaee, 2008). In each stage, language teachers have been assigned different roles regarding the dominant stream of thought.

2.1. The pre-modern era of ELT

During the Pre-modern era of ELT, the Classical Method, later known as the Grammar Translation Method (GTM), whose aim was the learning of classical languages like Latin and Greek through translation of literary texts and focus on rote learning of vocabulary and grammar rules, became the dominant method of teaching the language. The GTM focused on grammar, vocabulary memorization, reading and writing, with little or no attention given to listening and speaking skills. During the heyday of GTM in the late 1800s and early 1900s, language teachers were considered the sole authority in the classroom and used their own *intuition* to teach the language.

2.2. The modern era of ELT

During the Modern era of ELT, the professionals of the field were mostly preoccupied with the quest for the deceptive 'best' method of teaching language (McArthur, 1983) in terms of the theories, methods, and techniques which were stipulated in advance (Strevens, 1977) and generalized across various audiences from unknown locations. The prescription of the theories of English language teaching by professionals in Core countries such as the UK, USA, and Canada (Phillipson, 1986) for a variety of contexts around the world signaled the entrance of colonialism into the field of ELT.

2.2.1. The Native-like teacher

The Audiolingual Method, for example, was based on the basic premises of behavioristic psychology such as stimulus-response, habit formation, and reinforcement. During Audio- lingual Method (ALM), the English teachers were supposed to be as native-like as possible, the native English-speaking teacher was deemed as the best teacher, and the monolingual teaching of English as the perfect form of instruction.

2.2.2. The Consumer teacher

In the 1970s, an era referred to as the "spirited seventies" (Brown, 2002), the ALM waned in popularity and gave way to some new methods such as, to name just a few, the Silent Way, Suggestopedia, and Total Physical Response. Having made universal claims, these "changing winds and shifting sands" or 'designer methods' (Marckwardt, 1972), sought to discover the best method for teaching the English language (Brown, 2000) which could be prescribed for and generalized across a wide range of audiences and contexts.

In this view, teachers were considered to play the role of a technician and a classroom consumer in the sense that they should adhere to, and transmit, the professional knowledge to learners without having the right to change the content of information (Kincheloe, 1993). Put another way, the language teacher was primarily concerned with the passive transmission of the information which has already been agreed upon by experts in the field to language learners. That is to say, the situated, novel and context-specific experiences of language teachers were ignored to the extent that teachers were supposed to continually refer to the theoreticians' general prescriptions of what the best way for language teaching was. This strand of thought was deemed too inefficient to cater to the specific localities of language classrooms around the globe and, consequently, gave way to a reflective view of teaching in the postmodern era.

2.3. The post-modern era of ELT

Finally, during the postmodern era of ELT, the emergence of notions such as subjectivism, constructivism, relativism, and localism made the ELT professionals cast doubt on the validity of the mainstream Western scientific practice (Kuhn, 1962). Accordingly, during this period second language learning and teaching were considered complex, non-linear, dynamic, emergent, and unpredictable processes (Larsen-Freeman, 1997). Similarly, the modernist idea of looking for the 'best' method was severely criticized (Kumaravadivelu, 1994, Long, 1989; Pennycook, 1989; Prabhu, 1990; Richards, 1990, 2003; Stern, 1991) granted that the relationship between theory and practice could only be visualized by virtue of the immediate act of teaching (Widdowson, 1990).

Likewise, the native-like pronunciation was no longer the sole criterion for language proficiency (Kachru, 1990; Swales, 1993; Walker, 2001) and as a result, the ownership of the English language became de-nationalized and indigenized by the speakers of other English varieties (Kachru, 1992; Smith, 1976; Widdowson, 1994). When it comes to language teaching, likewise, the idea of the idealized native speaker as the best teacher has been deconstructed by many scholars such as Kramsch (1997), Paikeday (1985), Rampton (1990), and Phillipson (1992). These scholars argued that non-native teachers should abandon their undue pursuance of native identities and, instead they ought to focus upon accepting their own unique identities as non-native speakers so that they can put into practice their own language learning experiences to contribute to the promotion of language proficiency among learners of multiple linguistic, sociocultural, historical, national and religious identities.

Through introducing the notion of 'postmethod', Kumaravadivelu (1994, 2003) invited the ELT community to look for a meaningful and organized *alternative to method*, and not an *alternative method*, in order to decolonize the colonized ELT. In a similar vein of argument, Prabhu (1990), through what he called teachers' *sense of plausibility*, gave a high prominence to language teachers' subjective understanding of their own teaching as well as their local needs. In other words, English teachers were encouraged to become reflective practitioners (Kumaravadivelu, 1999).

2.3.1. The Reflective language teacher

In the reflective outlook to teachers, language teachers are no longer looked at as passive executors appointed by the professionals of the field to carry out their prescriptive rules of language teaching, but are empowered to reflect on, and be sensitive to, their own context-specific teaching practice. In fact, the idea that teachers need to become reflective practitioners was proposed to give priority to the fact that teachers need to be continually concerned with enhancing their research-based instruction, revising their lessons, and adapting their teaching practices to the specific contexts of language teaching and learning based on the feedback they receive from learners. Accordingly, several scholars such as, to name just a few, Cutforth (1999), Kincheloe (1991), Kumaravadivelu (1999), and Mena Marcos, Sanchez and Tillema (2011) widely accepted, and still use the notion of teacher reflection.

Such research-based reflection on the teaching practice has two interconnected manifestations: *reflection-on-action* in which teachers devise a lesson plan and, subsequently, pass judgment on the effectiveness of their teaching practices, and *reflection-in-action* in which teachers monitor their ongoing performance on the spot and make attempts to immediately accommodate and redress their teaching practice (Schon, 1983). Accordingly, teachers' engagement in action research has gained considerable momentum in the area of education (e.g., Argyris & Schon, 1974; Schon, 1983; Noffke, 1997; Zeichner & Liston, 1996; Hui & Grossman, 2008; Gordon & Schwinge, 2009). This research has been a venue for teachers to enhance the teaching process, modify their instruction, create collaborative environments, and simply put, to reflect on their own teaching and become reflective practitioners.

Likewise, in the realm of language teaching, action research has received more and more attention in recent years (e.g., Altrichter, Posch, & Chamot, 1995; Curry, 1996; Schmuck, 2006; Wallace, 1993). It was thus recommended that action research be incorporated into the language teacher education programs (Richards & Lockhart, 1994) so that teachers could enhance and enrich their teaching practices in response to the situated conditions and their continuously changing experiences (Curry, 1996).

2.3.2. The Participatory/Critical language teacher

Later in the era of postmodernism, some scholars of the field of language teaching such as Auerbach (1995), Kumaravadivelu (1999) and Pennycook (1999) who were largely inspired by Freire (1972), espoused the idea that any kind of pedagogy should challenge the ideological, sociopolitical, and historical forces with the aim of empowering learners to acquire the required knowledge and social skills to be able to function as critical agents in a society (Giroux, 1988).

In fact, under the rubric 'Critical Pedagogy', these scholars made attempts to give language teachers a critical and participatory role to become *transformative intellectuals*, whose primary obligation was to empower language learners and emancipating them from the hegemony of dominant ideologies. Such pedagogy is concerned with "connecting the word with the world," "recognizing language as ideology, not just as system," "extending the educational space to the social, cultural, and political dynamics of language use," and "creating the cultural forms and interested knowledge that give meaning to the lived experiences of teachers and learners" (Kumaravadivelu, 2006, p. 70).

In this view, language teachers, as agents of change and seekers of democracy in education, should therefore raise their sociopolitical awareness

via problem-posing activities in terms of a holistic approach to both educational advancement and personal transformation (Kumaravadivelu, 2003) in such a way that teachers and learners acquire a sense of ownership of their own teaching and learning rather than unduly relying on professional experts (Kincheloe, 1993).

3. Introducing Educational Language Teacher

Now, inspired by Pishghadam (2011) who states that ELT has already acquired a scientific and independent status among many other disciplines making it ready to be applied to and improve other domains of knowledge, we argue that it is time for a new role for language teachers to adopt. In fact, during this era of neo-colonization and globalization, in which the knowledge of the colonized countries is constantly being used to serve the colonizer's interests while the cultural identity of the colonized people are lamentably being compromised (Phillipson, 1988), and that the need for localization of knowledge and expertise is being felt more than any other time (Kumaravadivelu, 2006), language teachers are expected to become *Educational Language Teachers* who are not only experts in teaching language but also educational experts in the specific context they work. In other words, although reflective language teaching largely pervades the current practices in teacher education (Gimenez, 1999) and is considered by many scholars (e.g., Gordon, 2011; Johnson & Johnson, 1999; Mena Marcos, 2007; Richards, 2000; Tabachnick & Zeichner, 2002; Vieira & Marques, 2002) to be part and parcel of teacher development, we suggest that language teachers should not be limited to but should be empowered to move *beyond*, reflective language teaching towards gaining the relevant and sufficient caliber required for extending their professional identities by taking into account and trying to improve other domains of knowledge which, directly or indirectly affect learners' idiosyncratic lives.

Most recently, a new paradigm in second/foreign language studies, i.e. Applied ELT, which simply refers to the applications and contributions of ELT to other domains of knowledge, has been pioneered by Pishghadam (2011), and furthered by Pishghadam and Zabihi (2012) in order to breathe new life into the field of English language teaching and learning by giving it a more contributory and life-changing status, and inviting the professionals of the field to take a fresh look at its principles.

A key tenet of Applied ELT is that ELT, as a full-fledged and interdisciplinary field, is ready to be applied to and improve other domains of knowledge (Pishghadam, 2011). As such, the inclusion of learners' other characteristics such as motivation, emotional abilities, thinking styles, and values in language teaching programs is not considered an obstacle for language teaching and learning, but as a real strength. A number of research studies have been carried out in this regard coming up with the idea that, through the proper manipulation of the procedures in ELT classes, language teachers can contribute to other disciplines of knowledge. As a case in point, the field of psychology has received some useful implications from ELT in order for some psychological traits such as emotional intelligence (Hosseini, Pishghadam, & Navari, 2010), critical abilities (Pishghadam, 2008), and their national and cultural identities (Pishghadam & Saboori, 2011) to be improved.

Added impetus was given to the theory of applied ELT when Pishghadam and Zabihi (2012) brought forth the essential concept of *Life Syllabus,* suggesting that for a language course to be as efficient as possible it should incorporate the issues of concern in learners' life into the ELT curriculum, highlighting these aspects as well as the enhancement of learners' language proficiency. In a later extension of the theory of Applied ELT, Pishghadam and Zabihi (in press) have introduced *English for Life Purposes (ELP)* as a new concept in English language teaching. ELP offers a variety of topics for discussion which can enable the learners to compare their home culture with other cultures and project their unique identities. It not only mitigates the learners' anxiety, depression or other negative aspects of life but it would also enable the teachers to enhance the learners' emotional, intellectual, and motivational abilities while teaching them a second/foreign language.

Of course, the Applied ELT paradigm from which flow these essential changes in language planning and policy, language teaching and learning, syllabus design, materials development, and finally language teacher education informs these changes in myriad ways. For example, in the case of language teacher education, as is the primary concern of this paper, an emphasis on the *educational identity* of language teachers rather than their language-related native or professional identities demands a greater part on the side of the teachers that they should be trained to understand the psychological, emotional, social, economic, and even religious and moral needs of learners and design the syllabus according to those needs.

Fascinatingly, in accordance with the principles of Applied ELT discussed above, we further argue that language teachers should be

enabled to cross the boundaries of language teaching by virtue of extending their knowledge of other disciplines. The overall conclusion which our discussion here leads us to is that language teacher education has to prepare teachers for a dual role both as professionals in language-related as well as interdisciplinary issues. In fact, henceforth English language teachers ought to be regarded as *educational teachers* _ teachers who have gained a sense of agency and have become empowered to construct positive professional identities in different disciplines of knowledge other than language teaching. Our purpose here is thus to reallocate the role of the teacher from language facilitator and instructor to an *educational* language teacher, a role which may not be possible for every teacher to take on at the outset, hence the need for devising appropriate language teacher training courses.

4. Reorienting Teacher Training Courses in ELT

It has been and still is the common belief among ELT professionals that linguistic knowledge should form the central part of language teachers' knowledge base for teaching language (e.g., Baur, 2003; Yates & Muchisky, 2003; Zimmermann, 2003) to the extent that any language teacher education program was expected to train teaching trainees to become linguists (Leow, 1995). Later on, with the advent of Applied Linguistics, it was felt that other academic fields such as Sociology, Psychology, and Anthropology could provide useful implications for teachers and teacher educators towards the betterment of L2 teaching (e.g., Lafayette, 1993; Stern, 1983).

However, the theory of *Applied ELT* (Pishghadam, 2011) emerged with the aim of reversing the direction, ascribing a more contributory role to ELT. Put another way, so far, it has been the acceptable trend to employ the findings of other disciplines such as linguistics, sociology, psychology, neurology, etc. to enhance our understanding of teaching English. Now it is time for language teachers to play the role of a producer. In this view, language teachers play a vital role in building learners' social, cultural, and national identities. It is believed that every action on the part of ELT teachers would necessarily leave perennial steel prints on the slate of the mind of the learners and might play an important role in molding their life patterns as an individual, as a social member and as a citizen.

Be that as it may, if teacher education programs wish to prepare language teachers who are capable of becoming *educational teachers* in their classrooms and if learners ought to be exposed to language teachers of sufficient caliber, then these programs will have to include activities aimed at promoting this end. Thus it is recommended that a teacher training course should *not only* comprise a series of facts about phonetics, morphology, syntax, semantics, discourse analysis, etc., but should instead add knowledge of other academic fields to the knowledge base that language teachers are typically presumed to need in order to teach language so that language teachers, as educational experts, become empowered to exert their newly achieved educational identities to contribute to the disciplines they wish to enrich.

To give but one example of such training courses, if teacher educators want to add the knowledge of the field of psychology to *educational language teachers*' repertoire of disciplinary knowledge, they have to (a) be sensitive to a wide range of issues such as self-confidence, emotional intelligence, creativity, etc. within the field of psychology, (b) keep abreast of the effective strategies and activities developed elsewhere for the enrichment of these issues, and (c) share their own ideas and practices with other educators worldwide. This is undoubtedly a huge task for language teacher educators, considering the fact that the psychological demands of individuals are so wide in range, and as a result, they inevitably have to strive hard to handle all these issues. It thus behooves the professionals in language teacher education to prepare language teachers to get on with such a colossal task.

To summarize thus far, it is recommended that the professionals in language teacher education develop training programs in which teachers of English are empowered to gain expertise not only in language teaching but also in different other disciplines and in so doing, become more of an *educational teacher*, a critical and proactive educator rather than merely a language instructor.

5. Final Commentaries

In this paper, we were mainly concerned with introducing a new concept in language teacher education, drawing upon the previous roles ascribed to language teachers as well as our conceptions of the emergent need for language teachers to adopt a new role. Firstly, we referred to some stages in the development of ELT and the respective roles assigned to language teachers at each stage, and then argued that in an age of globalization and neo-colonization, language teachers by virtue of promoting their educational identity should be regarded as *educational language teachers*, i.e. critical, forethoughtful, and interdisciplinary educators who in addition to having a thorough command of language have a fair knowledge of other

disciplines rather than merely being reflective and critical language instructors.

It is important to note that the main rationale and source of inspiration for us to propose such a new identity for language teachers to take on has been the theory of Applied ELT brought forth by Pishghadam (2011). Therefore, obviously we are not generating new theories to the detriment or total negation of other theories but instead, we hinge upon, not annihilate, other theories and practices without which, we firmly believe, we could not have developed ours.

As such, the paper concludes with recommendations proposing ways in which language teacher education programs can be enriched by centering their attention on the promotion of teachers' professional identities while teachers, once their roles as *educational language teachers* have been established, i.e. when they have acquired a fair degree of mastery over those disciplines which ELT can enrich, decide how to focus more and more on the enhancement of learners' quality of life.

Therefore, we have made attempts to revitalize and reorient language teacher education by suggesting that the construction and development of language teachers' professional identity should empower them to become educational teachers. That is to say, we should encourage ESL/EFL teachers to develop an understanding of their own assets, values, and beliefs, enabling them to become, as was shown above, not only self-reflective practitioners and transformative intellectuals but also educational experts who very well know themselves and their own area of expertise as well as different disciplines of knowledge to which the field of ELT is able to contribute.

After the pioneering work of Pishghadam in 2011, inevitably years have to elapse for further research to emerge on the Applied ELT paradigm. His innovative stance appears to have encouraged a number of researchers and scholars to put the theory into practice, with all topics centering on the promotion of language learners' life qualities such as emotional abilities, ways of thinking, interpersonal competencies, self-confidence, motivation, creativity, etc. in a variety of domains ranging from language policy and planning, to materials development, syllabus design, and teacher education. In the area of language teacher education, these alterations epitomize an essential shift of focus from a *language-only* perspective to an *'education + language'* orientation with which teachers can enlarge their roles to be experts in teaching the language and at the same time in different other disciplines of knowledge.

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Understanding knowledge sharing intention in optometry practices: Examining the roles of extrinsic and intrinsic motivation

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Abstract: According the modern optometry industrial develop, the optometry employee in order to face the emerging turbulent environment, need to rely on information and knowledge from all aspects to operate for highly competitive optometry business domain, that is relate to the organization survive and remain invincible key factors. Therefore, prior study [1] believed that exploring the employee's professional knowledge how to interflow and whether willing to sharing expert experience to colleagues or not, that is important issue of the organizational knowledge management activity. This study examines employed for optometry industrial have professional capability worker include optometrist and opticians who antecedents influence to share professional knowledge for co-worker behavior intentions. Data gathered from 198 optometry industrial employees were employed to examine the relationships. The results indicate that organizational climate and self-efficacy have indirect effects knowledge sharing intention, while individual's knowledge sharing attitude and knowledge sharing subjective norm have direct effects knowledge sharing intention. [Ming-Tien Tsai, Kun-Shiang Chen. **Understanding knowledge sharing intention in optometry practices: Examining the roles of extrinsic and intrinsic motivation.** Life Science Journal, 2012;9(1):900-902.] (ISSN:1097-8135) http://www.lifesciencesite.com

Keywords: knowledge sharing, optometry, self-efficacy, organizational climate, perceived behavioral control

1. Introduction

The optometry is a professional behavior to solve patient abnormal visual based on the professional skills of the optical and visual sciences knowledge. According to the rapid development of modern optometry professional technical, the patient higher concern about for optometrist visual examination quality. However, the application and penetration of the optometry knowledge and research achievement in every domain of optometry business are related to affect the elevation of visual quality, which is highlight courses of regarding whole optometry future work. Thus, from a managerial point of view, the knowledge management defined that a method for simplifying and arising the process of sharing, distributing, creating, and effective use of organizational knowledge [2]. As a result, knowledge sharing is the mainly issue of knowledge management, and the process where people with others for mutually exchange itself knowledge and further jointly discover new knowledge [3]. Within organization engage effective interpersonal knowledge sharing is organized to enhance the core competitiveness and gain a competitive advantage the best knowledge management activities [4]. It should be noted that, there are many factors both internal and external the organization, directly or indirectly affect the knowledge sharing activities, such as personal skills, self-efficacy, motivation and organizational climate. Since individual's knowledge-sharing is not compulsory, but only by the way of encouraging and facilitating [5]. Organization to formal knowledge-sharing climate will be focus on play "knowledge possessor" role into a "knowledge provider" to strengthen the organization's competitive advantage, atmosphere by developing innovation atmosphere within organizational activities. Therefore, the factors influence success of knowledge-sharing activities affect by internal and external knowledge provider's behavior intentions. The research aims to discover whether internal and external factors would affect individuals' actual behavior intentions of sharing their knowledge. Namely, knowledge sharing intentions may probably influenced not only by individual beliefs but also by contextual factors.

Motivated by the above discussion, the study investigates and proposes individualš knowledge sharing motivations will be affected by regarding inter-personal beliefs—self-efficacy, and perceived behavioral control and inter-organization factors—organizational climate are identified in theory of planned behavior (TPB). Keeping the above in view, the research model of this study is based on above mentions, which is to combine the existing internal and external variables to explore the knowledge-sharing intentions, especially focused on Taiwan optometry industrial personnel. Based upon

above mentions, we proposal five hypothesis and depicts the research model in figure 1.

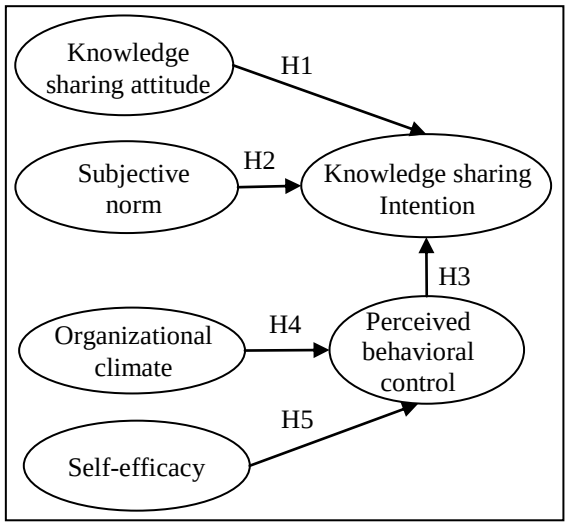

Figure 1 research model

2. Methodology

2.1 Sample and collection

In order to collect related data, a survey was applied in this study. After the pilot test, the formal questionnaire was delivered in many optical shops such as optical industry in all regions of Taiwan Business Association member stores to fill out this questionnaire. During October 2010 to December, 2010, total 600 questionnaires distributed, 225 responses were collected, 12 were eliminated because of incomplete answering or monotone answers among all responses. The purpose to delete the incomplete answering or monotone answers sample is that this research attempts to fine out the real motivations of sharing knowledge with people. This study has to delete these 15 invalid samples and adopt the other 198 valid samples for further analysis. The questionnaire was developed from the literature. In recent years, many studies have developed and validated instruments for measuring TPB constructs such as individual's self-efficacy and knowledge sharing intentions. Therefore, the items in the instrument were derived from the existing literature and slightly modified to suit the context. Each item was measured on a five-point Likert scale, ranging from "disagree strongly" (1) to "agree strongly" (5). Before conducting the main survey, both a pre-test and a pilot test were performed to validate the instrument. The pre-test involved ten respondents who had more than two years' experience in optometry work. Respondents were asked to comment on list items, such as the length, questionnaire format, and wording of scales. Respondent demographic characteristics and research subjects and data collection are shown in Table 1.

Table 1 Demographic details of the respondents (n =198)

Measure	Items	Frequency	Percentage (%)
Gender	Male	112	56.6%
	Female	86	43.4%
Age	21-25	10	5.05%
	26-30	50	25.3%
	31-35	95	50.0%
	36-40	35	17.7%
	41(or above)	8	4.0%
Education	Specialty school	60	30.3%
	Bachelor	118	59.6%
	Master	20	10.1%
Unit	Optical shop	155	78.28%
	Eye clinic	31	15.66%
	Hospital .	10	5.1%
	factory	2	1.0%
Position	Employee	163	82.3%
	Team leader	23	11.6%
	Manager	12	6.0%
Seniority	Under 3 years	30	15.1%
	3-5 years	25	12.6%
	5-10 years	80	40.4%
	10-15 years	50	25.3%
	15years (or above)	13	6.6%

2.2 Statistical analysis

Multiple linear regression analysis and factor analysis are used to develop models that predict optometry employees' knowledge sharing intentions and motivations from quantitative statistics analysis methods (e.g. SPSS 16.0 software).

2.3 Measure

This research in order to purify the measurement scales and to identify their dimensionality, principal components factor analysis was applied to condense the collected data into certain factors and combine the multiple regression analysis to estimate a series of interrelated dependence relationships simultaneously.

3. Results

3.1 Factor analysis, reliability and linear regression analysis

According to factor analysis, there have two essential criteria in terms of the values of factor of each factor loading are greater than 0.6 and the difference of factor loading between each other being larger than 0.3 are ensured in specification [6]. In the reliability analysis, Cronbach's alpha finding must be larger than 0.6.

Table 2 Factor analysis

	Factors						
							Cronbach's α
items	1	2	3	4	5	6	
PBC.4	.829	.167	.085	.193	.164	.193	PBC=0.900
PBC.5	.821	.114	.089	.240	.108	.056	
PBC.6	.794	.165	.214	.176	.140	.163	
PBC.3	.758	.063	.184	.222	.163	.182	
PBC.1	.657	.141	.015	.281	.136	.375	
SE.1	.087	.851	.117	.188	.031	.061	SE=0.909
SE.3	.210	.847	.163	.172	-.020	.111	
SE.4	.121	.829	.130	.168	.141	.132	
SE.2	.106	.829	.127	.148	-.006	.174	
OC.3	.129	.055	.846	.038	.103	.148	OC=0.864
OC.2	.083	.182	.840	.034	.186	.133	
OC.4	.129	.121	.748	.141	.243	.016	
OC.1	.133	.193	.716	.124	.246	.115	
ATT.3	.316	.190	.242	.761	.013	.095	ATT=0.862
ATT.2	.243	.261	.077	.759	.049	.183	
ATT.1	.204	.113	.128	.744	.096	.274	
ATT.5	.245	.216	-.047	.721	.014	.176	
SN.3	.174	-.080	.174	.128	.819	-.023	SN=0.846
SN.5	.118	-.045	.222	.050	.795	-.064	
SN.2	.176	.113	.131	.019	.783	.207	
SN.4	.072	.188	.216	-.048	.744	.274	
INT.3	.226	.171	.173	.226	.073	.807	INT=0.891
INT.2	.369	.168	.184	.281	.124	.723	
INT.1	.265	.224	.145	.332	.181	.711	

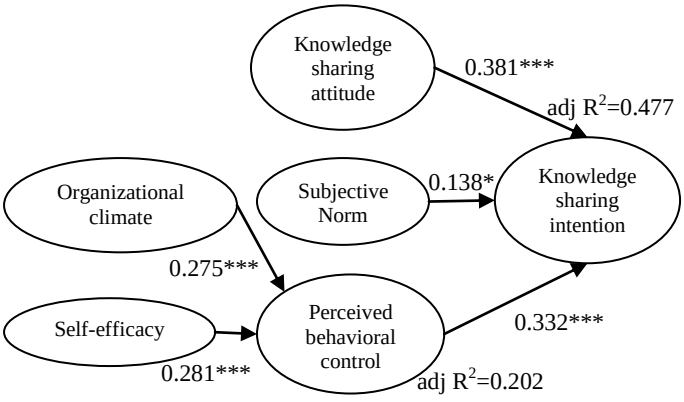

Fig. 2. Results of the hypotheses testing

Note: *:p<0..05; **:p<0.01; ***:p<0.001

The results of the factor analysis and reliability for each dimension are shown in Table2. From the multiple regression analysis to empirically investigating the relationships between constructs. The following relationships of constructs are tested which are related between constructs of organizational climate, self-efficacy toward PBC, and PBC, subjective norm, knowledge sharing attitude toward knowledge sharing intention. The results of the multiple regressions for each dimension are shown in figure 2.

4. Discussion

The research uses the organizational climate and self-efficacy as the antecedents to examine PBC which is a complete TPB theory. Further, also measurement of knowledge sharing attitude, subjective norm, knowledge sharing attitude and PBC produce indirect or direct influence knowledge sharing intentions in optometry practice. Due to result of empirical evidence, all hypothesis of this study are supported. The optometry industrial will be achieve more successful knowledge management (KM) by apply this research finding. At the same time, provided optometry supervisor clarify understanding interrupt factors of embedded mind of optometry employees' knowledge sharing intentions to develop the better-performing KM strategy accordingly.

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Phylogenetic subtyping of hepatitis C virus 5' UTR isolated in Egypt and the effect of 2 transitions in subdomain IIId on the apical loop structure

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Abstract: HCV 5′-UTR of 3 non-responding isolates (Sohag 1, 2 and 3), collected from Sohag-Egypt was compared with the 6 genotypes collected from GenBank and HCV database sequences included a responder isolate from Egypt. Multiple alignment comparison showed that TTGGGT sequence located in the IIId subdomain loop was conserved in all genotypes. Phylogenetic tree revealed that isolate Sohag 1 was clustered with the responder isolate within subtype 4a. Sohag 2 was clustered with isolates of subtypes g and o, however, isolate 3 was grouped with isolates from subtype 4a and q. Two transitions $T_{175}\rightarrow C$ and $C_{183}\rightarrow T$ were detected in the stem of IIId subdomain and were highly represented in genotype 2 followed by genotype 6: 1.0, 95.5, 2.0, 14.5, 0.0 and 33.5% for genotypes 1, 2, 3, 4, 5 and 6, respectively. The predicted secondary structure of HCV 5′-UTR showed that the formation of UUGGGU loop was affected by the 2 transitions $T_{175}\rightarrow C$ and $C_{183}\rightarrow T$. In the absence of these 2 transitions, the apical loop was replaced by double helix structure in most predicted folding. Taken together, the % of the 2 transitions in different genotypes and the modification in the apical loop structure could affect the response to therapeutic treatment.

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Keywords: Hepatitis C virus; 5` UTR; IIId subdomain; Secondary structure

1. Introduction

Egypt has the highest prevalence of Hepatitis C virus (HCV) worldwide, where it infects about 15% of the general population (Egyptian Ministry of Health, 2007). The infection with the HCV is the leading cause of chronic hepatitis worldwide, progressing to liver cirrhosis in approximately 20% of patients after 10 years and to hepatocellular carcinoma (HCC) in a subset of them with a yearly incidence of 3% (Zein, 2000).

HCV is an RNA virus and a member of the Hepacivirus genus classified into the Flaviviridae family. HCV presents high mutation rates and because of that it has been evolved to different genotypes based on nucleotide sequence heterogeneity and classified in six major genotypes and more than 80 subtypes (Mizokami *et al*., 1996; Robertson *et al*., 1998 and Simmonds *et al.,* 2005).

The 5′-UTR of HCV is an essential component of the internal ribosome entry site (IRES) that regulates Cap-independent translation of HCV (Wang *et al.,* 1994). Three domains: I, II, and III, are inside this region. Mutations in IIId domain disrupt IRES-mediated translation initiation and also affect the RNA structure, demonstrating the importance of correct RNA folding to IRES function (Jubin *et al*., 2000). Hazari *et al*., 2005 suggested that the antiviral action of IFN-a2b blocks IRES-mediated translation and this effect is the same among HCVs of other genotypes.

In this study, a comparison between our non-responder isolates with a responder one and a classification at the subtype level in the 5`-UTR of HCV was done. We analyzed the effect of 2 transitions exist in IIId subdomain of the IRES (Hazari *et al*., 2005 and Klinck *et al*., 2000) on its secondary structure.

2. Material and Methods

2.1. Samples

In a previous study, (Hemeida *et al*., 2010), We collected 92 HCV positive isolates from Center of Cardiac and Digestive System, Sohag, Egypt. The confirmed patients were received 12 vials of BEG IFN- 2a for 12 weeks (180IU/ml weekly) plus ribavirin (1000 mg for ≤ 75 Kg or 1200 mg for > 75Kg- Roche) and follow up by RT-PCR. The results showed that 67 patients (72.8%) responded to the treatment, while 25 patients (27.2%) were non responders. We selected 3 non responder isolates (Sohag 1, 2 and 3), then were sequenced and analyzed (Hemeida *et al*., 2010).

2.2. Sequence analysis

DNA sequencing of three random serum samples from HCV non-responder patients (sohag 1, 2 and 3) was carried out as previously described

(Hemeida *et al*., 2010). BLAST software (NCBI) was used to identify the similarity between isolates. was Our sequences were submitted to GenBank database. [GenBank: JQ228803–JQ228805 for isolates Sohag 2, 3 and 1, respectively]. Sequences of HCV different genotypes were retrieved from GenBank and HCV databases. An isolate responded to the pegylated IFN alpha-2a plus ribavirin treatment from Egypt was used in this study for comparison (Zekri *et al*. 2007). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura *et al*., 2007). Standard error estimates are shown and were obtained by a bootstrap procedure (500 replicates). Secondary structure of 5′-UTR of HCV was deduced using mFOLD software. Version 3.2 program(Zucker,1989) (http://mfold.bioinfo.rpi.edu/).

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3. Results

3.1. Multiple sequence alignment of HCV 5′-UTR

Our non-responder isolates (Sohag isolates) showed identity of 94-98% with those of GenBank databases. The alignment of the responder isolate against Sohag isolates (192nt) resulted in 8 variable sites (highlighted positions in is Fig. 1). The responder isolate was characterized by one insertion (T_9) and 2 deletions (C_{36} and A_{117}).

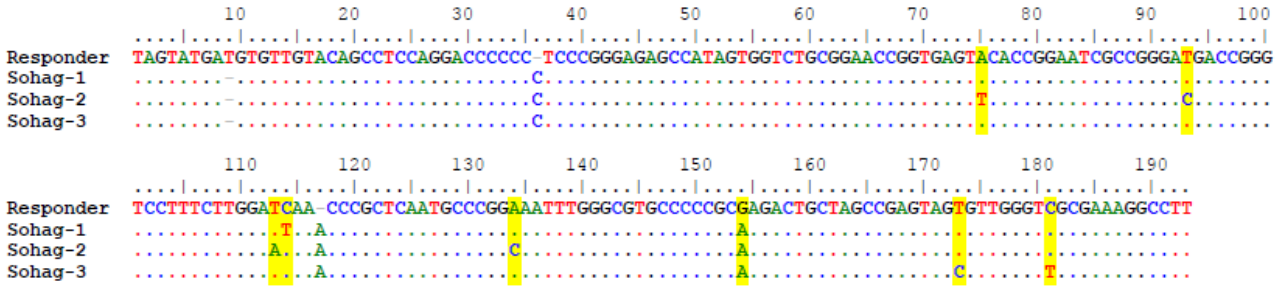

Figure 1. Nucleotide sequence alignment comparison of the HCV 5′-UTR region between responder and non-responders isolates.

Multiple alignment comparison with the 6 known genotypes retrieved from GenBank databse is shown in Fig. 2. Hyper variable region was located from nt 85 to 161 in the 5'UTR of different HCV isolates. Located in the IIRIS IIId subdomain loop, TTGGGT sequence at position 177-182 nt, this sequence was conserved in all genotypes (highlighted and boxed in Fig. 2). Two transitions T_{175}→C and C_{183}→T exist in the stem of IIId subdomain were highly represented in genotype 2 and 6. Using HCV database, we retrieved 200 sequences for each genotype to analyze the percentage of the T_{175}→C and C_{183}→T transitions in each genotype and it was as follow: 1.0, 95.5, 2.0, 14.5, 0.0 and 33.5% in genotypes 1, 2, 3, 4, 5 and 6, respectively. Also, the 2 transitions were detected in isolate Sohag 3 but not in Sohag 1, 2 or the responder isolate. Multiple alignment comparison against hepatocellular carcinoma (HCC), responder and non-responder isolates, collected from HCV database was done (Fig. 3). We noticed that the occurrence of the 2 transitions was not related to HCC or response to therapeutic treatment.

3.2. Phylogenetic subtyping of HCV 5′-UTR of Sohag isolates

According to the phylogenetic analysis, genotypes 1, 2, 3, 4, 5 and 6 were separated in 6 clusters (Fig. 4a). The Egyptian isolates (responder and non-responders) were clustered into genotype 4. Phylogentic relationship between our isolates and genotype 4 is shown in Fig. 4b. Sohag 1 and the responder isolates were clustered with subtype 4a. However, isolate Sohag 2 was clustered with isolate FJ462432 (4g), AB548316.1 (4o) and FJ462440 (4o). Isolate Sohag 3 was clustered with isolate FJ462434 (4q) and AB550017.1 (4a).

3.3. Secondary structure of HCV 5′-UTR

To investigate the effect of the 2 transitions T_{175}→C and C_{183}→T, located within IIId subdomain stem, the secondary structure was analyzed using mFOLD where the window parameter controls how many foldings will be automatically computed and how different they will be from one another. The secondary structure of the 5′-UTR III domain resulted in 4 stem loop subdomains IIIa-d (Fig. 5A). The absence of the 2 transitions T_{175}→C and C_{183}→T in IIId subdomain stem, affected the formation of the UUGGGU apical loop, it was replaced by double helix structure in most predicted folding (B and C, respectively).

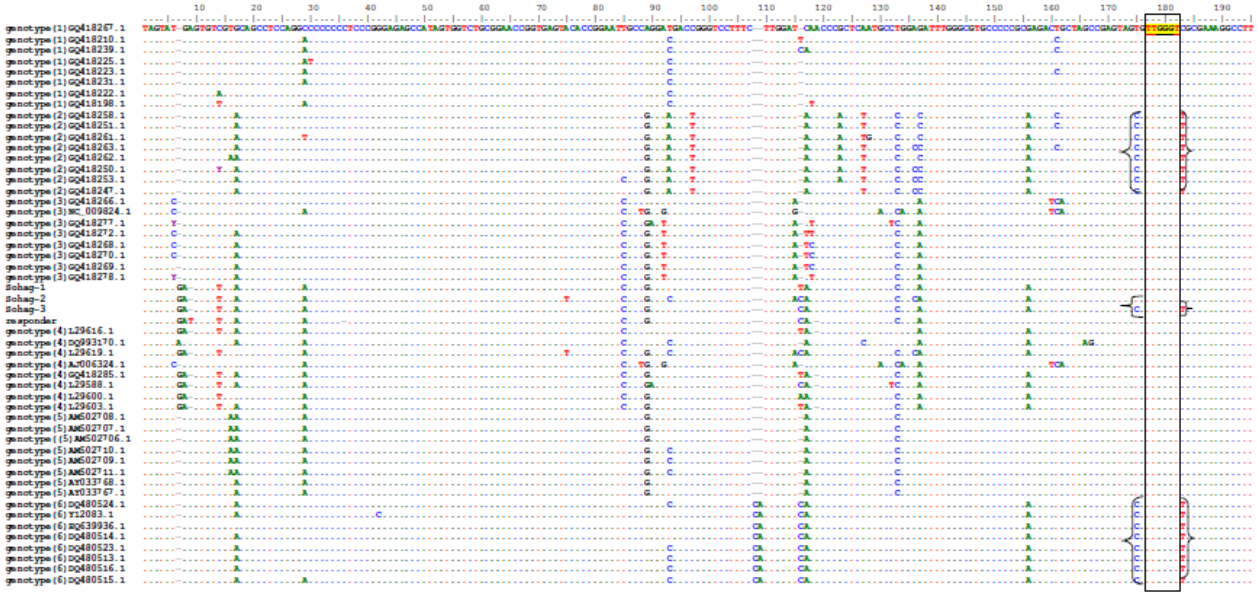

Figure 2. Multiple alignments of the nucleotide sequence of HCV 5′-UTR genotypes. The TTGGGT conserved sequences located in the IIRIS IIId (177-182nt) are boxed; T_{175}→C and C_{183}→T substitutions could be related to IIId loop formation are parenthesized.

Figure 3. Multiple alignments of the nucleotide sequence of HCV 5′-UTR using isolate Sohag 3 against hepatocellular carcinoma (HCC), responder (RS) and non-responder (NR) isolates. Arrows mention to the 2 transitions T_{175}→C and C_{183}→T.

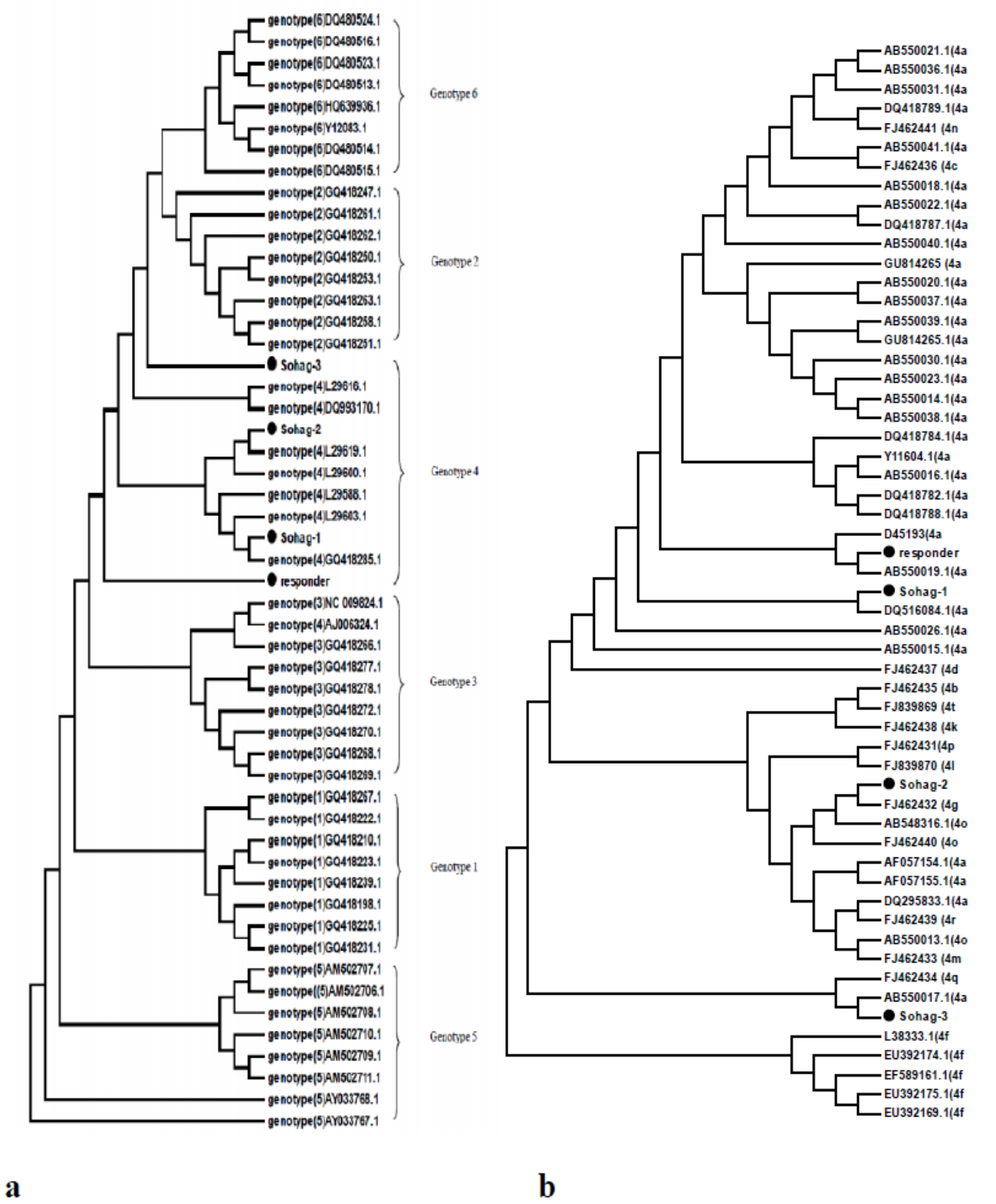

Figure 4. Rooted neighbor-joining tree of HCV 5′-UTR using genotypes 1-6 (a) and subtypes of genotype 4 (b). Bootstrapping of 1000 replicates was carried out.

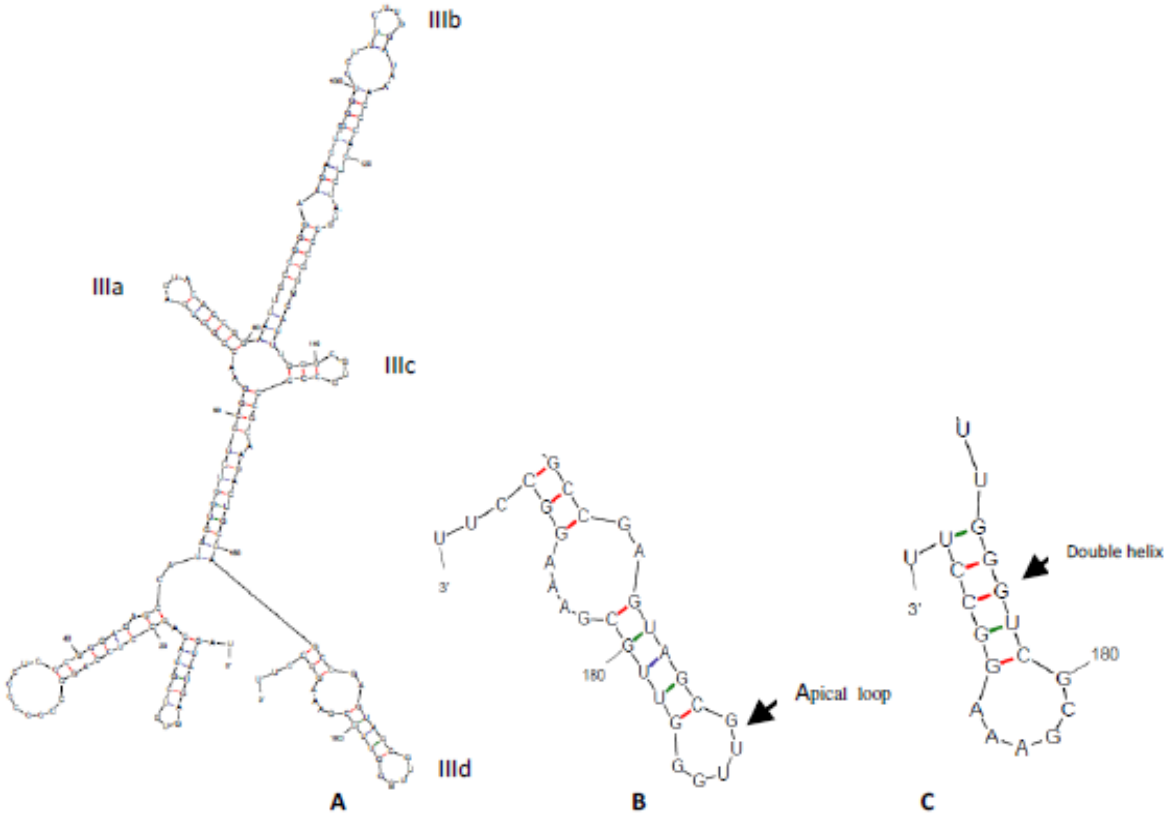

Figure 5. RNA secondary structures of the IRIS IIId subdomain of HCV5′ UTR isolates, predicted by mFOLD version 3.2 program. (A) Secondary structure of the entire IRIS III domain resulted in 4 stem loop subdomain (IIIa-d). The absence of the 2 transitions T_{175}→C and C_{183}→T in IIId subdomain stem, affected the formation of the UUGGGU apical loop, it was replaced by double helix structure in most predicted folding (B and C, respectively).

4. Discussion

We have previously reported the isolation, amplification and sequence analysis of HCV non-responder isolates (Sohag 1, 2 and 3) from Egypt and they were grouped with genotype 4 (Heimeda *et al*., 2010).

In the present study, sequence of 5′-UTR of the three non-responder isolates (sohag 1, 2 and 3) and one responder isolate reported by Zekri *et al*. (2007) were analyzed. Alignment comparison between the responder and non-responder isolates resulted in one insertion and 2 deletions in the responder isolate. The substitution G_{154}→A resulted in our non-responder isolates similar to results reported by Zekri *et al*. (2007). Anila *et al.* (2009) showed that the nucleotide substitutions within the HCV 5`UTR may influence the viral translation and its sensitivity to the antiviral action of interferon.

Extensive studies indicated the importance of HCV genotyping and subtyping in interferon treatment and progression of chronic liver disease (Dammacco *et al.,* 2000; Farci and Purcell, 2000 and Anila *et al*., 2009). Our non-responder and the responder isolates were clustered within genotype 4. Sohag 1 and the responder isolates were clustered with subtype 4a. Sohag 2 was clustered with isolates of subtypes g and o, however, isolate 3 was grouped with isolates from subtype 4a and q. Regrouping of some isolates was previously noticed with isolates genotyped as type 1a or 1b and were found to be wrongly subtyped (Stuyver *et al*., 1995).

Mutations in IIId domain disrupt IRES-mediated translation initiation and also affect the RNA structure, demonstrating the importance of correct RNA folding to IRES function. (Psaridi *et al*., 1999 and Jubin *et al*., 2000). Sequence comparison showed that apical loop nucleotides (UUGGGU) were absolutely conserved across HCV genotypes (Fig. 2), this was also reported by Jubin *et al*., (2000). This conserved sequence corresponds to position 262-270 of the strain H77, Honda *et al*. (1999). Two transitions T_{175}→C and C_{183}→T were highly

represented in genotype 2 followed by genotype 6 and were not found in the analysed isolates of genotype 5. Hazari *et al*., (2005) and Klinck *et al*., (2000) mentioned to the presence of these 2 changes in the primary sequence of IIId shows between the six major HCV genotypes. Dual substitution mutants within the IIId terminal loop demonstrated reductions in activity in the range of 18–42% for the U264:U269 series (Klinck *et al*., 2000). Prediction of secondary structure showed that the 2 transitions affect the formation of the apical loop of IIId subdomain. In the absence of these 2 transitions, the apical loop was replaced by double helix structure in most predicted folding. These 2 transitions could be related to the therapeutic response in genotype 2 and 6. Treatment with pegylated interferon and ribavirin resulted in a significantly higher rate of SVR in patients infected with genotype 2 and 6 than in those infected with the other genotypes (Dev *et al*., 2002, Fung *et al*., 2008, Hui *et al*., 2003 and Phillip *et al*., 2009). Our results showed that the occurrence of the 2 transitions was not related to HCC or response to therapeutic treatment.

This study revealed that our Egyptian isolates were clustered with subtypes 4a, g, o and q. A correlation between the two transitions $T_{175}\rightarrow C$ and $C_{183}\rightarrow T$ in IIId subdomain, the different genotypes and the secondary structure was detected. The 2 transitions were highly represented in genotype 2 followed by genotype 6 where a significantly high rate of SVR was detected for both genotypes. The formation of UUGGGU loop was highly affected by the presence of the 2 transitions $T_{175}\rightarrow C$ and $C_{183}\rightarrow T$. Taken together, the % of the 2 transitions in different genotypes and the modification in the apical loop structure could affect the response to therapeutic treatment.

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Ultrastructure of the Cellular Response of Rabbits' Gingivae to the Adverse Effects of Light Enhanced Bleaching

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Abstract: A total of 30 rabbits were selected. The animals were divided into equal 6 groups, 5 animals each. While a control group received no treatment (G1: normal), the animals of experimental groups (G2 to G6) were anesthetized and the labial gingivae of the upper and lower anterior teeth were painted with a layer of a mixture of a 35% hydrogen peroxide solution and a bleaching agent during the application enamel bleaching utilizing a plasma arc lamp for three intervals, 20 minutes each. The animals were sacrificed after five intervals: (24 hours: G2, one week: G3, two weeks: G4, one month: G5 and two months: G6) subsequently. After each period of investigation, the gingiva of the rabbits were carefully dissected and prepared for transmission electron microscopy examination. The results revealed that bleaching effects on gingival tissue elements were of various degrees cellular and nuclear affections. Moderate to severe cellular and nuclear injuries may be produced as an early response to the bleaching effect. Subsequently, tissue injuries were of various degrees involving the different gingival tissue elements.
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Keywords: transmission electron microscope, bleaching, Gingivae

1. Introduction

Since bleaching has become a popular procedure, the side effects of peroxides was of great interest in research.[1-5] Moreover, studies have been performed in order to evaluate the adverse effects produced during and after bleaching procedures on soft tissues.[6-10] The side/adverse effects with the various bleaching regimens studied included those of night guard vital bleaching and internal bleaching of endodontically treated teeth.[11-13] Damage of cellular and nuclear proteins and lipids was seen.[14,15] Potential adverse outcomes including co-carcinogenic effects with tobacco, and free radical generation and release have been reported.[16] Moreover, several studies have shown the involvement of reactive oxygen species including hydroxyl radical and hydrogen peroxide in colon cancer, breast tumors and stroke.[17-19] However, Cellular response to peroxides has been investigated in various studies.[12-28] Ultrastructurally, gingival tissues have the characteristics of keratinized epithelium.[29] Since there was no reports about the ultrastructural response of cellular structures of gingiva during bleaching, the current study seemed of interest. The ultimate goal of the current study was to gain insight into the effect of bleaching on rabbits' gingivae, by transmission electron microscopy (TEM), following enamel bleaching.

2. Materials and Methods

A total of thirty male New Zealand rabbits were selected from a reputable supply, the rabbit unit at the Faculty of Agriculture, Cairo University. The animals' weight ranged between 2.5-3 Kg with an age of 4-4.5 months. Two rabbits were kept in a separate cage, fed and maintained during the time of the study at a private animal housing unit. The rabbits were fed on a specific diet Ad libitum (about 150 gm per day) and water. Rabbits were left for a few days before the procedure to settle down. All animals were previously vaccinated and treated against scabies, coccidiosis and enteritis (viral hemorrhage diseases).

Treatment schedule;

The thirty animals were randomly divided into control and five experimental groups, 5 animals each. Animals in group (1) were received no further treatment. Animals in groups (2-6) were received the same treatment but sacrificed at different intervals (G2: 24 hours-treatment, G3: one week-treatment, G4: two weeks-treatment, G5: one month-treatment, and G6: two months-treatment). The labial gingivae of upper and lower anterior teeth of the rabbits in the experimental groups 2 to 6 were painted, using a brush, with a mixture of 35% hydrogen peroxide solution and the bleaching agent during the application of enamel bleaching according to the manufacturer's instruction. The mixture on the tooth surface and gingiva was the exposed to a bleaching light source (Wave light, Schein, Melville, NY, USA) that contains a plasma arc

lamp. All the upper and lower incisors teeth and labial gingivae were exposed to light enhanced bleaching three times, 20 minutes each according to the technique described in previous studies.[4,5] Animals were inspected regularly before and after the application of the bleaching procedures and throughout the experimental periods (Fig.1). The animals were sacrificed at the termination of the aforementioned periods.

Preparation of the specimens for TEM examination

After each period of investigation, the rabbits were sacrificed, the gingiva of the rabbits were carefully dissected with a sharp scalpel No 15 and removed (Figs. 3 and 4). The gingival samples were cut into very small cubes nearly 1mm x 1mm and rabidly immersed in labeled jars of a mixture of 2.5% glutaraldehyde and 10% formaldehyde (F/G solution). Specimens were kept in FG solution for 24-48 hours in dried cool place. Specimens were then washed several times in phosphate buffer solution with pH 7.2-7.4. The specimens were post-fixed in 1% osmium tetroxide for one hour, and washed again in phosphate buffer. The specimens were loaded in ascending concentrations of ethyl alcohol for dehydration. After complete dehydration, the specimens were embedded in (EPON 812) using flat rubber moulds. Curing of EPON 812 moulds were done to obtain the specimen blocks. Semi-thin sections were cut with a diamond knife, mounted on glass slides and stained with 1.0% toluidine blue for light microscopic examination. The area of interest was selected for ultra-thin sectioning. The cut sections were stained with uranyl acetate and lead citrate to be examined with TEM (Electron Microscope 1010, Japan).

3. Results

Control group

Semi-thin section examination of toluidine blue stained gingival specimens of this group, revealed the configuration of both epithelial and connective tissue cell layers. The gingival epithelium was appeared as stratified, squamous keratinizing epithelium, which consists of a basal layer, a spinous layer, a granular layer and a superfacial cornified layer (Fig.6).

Ultrastructural features, using transmission electron microscope (TEM) revealed that the basal cells are columnar in shape with well-defined cell membrane. Their nuclei were elongated, centrally located, with homogenous chromatin distribution and condensation near the nuclear membrane. The nuclei were surrounded by clear regular double nuclear membrane. The rough endoplasmic reticulum (RER) was well developed, with flattened and parallel cisternae and arranged in the perinuclear region of the basal cell layer. Abundant mitochondria were detected showing their typical structure, which is a double membrane with internal cristae. They appeared spherical and/or elongated in the basal cell cytoplasm. Some of them appeared swollen and lost their internal cristae. Numerous junctions were seen between adjacent basal cells mostly desmosomes. The spinosum cells were arranged above the basal cells and attained polyhedral outline. Their nuclei were voluminous, with irregular nuclear membrane and peripheral chromatin distribution. Nucleolus was very evident in this layer. Their cytoplasm contained some mitochondria, RER and small electron dense granules. The next overlying layer is the granulosum cell layer. It was characterized by the presence of flattened elongated cells with a wide centrally located nucleus. Superficially, the cornified layer was seen as flattened layer of cells with relatively narrow intercellular spaces.

The junction between a basal cell and the connective tissue of the underlying lamina propria was studded with numerous hemidesmosomes (HD) and is connected to it by a basal lamina (BL). The basal lamina appeared intact, consisted of an electron-dense layer, the lamina densa (LD) and an electron-lucent layer, the lamina lucida (LL). Anchoring fibrils (AF) extended from the undersurface of the lamina densa into the lamina propria. Densely packed groups of transversal and longitudinal collagen fibers were seen. A large collagen fiber (CF) consisting of hundreds of collagen fibrils is outlined. Fibroblasts were seen scattered throughout the connective tissue. They were irregularly shaped cell. Their nuclei were elongated and/or fusiform with normal chromatin distribution. Moreover, the cytoplasmic extensions of fibroblasts were clearly apparent and connected with each other by intercellular junctions. Some fibroblasts had intracellular vacuoles containing collagen fibrils. Vascular components revealed that blood vessels are lined with endotheliocytes and red blood corpuscles (RBCs) are seen in the vascular lumen (Figs. 7-10).

Group 2

By comparing this group with the control group, serious ultrastructural changes had occurred throughout the gingival tissue cell layers. The stratified epithelium cell layers were markedly altered. The arrangement of the basal cell layer was more or less clear. However, obvious destruction of intercellular cell junctions in basal cell layer and wide extracellular matrix were observed. Intracellulary, the basal cells showed varied degrees of cellular activity. Most of them showed some degree of pleomorphism with irregular cell membranes. The cytoplasm of the basal cells showed severe vacuolization and hyalinization. Mitochondria appeared rounded and swollen with wide spread of crystolisis. Also, RER had become disrupted, dilated with much loss of their ribosomes. The nucleus appeared enlarged with irregular nuclear membrane. Severe peripheral and central nuclear chromatin clumping was detected. In some cells the nuclear

membrane became ill-defined with severe chromatin condensation. Nuclear mitosis and activity could be seen clearly in basal epithelial cells of this group.

The spinosum cells were preserved their polyhedral shape but with less volume and wide extracellular matrix. The nucleus appeared irregular with central chromatin condensation. The cytoplasm showed vacuolization and many scattered swollen mitochondria. The granulosum layer had the same characteristic outline as seen in the control group but with wide extracellular matrix. Vacuolization and numerous swollen mitochondria were detected in the cytoplasm. The nuclear membrane was irregular with marked peripheral and central chromatin condensation. Superficially, the cornified layers were partially separated from each other and form the granular cell layer. A clear wide cleft was detected between the keratin layer and the granular cell layer in many areas of this group samples. The basal lamina in this group was markedly interrupted along its course. Also the anchoring fibers were not detected clearly in samples of this group. The ultrastructural alterations had progressed already to the subepithelial connective tissue. Such alterations were represented by clear areas of hyalinization and vacuolization with the presence of inflammatory cells in between the transversal and longitudinal collagen fibers. Fibroblasts appeared shrunken, with marked reduction in cytoplasmic and nuclear volume. Numerous swollen mitochondria were condensed in the cytoplasm. Partial loss of the intercellular junctions between the fibroblastic cell processes (Figs. 11-14).

Group 3

Various grades of ultrastructural alterations had expressed in different gingival layers of this group samples. The basal cell layer of gingival epithelium showed obvious intracellular changes. Mitochondria appeared large, swollen with loss of its internal cresent. The RER showed clear hyalinization and loss of their ribosomes. The nucleus appeared moderate in size, with irregular nuclear membrane with central and peripheral chromatin condensation. Nuclear mitosis was very dominant in this group sample. Wide intercellular cell spaces were very apparent between the basal cell layers. The spinosum and granulosum cell layers preserve their main characteristic orientation in this group, however they had noticeable wide extracellular matrix. Intracellulary, the cytoplasmic vacuolization and mitochondrial enlargement were common feature in these cell layers. The nuclear alterations such as peripheral chromatin condensation and irregular nuclear membrane were seen clearly in both spinosum and granlosum cell layers. Some parts of the cornified cell layer were completely lost, other parts appeared intact without evidence of superficial separation. The basal lamina was detected in favorable condition with its lamina lucida and lamina densa layers. However, it expressed some minute sporadic areas of interruptions which were detected only on higher magnifications. The anchoring fibers were well defined in the underlying connective tissue (C.T). The lamina propria showed noticeable dilatation in blood vessels. No sign of hyalinization was detected in the lamina propria of this group. Some vacuolization could be seen between the numerous collagen bundles. Fibroblasts appeared scattered in the lamina propria, they showed small irregular nuclei with peripheral chromatin condensation. Their cytoplasmic organelles appeared more or less of regular appearance (Figs. 15-19).

Group 4

In this group, the gingival epithelium had different ultrastructural features than that of the previous groups. The basal cell layer of the gingival epithelium exhibited nearly regular orientation. They showed irregular cell membranes and enlarged nuclear-cytoplasmic ratio. Their nuclei expressed irregular nuclear membrane, central and peripheral chromatin condensation. No signs of nuclear mitosis were detected in this sample. Mitochondria showed hyalinization and loss their internal crisente. RER appeared with detached ribosomes. The intercellular spaces are still wide and little desmosomes could be detected between the basal cells. The spinosum, granulosum and the cornified cell layers expressed almost the same as those of group 3, while the deeper layer of the cornified part retained their nuclei. The basal lamina appeared intact along its course, with clear lamina lucida and lamina densa. The anchoring fibers could be seen clearly in such group sample. The lamina propria showed mild vasodilatation and hyalinization. Numerous fibroblasts had somewhat preserved cytoplasmic organelles (Figs. 20 and 21).

Groups 5 and 6

The last two groups were almost with similar features in various cell layers with noticeable increase in the epithelial thickness compared to that observed in the previous groups. The basal cell layer of gingival epithelium had regular orientation. Their cell membrane showed more or less regular and smooth outline. The nuclei appeared clear, open faced with regular nuclear membrane and chromatin distribution. Intercellular cell junctions, mostly desmosomes, were numerous and dominant especially between the basal cells. The spinosum cells retained their polyhedral shape. The intercellular spaces appeared edematous and vacuolated. Their cytoplasm expressed less vacuolization than that of the previous group. Their nucleui showed regular nuclear membrane and peripheral nuclear chromatin condensation. The granular cell layer showed regular orientation. However, the extracellular spaces were still evident. The cornified layer appeared regular and their basal part retained some nuclei. No separation could be seen

between the cornified layer and the underlying granular cell layer. The basal lamina of the gingival epithelial layer was intact and definite, neither perforations nor interruptions were noticed in both lamina densa and lucida layers for these samples. The lamina propria of these samples appeared in much favorable condition than the previous samples regarding tissue hyalinization and vasodilatation. Fibroblasts were numerous and clear in these samples. Their cytoplasmic contents appeared more preserved. The nucleus appeared elongated, open faced with regular nuclear membrane and peripheral chromatin condensation. The intercellular junctions between their cytoplasmic processes could not be detected clearly in these samples (Figs. 22 - 29).

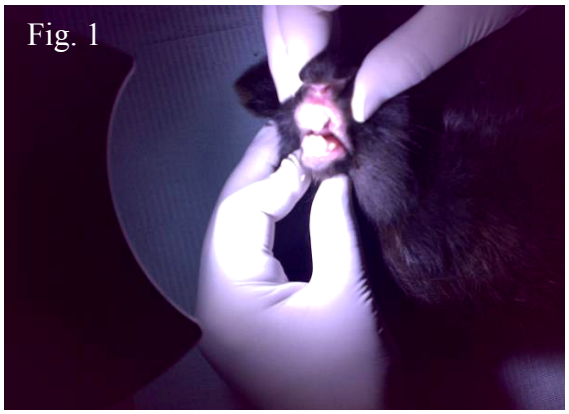
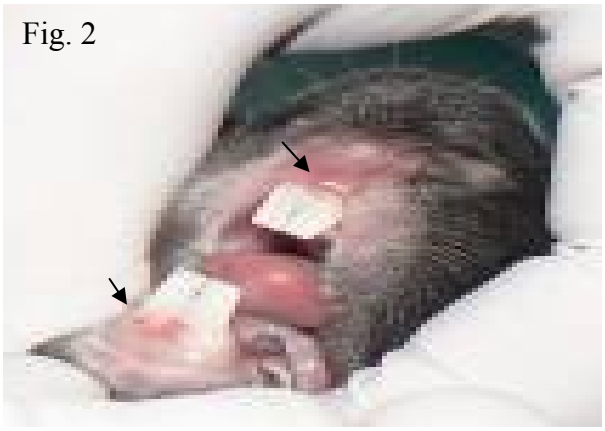
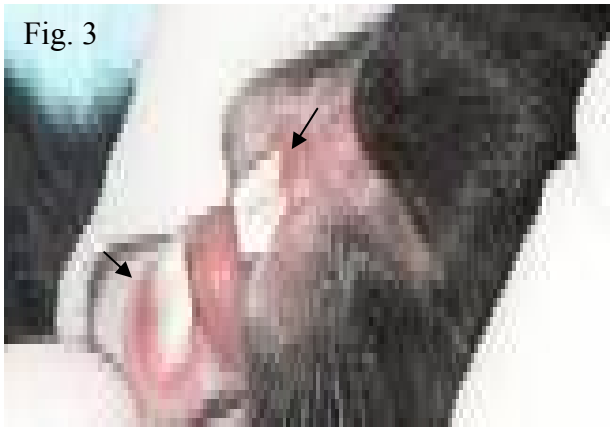
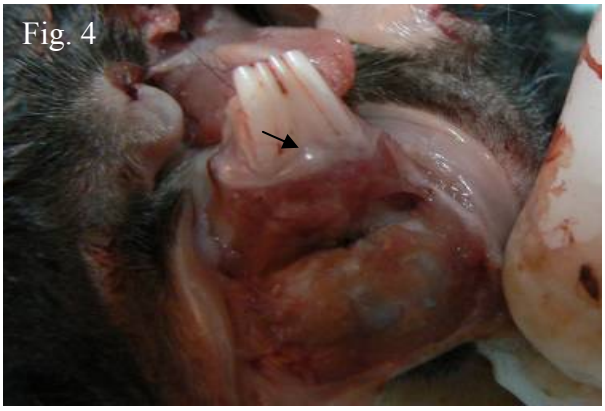


Fig. 1: Photomicrograph of rabbit's anterior teeth and gingiva during exposure to a bleaching light source that contains a plasma arc lamp .





Figs. 2 &3: Photomicrographs of rabbit's gingival tissue after exposure to the bleaching light source. Gingival tissue appeared edematous and inflamed (arrows).



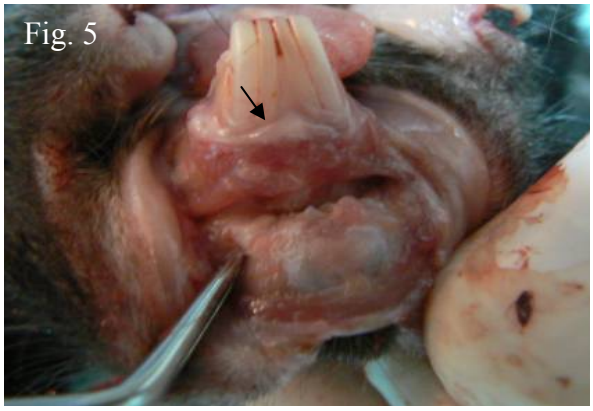


Fig. 4 & 5: Photomicrographs of rabbit`s gingiva (arrows) following mucoalveolar flap just prior to gingival tissue excision.

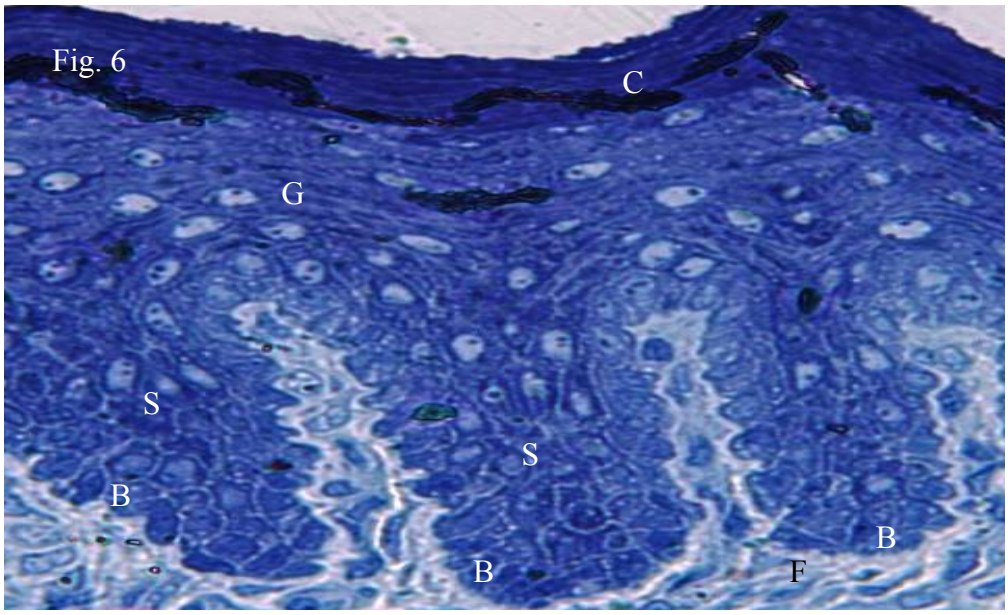


Fig. 6: Semi-thin section through rabbit's normal gingiva (group 1) showing basal cells (B), spinous cells (S), granular cells (G) and a superfacial cornified layer (C). Note fibrous connective tissue containing spindle-shaped fibroblasts (F). (Toulidin blue stain X 400 original magnification).

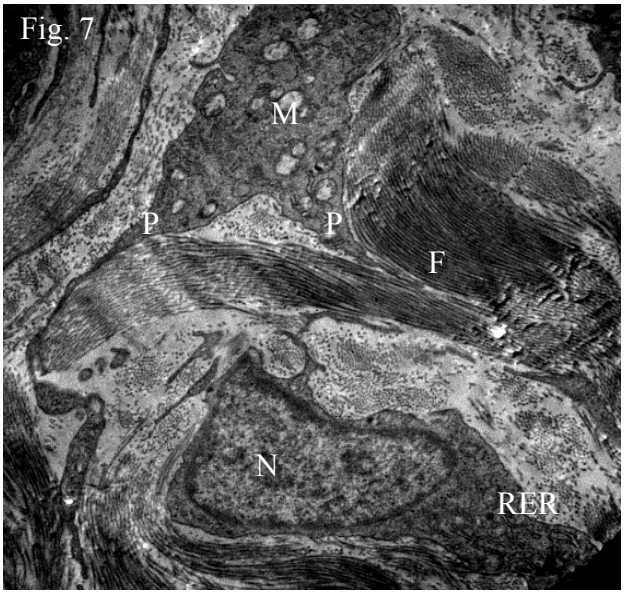


Fig. 7: Electronmicrograph of group 1,showing lamina propria of rat gingival tissue. (N) nuclus of fibroblast, (M) mitochondria, (P) cytoplasmic process, (RER) rough endoplasmic reticulium and (F) collagen fibers. X 8000 original magnification.

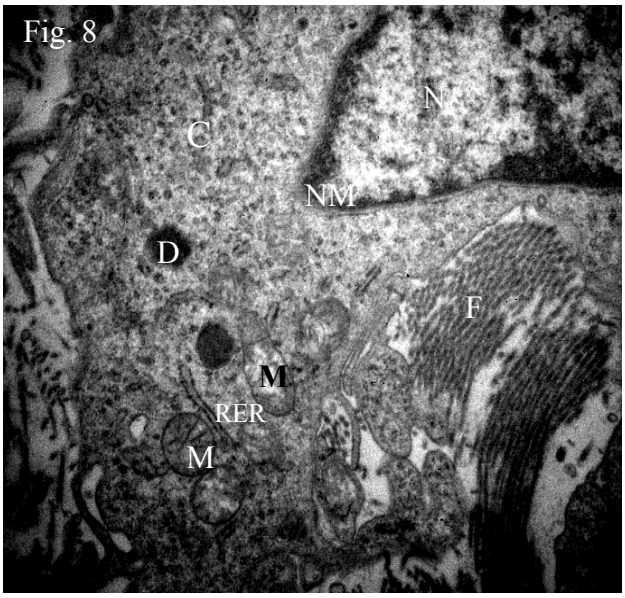


Fig.8: Elecronmicrograph of group 1, showing fibroblastic cell. (N) nucleus, (M) mitochondria, (RER) rough endoplasmic reticulium, (D) electron dense spherical body containing fibers and (F) collagen fibers extracellularly. X 20000 0riginal magnification.

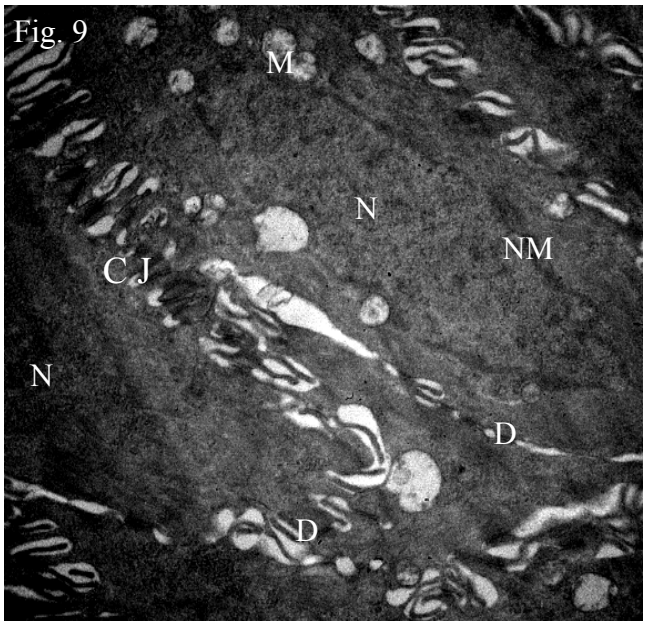


Fig. 9: Electronmicrograph of group 1, showing the basal cells of rat gingival epithelium. (N) basal cell nucleus, (NM) its nuclear membrane, (CJ) cell junctions and (D) desmosomes between basal cells. X 12000 original magnification.

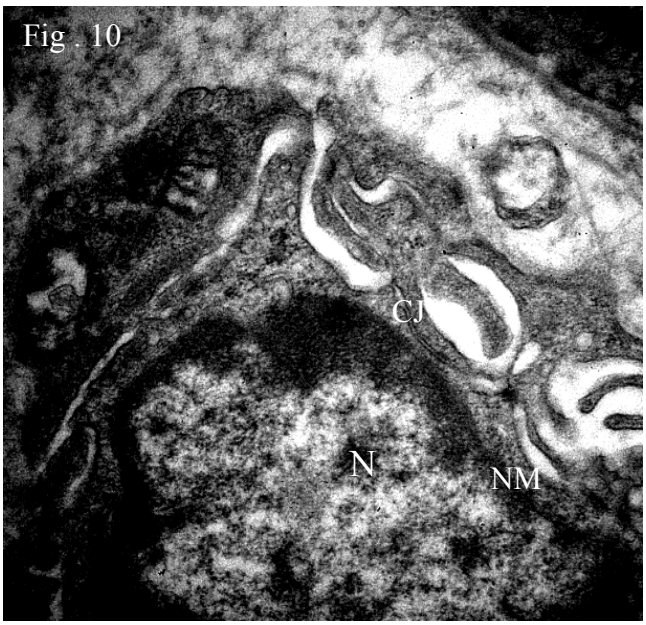


Fig. 10: Electronmicrograph of group 1,showing basal cell with high magnification. (N) its nucleus,(NM) its nuclear membrane and (CJ) cell junctions. X 30000 original magnification.

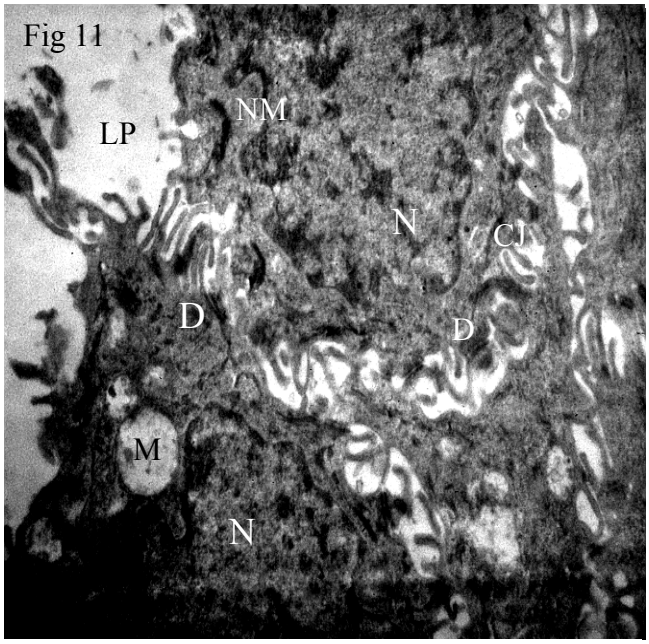


Fig. 11: Electronmicrograph of group 2, showing basal cells of gingival epithelium. (N) nucleus, (M) mitochondria, (D) desmosomes and (CJ) cell junctions. X 15000 original magnification.

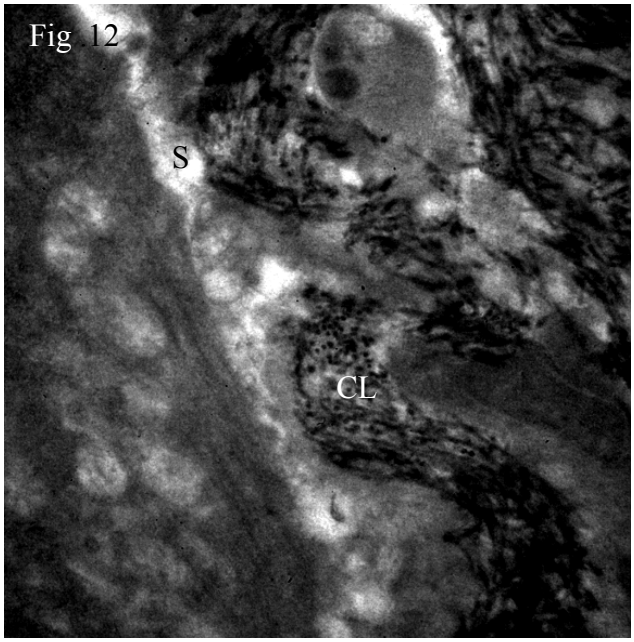


Fig. 12: Electronmicrograph of group 2, showing superfacial cornified layer of gingival epithelium.(CL) cornified layer and (S) separation area. X 20000 original magnification.

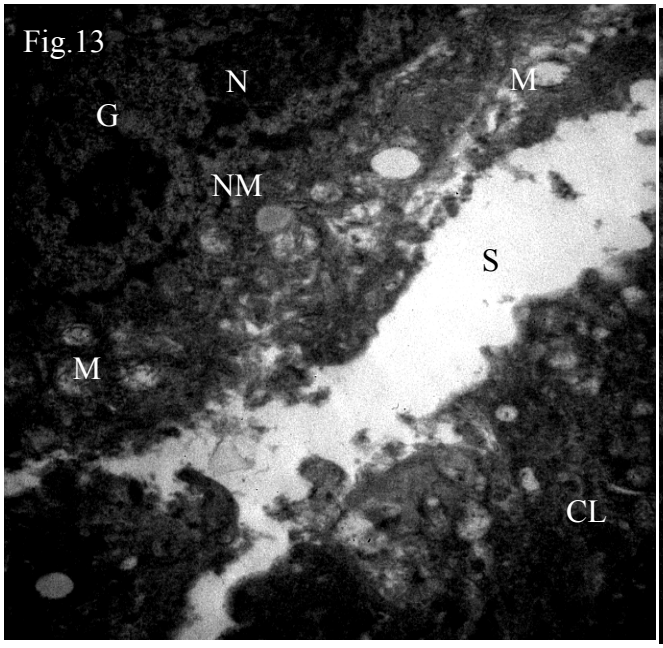


Fig.13: Electronmicrograph of group 2,showing the separation cleft in the superfacial part of gingival epithelium. (G) granulosum layer,(N) nucleus, (NM) nuclear membrane, (M) mitochondria and (CL) cornified layer. X 15000 original magnification.

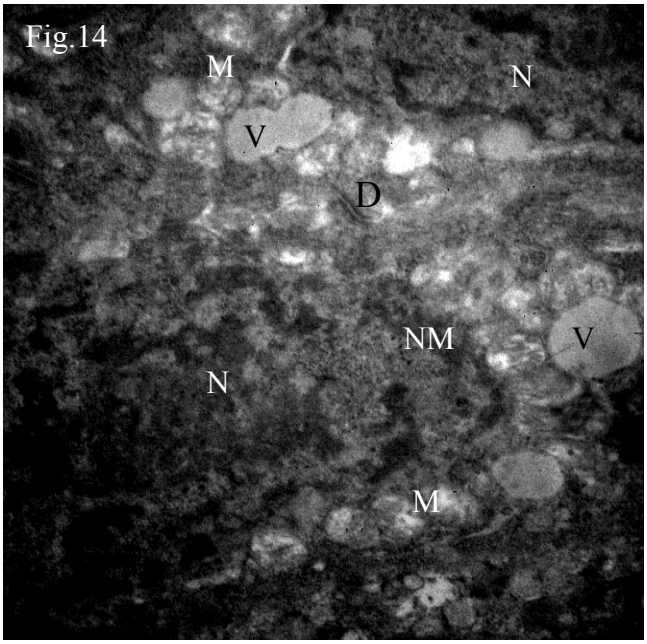


Fig.14: Electronmicrograph of group 2, showing the granular cell layer. (N) nucleus, (NM) nuclear membrane and (M) mitochondria, (V) intracellular vacule and (D) desmosome. X 15000 original magnification.

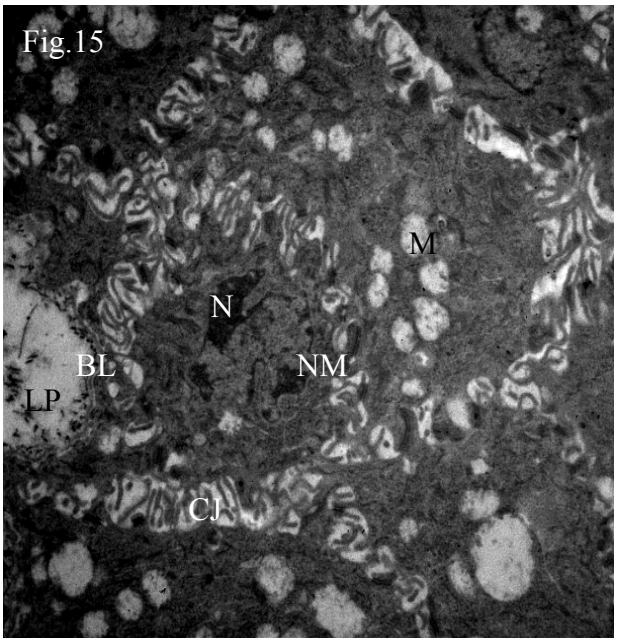


Fig.15: Electronmicrograph of group 3, showing basal cells of gingival epithelium.(N) nucleus, (NM) irregular nuclear membrane, (M) mitochondria, (CJ) cell junctions, (BL) basal lamina, and (LP) underlying lamina propria. X 8000 original magnification.

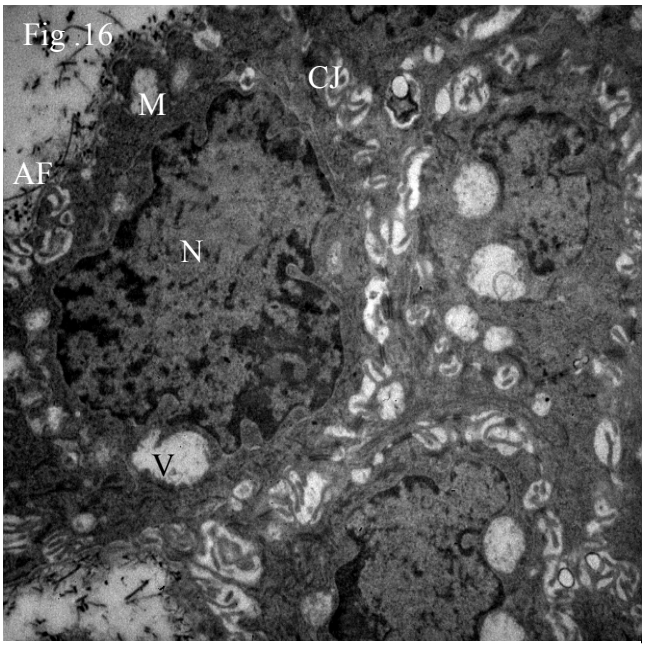


Fig.16: Electronmicrograph of group 3,showing higher magnification of basal cells.(N) nucleus, (V) intracellular vacule, (CJ) cell junctions, (D) desmosomes, (M) mitochondria and (AF) anchoring fibers. X 15000 original magnification.

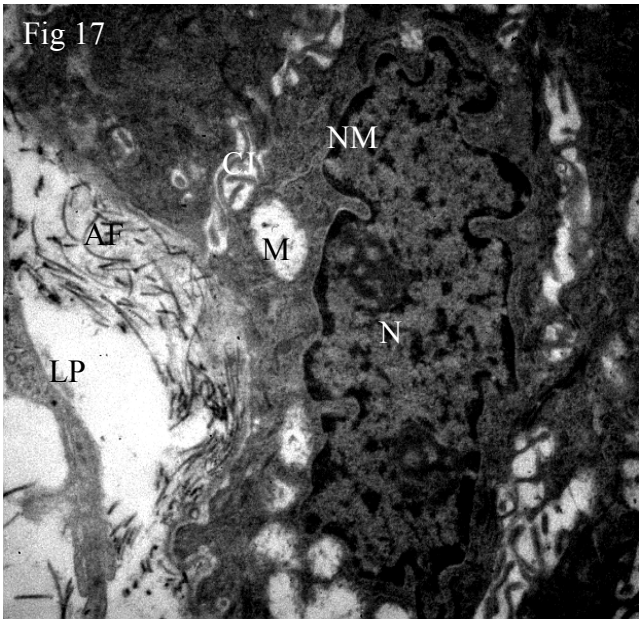


Fig. 17: Electronmicrograph of group 3, showing basal cell. (N) nucleus, (NM) nuclear membrane, (M) mitochondria, (CJ) cell junctions and (AF) anchoring fibers. X 15000 original magnification.

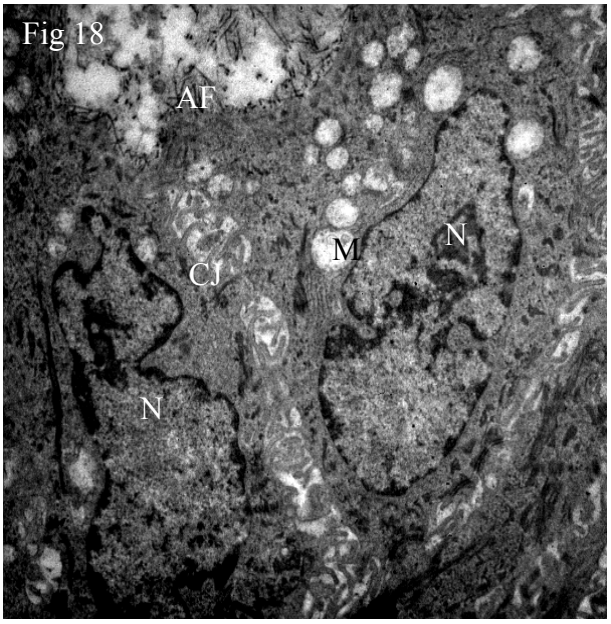


Fig.18: Electronmicrograph of group 3,showing nuclear mitosis in basal cells. (N) nucleus with central constriction, (M) mitochondria,(CJ) cell junctions and (AF) anchoring fibers. X 10000 original magnification.

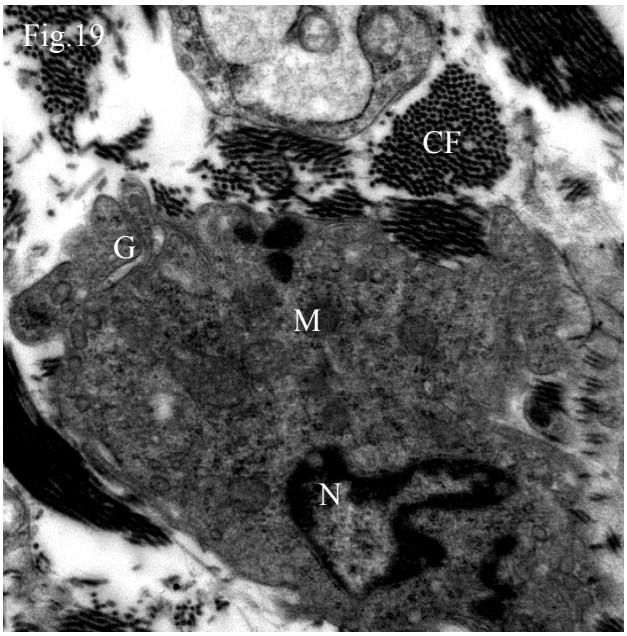


Fig. 19: Electronmicrograph of group 3, showing fibroblastic cell in gingival lamina propria. (N) nucleus, (M) mitochondria, (G) golgi and (CF) collagen fibers. X15000 original magnification.

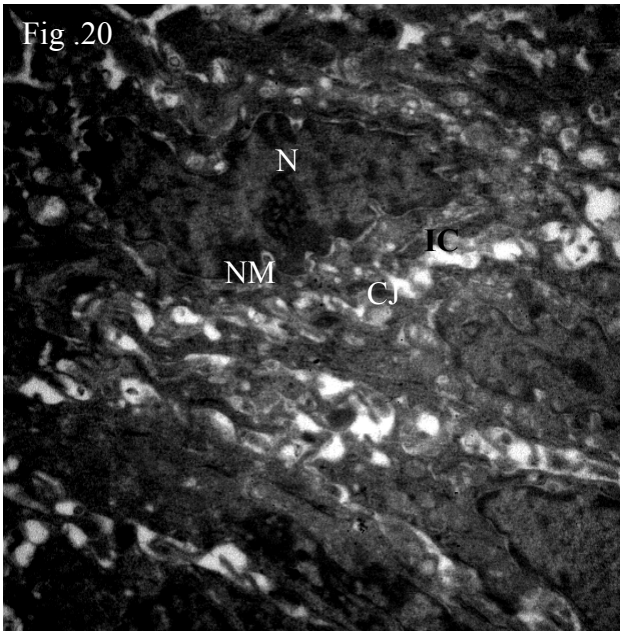


Fig. 20: Electronmicrograph of group 4, showing basal cell layer of gingival epithelium. (N) nucleus, (NM) nuclear membrane, (CJ) cell junctions and (IC) intercellular spaces. X 12000 original magnification.

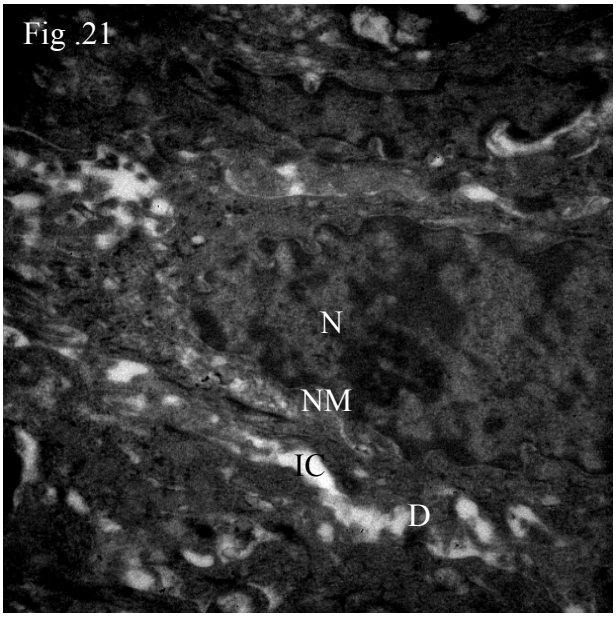


Fig. 21: Electronmicrograph of group 4, showing basal cell of gingival epithelium. (N) nucleus, (NM) nuclear membrane, (IC) intercellular spaces and (D) desmosomes. X 15000 original magnification.

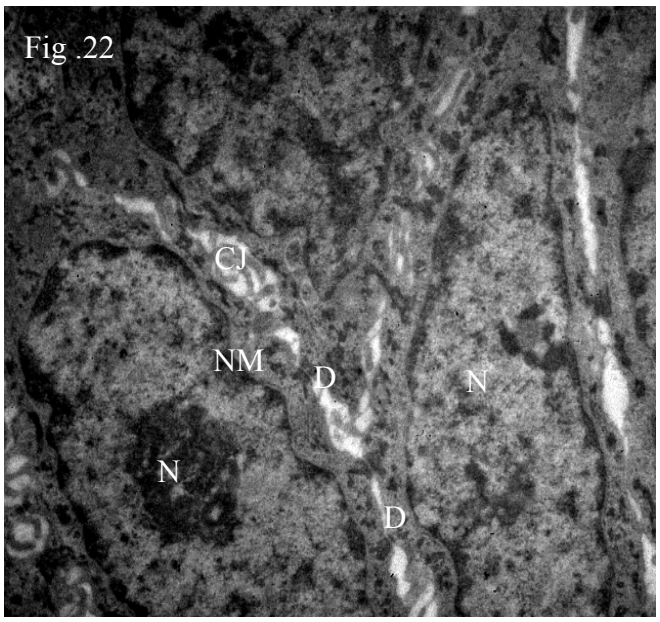


Fig. 22: Electronmicrograph of group 5, showing basal cell of gingival epithelium. (N) nucleus, (NM) nuclear membrane , (CJ) cell junctions and (D) desmosomes. X 15000 original magnification.

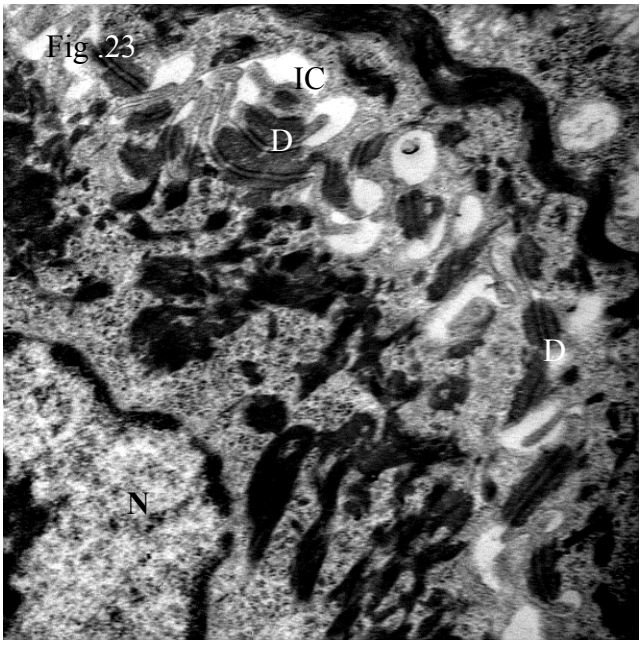


Fig. 23: Electronmicrograph of group 5, showing higher magnification of basal cell of gingival epithelium. (N) nucleus, (NM) nuclear membrane and (D) desmosomes. X 25000 original magnification.

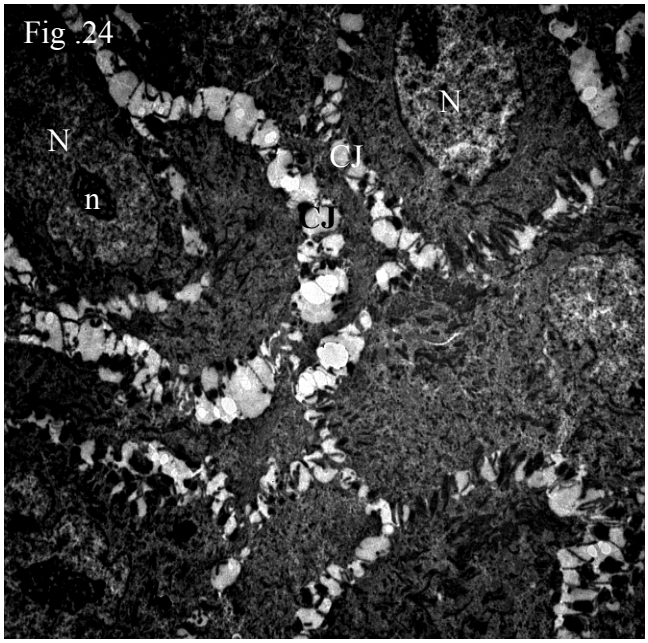


Fig. 24: Electronmicrograph of group 5, showing spinosum cell layer of gingival epithelium. (N) nucleus, (n) nucleolus and cell junction (CJ). X 8000 original magnification.

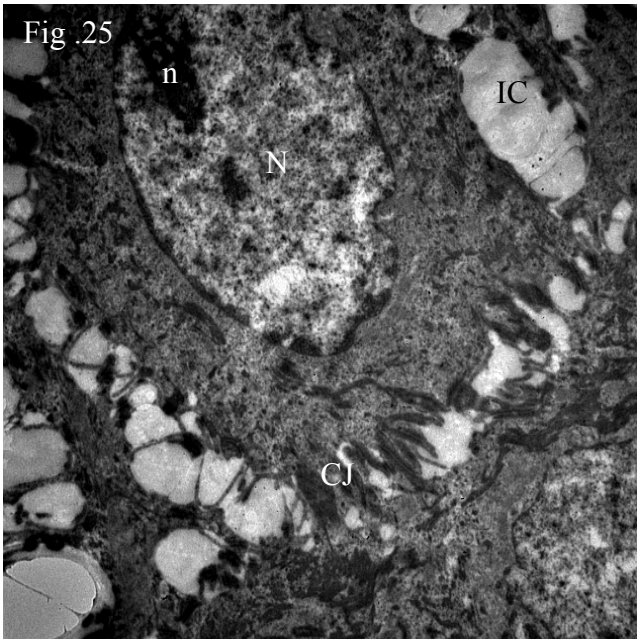


Fig. 25: Electronmicrograph of group 5,showing higher magnification of spinosum cells.(N) nucleus, (n) nucleolus , (CJ) cell junctions and (IC) intercellular spaces. X 15000 original magnification.

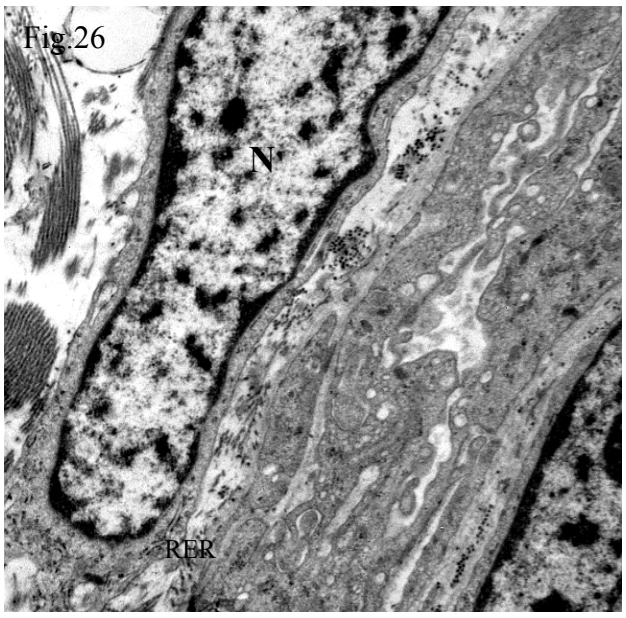


Fig.26: Electronmicrograph of group 5, showing fibroblastic cells of gingival lamina propria. (N) nucleus and (RER) rough endoplasmic reticulum. X 20000 original magnification.

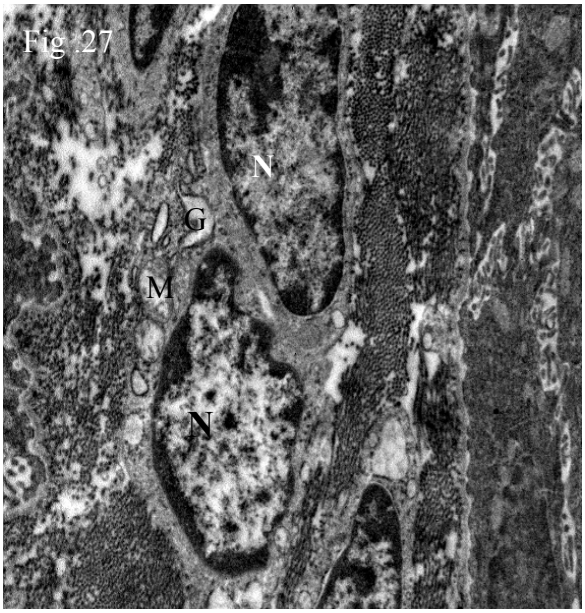


Fig. 27: Electronmicrograph of group 5, showing fibroblasts . (N) nucleus, (M) mitochondria and (G) golgi. X 15000 original magnification.

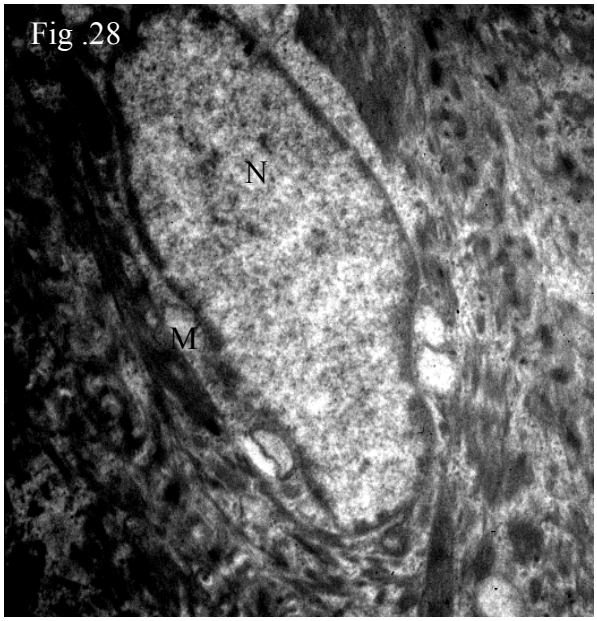


Fig. 28: Electronmicrograph of group 6, showing basal cells of gingival epithelium. (N) nucleus and (M) mitochondria. X 20000 original magnification.

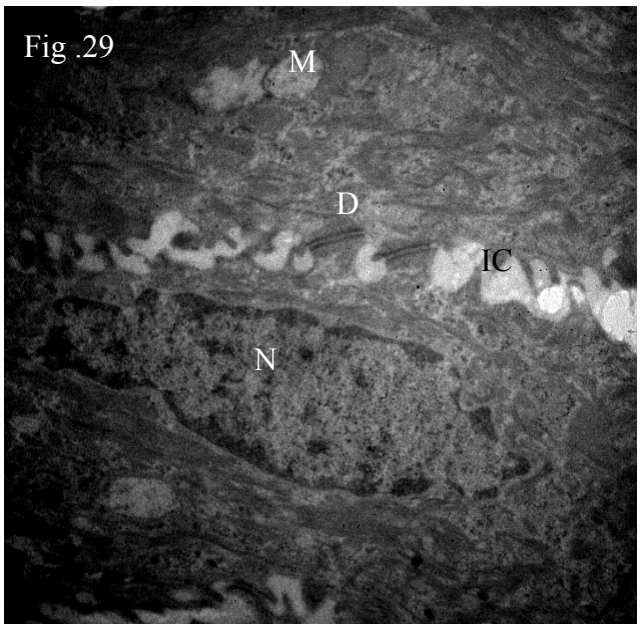


Fig. 29: Electronmicrograph of group 6 , showing basal cell of gingival epithelium . (N) nucleus, (M) mitochondria, (D)desmosomes and (IC) intercellular spaces. X 15000 original magnification.

4. Discussion

Much debate still surrounds many of the noted tooth bleaching side effects/adverse effects. Qualifiers having been outlined, it has found that the most published side effects fall into various categories including tooth sensitivity, gastrointestinal mucosal irritation, changes to enamel and dentin hardness and surface structure, changes associated with internal bleaching. Gingival irritation is another common side effect that is experienced during a tooth whitening treatment. Gingival irritation may be chemically or physically induced teeth whitening problem.

In the present study, almost similar cellular morphological findings of the gingiva of the male Germany rabbits to that reported in human gingiva. The pattern of mucosa of the gingiva (epithelium and subepithelial connective tissues) seen in the low power displayed similar features that are commonly encountered in the human gingiva. Moreover, at a high magnification micrograph, details of membranous and various cellular components and structures revealed similar findings as in the human.[29] These results indicate that the rabbits` gingiva is a successful model for this experiment.

The present study revealed that the effects of bleaching agents on soft tissues in the mouth may adversely affect not only the gingival epithelium but also the subepithelial tissue elements if they gain access to the underlying gingival connective tissue. The present TEM results revealed that hydrogen peroxide in a combination with heat generation caused early alterations in gingival mucosa at the 24 hours (group 2) following the bleaching application compared to group1. This indicates that the gingival tissue did not remain healthy. Serious ultrastructural changes encountered epithelial cell pattern and morphology, in addition to the extracellular matrix and cytoplasmic alterations. The cytoplasmic organelles including mitochondria, RER, ribosomes revealed some sort of distortion. This distortion extended to include nuclear membrane and chromatin in addition to the mitotic figures. Separation between the cell layers with appearance of clefts was obvious. Moreover, similar degenerative observation was seen in the fibroblasts including reduction in cytoplasmic and nuclear volume with partial loss of the intercellular junctions between the fibroblastic cell processes. The presence of inflammatory cells in between the transversal and longitudinal collagen fibers was prominent feature. Similarly, gingival tissues of dogs respond to a continuous application of 1% H_2O_2 solution over 48 hours. Features of edema, followed by epithelial vacuolisation and finally destruction and sloughing of the cornified layer were reported. A

cellular response similar to that in acute inflammation occurred. In addition, increase in vascular permeability is likely, as there is severe edema, a large number of acute inflammatory cells, hemoconcentration in blood vessels and presence of fibrin strands were seen.[20] At a cellular level, Schraufstatter *et al.*[21] demonstrated an induction of poly-ADP-ribose polymerase activation followed by NAD depletion and a fall in ATP, resulting eventually in cell death

In general, the results of the present study are considered as representative with the most commonly observed clinical effects of treatments with tooth whiteners of oral mucosa in some.[6,10,12] Some patients have also reported burning palate, throat and gingiva.[18] Several simultaneous developments to cause any observable effects: direct contact of hydrogen peroxide with tissues, the failure of normal human antioxidant defenses, the access of free radicals to target DNA, and the failure of damaged DNA to repair itself. [15] As hydrogen peroxide is capable of producing free radicals (oxygen species with an unpaired electron) which are highly reactive, it can damage proteins, lipids, and nucleic acids.[10]

The mechanism by which epithelial cells are irritating could be explained as the initial diffusion of peroxide into and through the epithelial layers reach the connective tissue elements by the oxidizing hydrogen peroxide irritation that is hypothesized to originate from the dehydrating effects of ingredient used to carry the active bleaching ingredient The irritation is presumed to result from escape of the bleaching material into the gingival margin area where salivary flow is typically low, allowing the material to sit relatively undisturbed. The hydrogen peroxide can cause an acute inflammatory reaction and some of the other components of the bleaching solution can dehydrate tissues. Noticeably, gingival irritation was much more likely to occur when the overlying epithelium was abnormally thin or permeable.[22, 27, 28] that was detectable in group 2 where the basal lamina was markedly interrupted along its course. Also the anchoring fibers were not detected clearly in samples of this group.

The present TEM results revealed that hydrogen peroxide in a combination with heat generation at one week period (group 3), comparing to group 2, and revealed moderate cellular and nuclear alterations. Nuclear mitosis was very dominant. Although, the basement membrane was in favorable situation with well defined anchoring fibrils into the underlying connective tissue, the subepithelial connective tissue elements showed noticeable dilatation of vascular channels. This indicates to the incidence of adverse reactions to include the subepithelial connective tissue elements through a permeable basement membrane.

At two weeks period (group 4), the TEM results revealed that hydrogen peroxide in a combination with heat generation revealed minimal alterations, comparing to group 3. The basal cell layer of the epithelial layer exhibited nearly regular orientation with irregular cell membrane and enlarged nuclear-cytoplasmic ratio. Interestingly, the concomitant features of mitosis of the previous groups are not present. Mitochondria showed severe hyalinization and loss their internal crisente. RER appeared with detached ribosomes. The intercellular spaces are still wide and nearly nil desmosomes could be detected clearly between the basal cells. The anchoring fibers could not be seen clearly as in group 3. The superfacial part of the cornified layer showed complete separation in some areas, while the deeper layer retained their nuclei. The remaining cell layers of gingival epithelium and lamina propria attained nearly the same ultrastructural features as seen in group 3. Similarly, Up to two weeks, Matis *et al.*[25] reported 79% incidence of gingival sensitivity. Contrarily, Nathoo *et al.*[24] reported no adverse effects of 2-weeks bleaching with 10% carbamide peroxide. Also, Kowitz *et al.*[23] reported almost no adverse events were reported during this 2-weeks exposure except 1% tooth sensitivity.

The present TEM results revealed that hydrogen peroxide in a combination with heat generation at one month-period (group 5), compared to group 4, revealed minimal alterations. The basal cell layer almost had regular orientation. Their cell membrane showed more or less regular and smooth outline. Their nuclei were markedly open faced with regular nuclear membrane and chromatin distribution. The intercellular spaces were still present but lesser than in the group 4. Intercellular cell junctions, mostly desmosomes, were numerous and dominant especially between the basal cells. The spinosum cells retained their polyhedral shape. The intercellular spaces appeared edematous and vacuolated. Their cytoplasm expressed less vacuolization than that of the previous group The nuclei showed regular nuclear membrane and peripheral nuclear chromatin condensation. The granular cell layer showed regular orientation. However, the extracellular spaces were still evident. The cornified layer appeared regular and their basal part retained some nuclei. No separation could be seen between the cornified layer and the underlying granular cell layer, compared to that seen in samples of groups (2).The fibroblastic cell in the lamina propria appeared in much favorable condition than the previous group, the cytoplasmic contents appeared more preserved. The nucleus appeared elongated, open faced with regular nuclear membrane and peripheral chromatin condensation. The intercellular junctions between their cytoplasmic processes could not be detected clearly in this sample. Similarly, Up to one month: a 3-weeks exposure to 10% carbamide peroxide, 95 of the subjects reported tooth sensitivity and 32% reported minor oral discomfort.[8] Contrarily, Kozlovsky *et al.*[9]

reported that none of the subjects had oral soft tissue irritation.

The present TEM results revealed that hydrogen peroxide in a combination with heat generation at two months-period (group 6), comparing to group 5, revealed almost normal finding, comparing to group 1. Contrarily, Beyond one month, A whitening product with 10% carbamide peroxide was used for 5 weeks on 5 women smokers and 6 women who were not smokers. The authors found with the use of biopsies an increase in the thickness of the epithelium producing an increase in cellular proliferation in the basement and parabasal membranes of the gingival epithelium. The authors pointed out that it is not possible to conclude that 10% carbamide peroxide is carcinogenic in clinical situations, but, it was possible to observe that it alters cellular proliferation and consequently, it could act as a tumor promoter.[16]

The gradual decreasing of the irritating effect of bleaching on gingival tissue elements through the experimental periods is in consistence with other studies where the adverse effects were transient.[23-25] This suggests that bleaching has only a limited and/or reversible. This can be attributed as: in gingiva, there is sufficient antioxidant defensive mechanisms that protect the tissue from radicals generated from the reaction of hydrogen peroxide, and defense mechanism of the gingiva would significantly reduce available levels of hydrogen peroxide. The reason why hydrogen peroxide is considered as a risk factor to our health is because it is a highly oxidative compound and easily decomposes into hydroxyl radicals. As a free radical with an unpaired electron, the hydroxyl radical readily attacks other molecules in its proximity and produces a new free radical and so on. The resulting damage, referred to as oxidative stress, leads to molecular and cellular dysfunction. The destruction of essential macromolecules by oxygen-based reactants is the basis of some diseases and is also believed to be involved in the processes of aging.[26]

In conclusion, caution should be exercised with the application of peroxide products used for bleaching due to the possibility of chemical irritation of oral soft tissues with injudicious use. The volumes of material and application times should be controlled carefully. It is recommended to use isolation because it is vital in the chair side process to prevent tissue irritation. Similarly, retractors are important to provide complete protection of the lips and soft tissue while allowing access to the facial side of teeth and gums.

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