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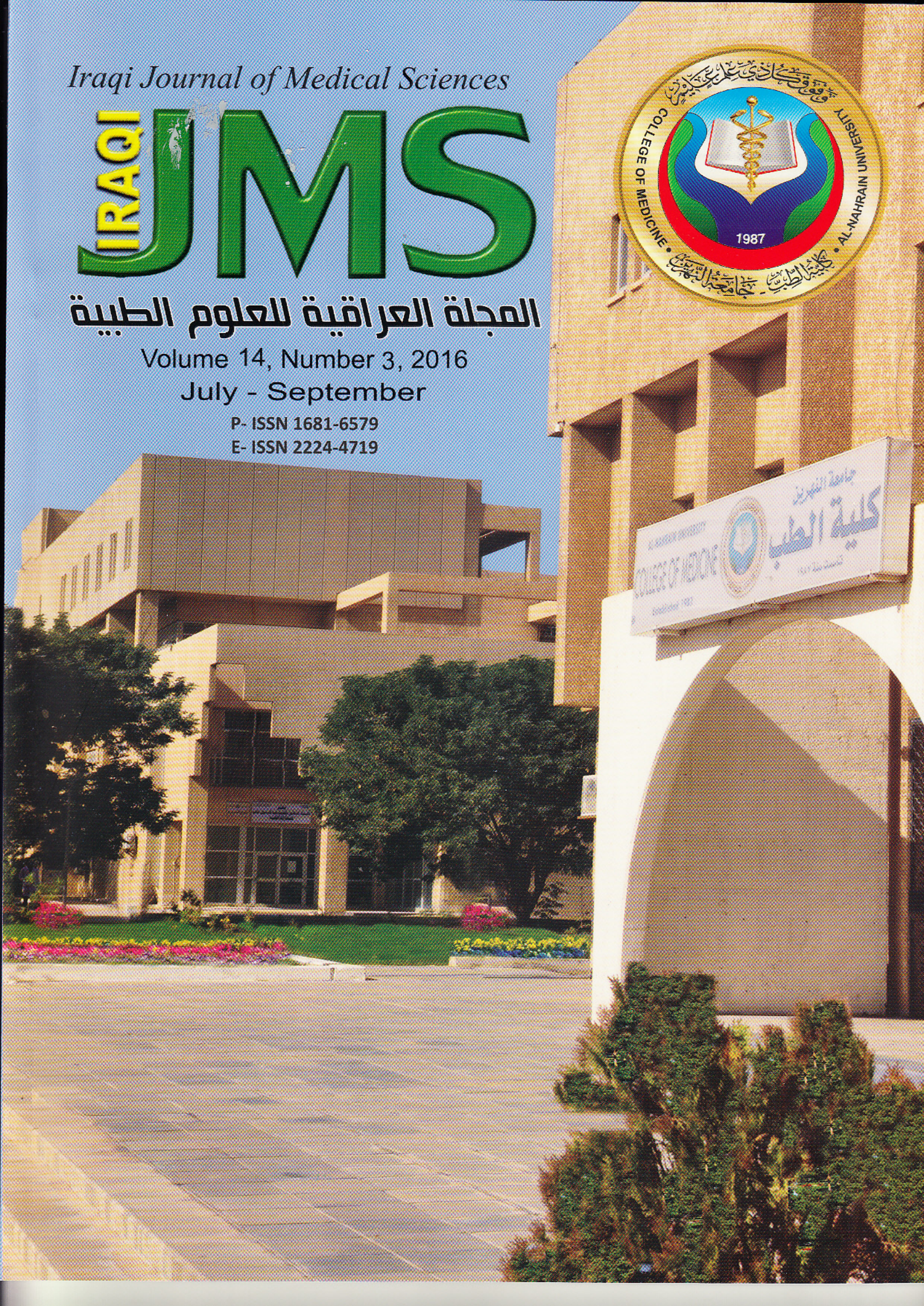

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Iraqi Journal of Medical Sciences is published by College of Medicine, Al-Nahrain University. It is a quarterly multidisciplinary medical journal. High quality papers written in English, dealing with aspects of clinical, academic or investigative medicine or research will be welcomed. Emphasis is placed on matters relating to medicine in Iraq in particular and the Middle East in general, though articles are welcomed from anywhere in the world.

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Iraqi Journal of Medical Sciences (Iraqi JMS) is a periodic, peer-reviewed journal published quarterly by College of Medicine, Al-Nahrain University. Iraqi JMS publishes manuscripts in all fields of health and medicine written in English.

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3. Chapter in book: Phillips SJ, Whisnant JP. Hypertension and strock. In: Laragh JH, and Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

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New Human Body Parts Discovered

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Abstract

Reports about new discoveries in human anatomy could help in the progress of medical care in term of diagnosis and management. Among the many of these was the hidden system of vessels discovered in the human brain in 2015, the anterolateral ligament of the knee joint describe in 2013, Dua's layer discovered in the human cornea in 2013, and the description of the anatomic structure of the G-spot done in 2012. These new part of the human body may be more properly considered as newly discovered variations in the human body.

Keywords: Anatomy, human discoveries, brain lymphatics, knee joint, Dua's layer, G-spot.

Introduction

Human anatomy had been studied in details since hundreds of years; physicians always thought that they know everything about human anatomy. However, the reports of new discoveries in human anatomy proved that there is always something more to learn that could help patients and doctors [(1)]. The knowledge of newly discovered human morphology is important to improve diagnostic and interventional performance for imaging techniques such as echocardiography, magnetic resonance imaging, computerized tomography, endoscopy; open and laparoscopic surgery [(2)].

This article presents 4 of the most recent anatomical discoveries, their regional variation provoked interest to encourage anatomists and clinicians to be aware of the existence of other new human body parts.

Lymphatic vessels of the human brain

The most recent of these new human body part discovered at the University of Virginia in (2015) was a hidden system of vessels discovered in the human brain, which drain lymphatic fluid from the brain to the surrounding lymph nodes. After this new discovery, neuroimmunologists are trying to investigate the mechanisms of brain drainage and inflammation, and a new map of lymphatics was drawn. This newly discovered lymphatic vessels in the brain were found in the dural sinuses draining blood from the internal and external veins of the brain into the internal jugular veins, their locations were nearer to the major blood vessel [(3)].

Before that discovery, the central nervous system was considered to lack lymphatic vasculature, which has raised long-standing questions about how cerebral interstitial fluid is cleared of waste products [(4)]. After centuries, it was presumed that the lymphatic system simply didn't extend to the brain. The discovery of hidden system of lymphatic vessels in the human brain may help in understanding and treating many disorders, including multiple sclerosis, Alzheimer's disease, and Parkinson's disease. New plans are now established to investigate variables aspects of this issue [(5)].

Anterolateral ligaments of the knee joint

Another new human body part discovered at the University Hospitals Leuven in Belgium in (2013) was a ligament in the knee, which appears to play a role in patients suffering from a tear in their anterior cruciate ligament. This ligament was described as the anterolateral ligament, it was found in 97% of all cases. The presence of this ligament was hypothetized in 1879 as an unknown ligament in the knee. Patients who had undergone repairs to their anterior cruciate ligament were experienced trouble with the knee giving way mid-motion even after the surgical recovery appeared to be complete. Patients whose knees remained unstable, a condition known as "pivot shift," were found to have damage in the anterolateral ligament. It was suggested that the close association of the femoral origins of the fibular collateral ligament and anterolateral ligament allowed referral to both these ligaments as one structure, the lateral collateral ligament complex. The anatomic location of the anterolateral ligament suggested that the structure of this ligament may be an important stabilizer for internal rotation [(6)].

The anterolateral ligament arises from the lateral femoral epicondyle, proximal and posterior to the popliteus tendon, and with posterior fibers blending with the proximal fibular collateral ligament. The anterolateral ligament has a strong connection with the periphery of the body of the lateral meniscus by way of its meniscofemoral and meniscotibial components. Distally, the ligament inserts adjacent to head of fibula, with distal flaring clearly apparent. There is no connection of the ligament with the lateral capsule proximally or with the iliotibial band distally [(7)].

Dua's layer of the human cornea

Histological new discovery at the University of Nottingham in England in (2013) was a strong, impervious to air layer of 15 microns thickness in human cornea. This microscopic layer had been called as Dua's layer following the name of the scientist discovered it. It was seen at the back of the cornea between the corneal stroma and Descemet's membrane, and it was considered as the sixth layer of the cornea. Prior to this discovery, the cornea was believed to have only five layers (from the outside in); the corneal epithelium, Bowman's layer, the corneal stroma, Descemet's membrane, and the corneal endothelium [(8)].

It was confirmed that the tear in Dua's layer of the cornea is the cause of corneal hydrops (a disorder that leads to fluid buildup in the cornea). The discovery of Dua's layer makes the eye surgery safer and simple; it could dramatically improve outcomes of corneal grafts and transplants. Eye surgeons taking advantage of Dua's layer by injecting air bubbles needed during some surgeries under the layer rather than above it, where there is a chance of air causing damage to the corneal stroma [(9)].

Anatomical structure of G-spot:

The anatomic structure of the G-spot has not been documented till 2012, at that year, the G-spot had been distinguished as an anatomic structure that is located on the dorsal perineal membrane, 16.5 mm from the upper part of the urethral meatus, and creates a 35° angle with the lateral border of the urethra. The lower pole (tail) and the upper pole (head) were located 3 and 15 mm next to the lateral border of the urethra, respectively. Grossly, the G-spot was described as a well-delineated sac with walls that resembled fibroconnective tissues and resembled erectile tissues. The superior surface of the sac had bluish irregularities visible through the coat. Upon opening the sac's upper coat, blue grape-like anatomic compositions of the G-spot emerged with dimensions of length (L) of 8.1 mm × width (W) of 3.6-1.5 mm × height (H) of 0.4 mm. The G-spot structure had three distinct areas: the proximal part (the head) L 3.4 mm × W 3.6 mm, the middle part L 3.1 mm × W 3.3 mm, and the distal part (tail) L 3.3 mm × W 3.0 mm. From the distal tail, a rope-like structure emerged, which was seen for approximately 1.6

mm and then disappeared into the surrounding tissue [10].

Finally, the question now, are these discovered human body parts new?

The answer to this question should probably considere that the body is formed of complex structures, with extraordinary variations seen in some individuals [11]. One example: An arm muscle known as the palmaris longus simply is absent in 16% of the population [12]. Other example: The plantaris muscle of the leg is absent in about 14% of people [13]. These new part of the human body may be more properly considered as newly discovered variations in the human body.

Anatomical variations may influence predisposition to diseases, symptomatology, clinical examination, investigation and patient management including operative surgery [14]. Indeed, there are reports that a substantial proportion of clinical malpractice may be attributed to ignorance of anatomical variations. This realization has informed inclusion of anatomical variations among the aims to be considered in medical curricula [15].

It is of significantly important not to consider that human already had a complete “map” of the human body. The history of human anatomy was always witness new discoveries .

The human anatomy departments should not be closed, and the fact that medical students today learn about the human body only from books and computer simulations is a bad sign concerning medical progress.

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Preterm Births among Women with Short Birth Interval in Two Hospitals in Baghdad /Al-Karkh

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Abstract

Background The World Health Organization had recommended that individuals and couples should wait for at least (2-3) years between births in order to reduce the risk of adverse maternal and child health outcomes.

Objective The current study was an attempt to measure the rate of preterm births among women with interbirth intervals of less than three years. It also attempted to describe some socio-demographic characteristics of these women with short intervals.

Methods A cross-sectional study was conducted in two hospitals at Baghdad / Al-Karkh district with a total 360 women were interviewed at the delivery room using a questionnaire specially prepared for this purpose during the period from the 1st of April to the 31st of May 2009.

Results The mean interbirth interval for the sample was 20.4±6.43 months gave a rate of preterm births of 17.8% with a significant association between short intervals and high occurrence of preterm births. It was found that women with short interbirth intervals were mostly of younger age, housewives, low educated, not using contraceptives, had a female baby in the last delivery before the current one and their monthly income was 500000 Iraqi dinars or less (about 385 USD).

Conclusions The rate of preterm births in women with short birth intervals less than three years was relatively high and significantly associated with short birth intervals.

Key words Preterm births, Short birth interval, Socio-demographic characteristics

List of abbreviation: WHO = World Health Organization, USAID = United States Agency for International Development, UNICEF = United Nations International Children's Emergency Fund, USA = United States of America, DHS = Demographic and Health Surveys

Introduction

Birth spacing is the practice of timing the period between births [1], while birth interval is the period between two consecutive live births, from birth date of a child to birth date of the other [2]. Preterm birth is defined as gestational age at birth of less than 37 completed gestational weeks [3]. The World Health Organization (WHO) and other International Organizations recommended that individuals and couples should wait for at least (2-3 years) between births in order to reduce the risk of adverse maternal and child health outcomes. Recent studies supported by USAID, UNICEF and others suggested that interval of (3-5 years) might help to reduce these risks [4]. Children born close together have long been associated with an increased risk of adverse health outcomes, including increased perinatal, infant, child and maternal morbidity and mortality [5,6]. Preterm birth is one of the risks of perinatal outcomes, and it is the single most important cause of perinatal mortality in North America and Europe [7,8], also it is responsible for nearly half of all cases of congenital neurological disabilities, including cerebral palsy, in addition, there was increased risk of

breathing, immune system, vision and hearing problems [9].

Regarding mother health a comparison of mothers with less than (15) months birth intervals with those of (27-32) months birth intervals revealed that there are increased risk of maternal death, third trimester bleeding in pregnancy, premature rupture of membrane, anemia and puerperal endometritis [10,11].

A variety of demographic and socioeconomic characteristics influences women's spacing practices including younger age [1], no or less educated [12], unemployment [13], and living in rural areas were more likely to have birth intervals shorter than three years [1].

In Iraq, a study was conducted in Mosul, in the north of Iraq in 2004 found a significant association between preterm birth and women who conceived at shorter birth interval [14].

The current study aimed to measure the rate of preterm births among women with interbirth intervals of less than three years. It also attempted to describe some socio-demographic characteristics of these women with short intervals

Methods

A cross-sectional study was conducted on 360 ladies with birth intervals less than three years, attending the delivery rooms of Al-Imamein Al-Kadhimein Medical City and Al-Shaheed Al-Hakeem General Hospital in Al-Shoulaa, at Al-Karkh district in Baghdad, from the 1st of April 2009 to the end of May 2009. A consecutive sample of all the women who fulfilled the inclusion criteria were included in the study and those were; women at age (18-45) years, had birth interval of less than 3-years during her current pregnancy, and with singleton pregnancy. Women with hypertension, diabetes, smokers, had any congenital abnormalities in the current delivered baby and her previous pregnancy before the current one ended with abortion were excluded from the study. A questionnaire included demographic information of the mother as age, occupation, residence, years of education, and monthly income was used. Also obstetrical data related to date of last menstrual period, using of contraception before this pregnancy, number and sex of the previous lived children and date of birth of the last child before the current pregnancy. The confirmation of gestational age was done by estimation of expected date of delivery, ultrasound report at first trimester and obstetrician / pediatrician notes.

Statistical analysis was done using SPSS (statistical package for social sciences) version (16) computer software. A level of significance of less than 0.05 was considered as statistically significant.

Results

The mean interbirth interval for all the participants was (20.4±6.43) months with a range of (9-35) months. The mean age of the women who participated in the study was (27.6±6.5) years with 81.7% of them were 34 years old and younger, about 63.6% of the women had education of less than secondary school level. The mean gestational age for the delivered infants of the whole sample was (38.2±2.5) weeks with a range of (26-41) weeks (Table 1). Most of the ladies (82.2%) included in the study were unemployed, (91.7%) of them lived in urban areas, and more than two thirds of them were not using contraception before conceiving the current pregnancy. More than half (55.6%) of the women had a female sex baby in the last birth order before the current pregnancy, and about three quarters of the sample (76.1%) had income less or equal to 500000 Iraqi Dinars per month (385 USD) in spite of it ranged from 50000 to 2250000 Iraqi Dinars per month (Table 2).

Among this sample 64 women (17.8%) gave preterm labor, and the remaining 296 (82.2%) gave full term labor (Figure 1).

There were no significant difference found regarding the mean age, years of education and income of mothers for both groups (P=0.895, P=0.6 and P=0.72) respectively (Table 3).

Also regarding mother occupation, the use of contraception, and the sex of the last baby before the current pregnancy there were no significant difference between term and preterm birth. (P=0.34, P=0.12 and P=0.07) respectively, however, there was a significant association between the preterm deliveries and living in rural areas P=0.005 (Table 2).

Comparing women who had preterm with those with term deliveries; there was significant difference in the mean interbirth interval between them (18.8±5.43 versus 20.7±6.58 months respectively, P=0.03) (Table 4).

Table 1. Distribution of the sample according to some continuous demographic variables

Variables	Mean	SD	Range
Age of mother (years)	27.6	6.46	(18-45)
Education of mother (years)	8.75	4.48	(0-18)
Income per month (ID)	421670	262807	(50000-2250000)
Gestational age (weeks)	38.2	2.52	(26-41)
Interbirth interval (months)	20.4	6.43	(9-35)

Table 2. Relation between preterm and term birth regarding mother occupation, residence, the use of contraception and the sex of the last baby before the current delivery and their distribution percent

Variable		Preterm Number (%)	Term Number (%)	Total Number (%)	Significance
Occupation	**Unemployed**	50 (16.9%)	246 (83.1%)	296 (100%)	X^2 =0.894
	Employed	14 (21.9%)	50 (78.1%)	64 (100%)	P= 0.34
Residence	**Urban**	53 (16.1%)	277 (83.9%)	330 (100%)	X^2=7.988
	Rural	11 (36.7%)	19 (63.3%)	30 (100%)	P=0.005
Contraception use	**Used**	10 (12%)	73 (88%)	83 (100%)	X^2=2.423
	Not used	54 (19.5%)	223 (80.5%)	277 (100%)	P=0.12
Sex of last baby	**Male**	20 (12.5%) 42	140 (87.5%)	160 (100%)	X^2=3.29
	Female	(21%)	158 (79%)	200 (100%)	P=0.07

Table 3. Significant association regarding age, years of education and income of mothers between preterm and term births

Mother variables	Preterm Mean (±SD)	Term Mean (±SD)	Significance
Age (years)	27.5 (±6.47)	27.6 (±6.46)	t-test=0.132 P=0.895
Education (years)	8.5 (±4.79)	8.8 (±4.42)	t-test=0.497 P=0.6
Income in ID per month	410940 (±381046)	423990 (±230246)	t-test=0.360 P=0.72

Table 4. The mean interbirth interval of women with term and preterm deliveries

Interbirth interval	Preterm Mean (±SD)	Term Mean (±SD)	Significance
Interbirth interval (months)	18.8 (±5.43)	20.7 (±6.58)	t-test=2.209 P=0.028

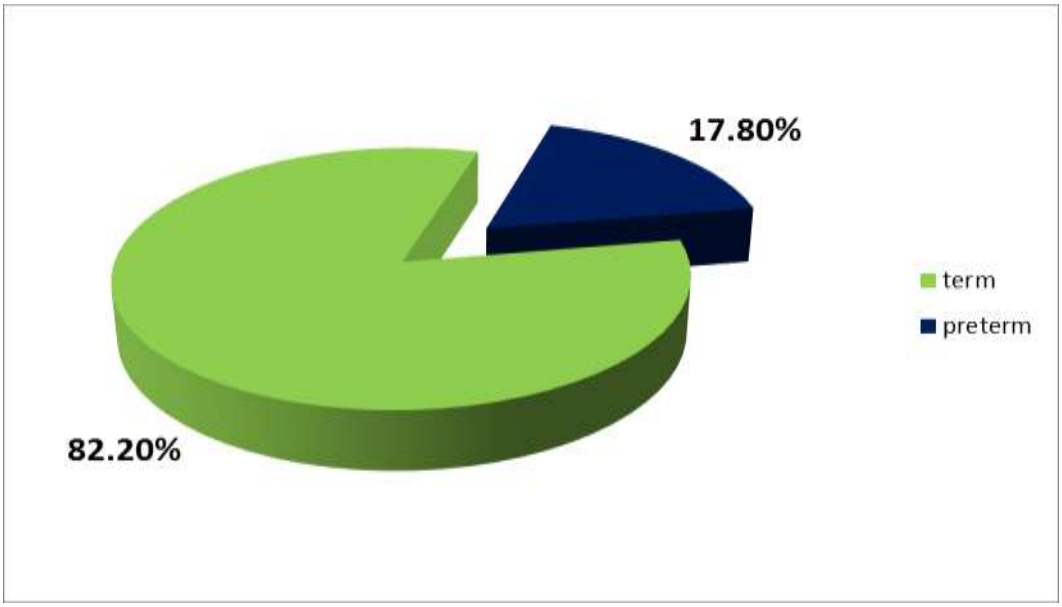

Fig. 1. The rate of preterm birth among the sample

Discussion

Several studies have shown that women with short interval pregnancies are at increased risk of complications and preterm birth was one of these complications [6,15,16]. The largest proportion of women with short birth intervals of less than three years were reported from the developing countries of the Middle East region, such as Jordan and Yemen, as well as from Turkmenistanin Central Asia [1]. While in Iraq, the problem of prematurity is becoming an ever growing one [17], no previous published researches tried to identify the rate of preterm birth in short interbirth intervals.

The rate of preterm birth in this sample of women with short interbirth intervals was (17.8%), and this is higher than general preterm birth rates recorded in developed and many developing countries as well as more than preterm deliveries in general population of Iraq (6.5%) [18].

The United States National Center for Health Statistic reported that preterm birth rates in USA is (12.7%) [19]. This study found that the mean birth interval of women with preterm deliveries was significantly less than those with term deliveries, and this is similar to many previous studies like studies done on Emirati women in 2002 and in Gaza strip in 2007 which found increase occurrence of preterm birth with short interbirth intervals duration [1,6,20,21]. In this research, it was also found that the women with short birth intervals were mostly young of 34 years or less, housewives, and of low educational level "not reach the secondary school", these findings are similarly reported by many countries like Egypt, Jordan and Turkey through the DHS data [1]. Although most of the women included in this study lived in urban

areas, but the study found that there was a significant association between living in rural areas and the occurrence of preterm births and this finding may be attributed partly to shorter interbirth intervals and found in previous other studies [16,22,23]. No significant association was found between the age of mother and having preterm births unlike previous studies, which found that both extremes of age less than 18 and more than 45 were associated with increased rates of preterm births [24,25]; and this is the reason for selecting the sample with exclusion of very young or old aged women to counteract the confounding effect of age that can contribute for preterm deliveries. Despite there is a known relation between maternal occupation and education with the occurrence of preterm births as preterm births increase in employed women specially with heavy manual work and with illiteracy [22,23], but in this study there was no significant association between occupation and education of mother and the occurrence of preterm births. No significant relation found between the income and the occurrence of preterm births and this was also found in previous studies which found no effect of socioeconomic status of the couples on the rate of preterm births [26], while other studies found the reverse as there was association between the preterm births occurrence and socioeconomic status of the family [27,28].

It can be concluded from this study that the rate of preterm births in women with short birth intervals of less than three years was relatively high and significantly associated with short birth intervals.

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To all participants in this study .

Author contributions

Dr. Atheer J Al-Saffar (Design, Methodology and Manuscript writing), Dr. Nibras Alaa Hussain (protocol preparation, data collection, literature review, and manuscript draft writing).

Conflict of Interest

The authors have no conflicts of interest.

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Rates of Cesarean section in Al-Imamein Al-Kadhimein Medical City

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Abstract

Background Caesarean section rates have been increasing dramatically in the past years in Al-Imamein Al-Kadhimein Medical City.

Objective To determine the rate of caesarean section and to analyze the indications, so as to introduce measures to control the caesarean section rate.

Methods This retrospective study was conducted in Obstetrics and Gynecology Department at Al-Imamein Al-Kadhimein Medical City from 1st Jan 2012 to 30th Apr 2013. In this study, clinical records of all the patients who underwent caesarean section were analyzed. AII cases who were underwent caesarean section whatever indication and regardless the type of caesarean section (elective, urgent, scheduled, emergent) were included in this study. Clinically diagnosed cases of ruptured uterus proved on laparotomy were excluded. Data was analyzed on SPSS version 17, Microsoft excels 2010 and frequency as well as percentages were calculated.

Results There were 10,354 deliveries during the study period (16 months) and 5897 of these were caesarean section. The rate of caesarean section was 56.95%. Scheduled caesarean section was 49.31%, elective caesarean section 43.89%, urgent caesarean section 4.32% and emergency caesarean section 2.48%.

Conclusions Caesarean section rate was high (56.95%) in Al-Imamain AlKadhimein Medical City, the majority of patients who underwent caesarean section were scheduled and elective caesarean section. The commonest indication was repeat caesarean section.

Key words Cesarean section, Indications, Frequency

List of abbreviation: CS = Caesarean section, RCU = Respiratory care unit, CEFM = Continuous electronic fetal monitoring, VBAC = vaginal birth after a previous CS

Introduction

For most of the 20th century, caesarians were a rarely used procedure; done only in truly life-threatening situations after all other options had been exhausted. The risks from the operation were so significant that doctors were very reluctant to use it without true need. As technology improved and caesarians became safer, doctors started doing more and more of them [1].

Caesarean section (CS) is a relatively common procedure in modern obstetric practice; some of the obstetricians consider it to be quite simple, efficient, safe and psychologically well-tolerated procedure and far superior to secondary interventions such as vacuum delivery or forceps delivery [2].

However, CS rates vary worldwide, ranging from approximately 10% in Sweden to about 80% in private-sector hospitals in Brazil [3].

High rates are reported from regional, tertiary public and private hospitals. These high rates are probably due to large proportions of high-risk patients attending tertiary and regional

public hospitals and financial benefits in private sectors.

The CS epidemic is a reason for immediate concern and deserves serious international attention. The procedure is not benign and needs to be performed only when circumstances distinctly require it [4].

Is CS safe as we think?

The possibility that indiscriminate use of CS can have a negative impact on maternal and neonatal health has been raised [5]. Even though caesareans are associated with higher rates of complications than vaginal births, they are becomingly increasingly common. Problems range from infections, including the more serious antibiotic-resistant ones, to severe bleeding, prematurity, respiratory problems for the baby, and more complications with subsequent pregnancies. There is even a small but measurably higher risk of death for the mother [6].

A fourfold increase in maternal mortality rate associated with CS was observed even after controlling for medical and obstetric complications, maternal age, and preterm delivery [7]. Even elective CS had a 2.84 fold greater chance of maternal death as compared to vaginal birth. In UK, a twofold increase in mortality with CS was detected [8].

Recurrent CS, scar rupture and hysterectomy are some of the future important risks. Previous CS increases the risk of multiple placental abnormalities like placental abruption, placenta previa, and adherent placentation in subsequent pregnancies [9]. The leading indication for cesarean hysterectomy in USA is placenta accrete, increta or percreta [10]. As the incidence of CS continues to rise worldwide, the problem of placenta previa and placenta accreta is likely to become more common. Obstetricians should be ready to face these future consequences of today's decision of performing CS [11].

Incidence

The consensus recommendation for optimal CS rate of 10-15% was made by world health organization (WHO) in 1985 [12]. The limitation issue is being debated by professionals and women's groups in most parts of developed world based on risks and benefits [13].

Efforts to bring down the rate have failed and it is on a steady rise. Caesarean section rates are high and continue to rise in developed countries [14]. However, the impact of guidelines and recommendations in curbing their growth has been limited: in 1985, representatives of a study group convened by the World Health Organization wrote, "there is no justification for any region to have caesarean section rates higher than 10-15% [12]. Although, levels of 10-15% were considered high but acceptable at the time, average caesarean rates in most developed regions (with the exception of Eastern Europe) now exceed 20%; the recommendation thus appears to have been largely overtaken by events [15].

In Sweden, Denmark and Netherlands, the CS rate is still close to 10% with some of the world's lowest maternal and perinatal mortality rates [5]. In 2001, an estimated 21.4% of all deliveries in England and Wales were by CS, a fivefold increase since 1971 [16]. In 2007, nearly one-third (32%) of all births were cesarean deliveries in USA [17].

Since 1996, CS deliveries have increased by more than 40% [18]. This may be due to more conservative clinical practice and legal pressures [19].

The rising trend in CS is definitely not limited to USA and UK. In Ireland, the CS rate now exceeds 26% [20].

In Saudi Arabia the overall CS rate significantly increased by 80.2%, from 10.6% in 1997 to 19.1% in 2006 [21].

Regarding our country, almost 26 % of Iraqi women giving birth at public hospitals do so via CS according to the 2010 health report for the Iraqi Health Ministry [22]; and in 2012, the rate was further increased to 29.25% [23].

WHO has since finessed its position on CS rates by stating that the most important issue is that every woman who needs a CS should have one. It acknowledges that there is little scientific evidence to support a 15% CS rate [24].

Against this background, this study was done to assess the indications of CS & caesarean delivery rates in Al-Imamein AL-Kadhimein Medical City in order to identify patient groups with an increasing risk for CS.

Methods

A retrospective analysis of 10,354 deliveries was carried out at Al-Imamein Al-Kadhimein Medical City, (which is a tertiary teaching center with a respiratory care unit (RCU) unit contain 24 RCU beds and maternity unit consist of maternity and gynecology ward with 124 beds), to examine the factors responsible for the high CS rates during the period from 1st Jan. 2012 to 30th Apr. 2013.

It included all the pregnant ladies (booked, non-booked) who were admitted through emergency or outpatient department. According to urgency of CS they were grouped into four categories: urgent, emergency, scheduled and elective CS [25]. Distribution of patients who had 1st CS according to parity (primigravida or multigravida) and percentage of recurrence of CS, assessment of patients who achieved vaginal delivery according to their parity and history of previous one CS then the major and miscellaneous indications of CS were categorized. The patients with clinical diagnosis of ruptured uterus, which later proved on laparotomy, were excluded from the study). The data was analyzed on SPSS version 17 and microsoft excel 2010 and frequency and percentages were calculated.

Results

The caesarean section rate (CSR), in general, was 56.95% births. (Fig. 1), and 48.8% of the caesareans were scheduled procedures, 43.26% elective, 4.95% urgent and 2.98% emergency (Fig. 2).

The caesarean section rate was high among primigravida (53.08%) versus 46.92% vaginal delivery (Fig. 3), and the rate of 1st CS in primigravida was 48.69% and 51.31% in multigravida (Fig. 4).

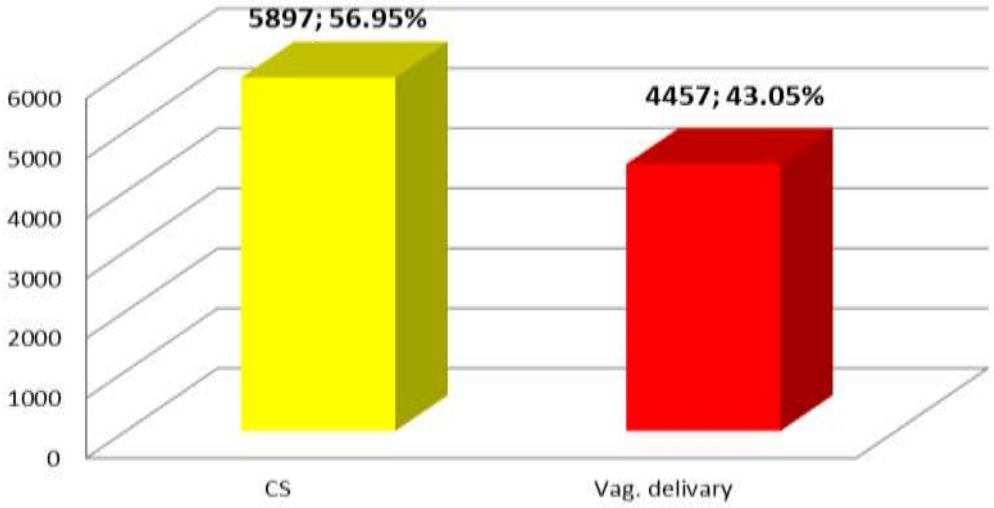

Fig. 1. Rate of CS and vaginal delivery

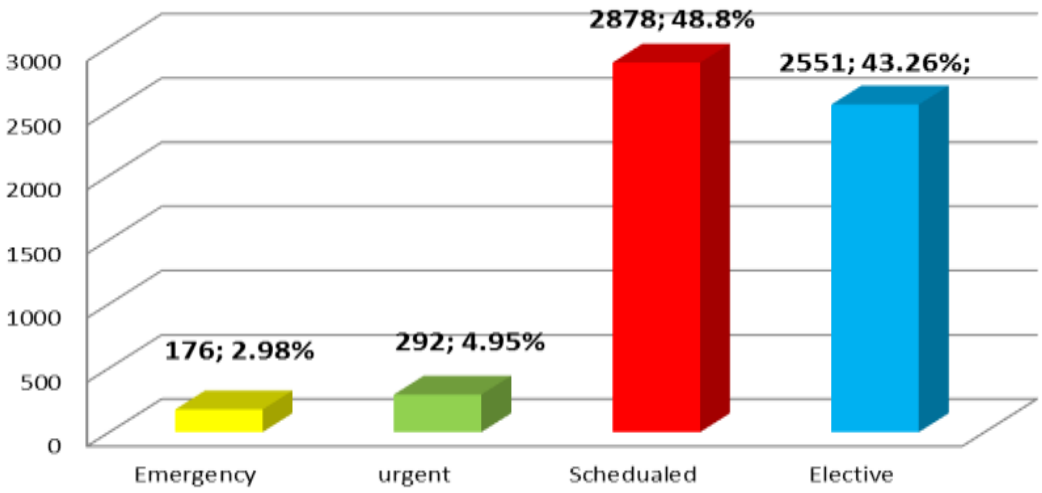

Fig. 2. Distribution of CS according to type

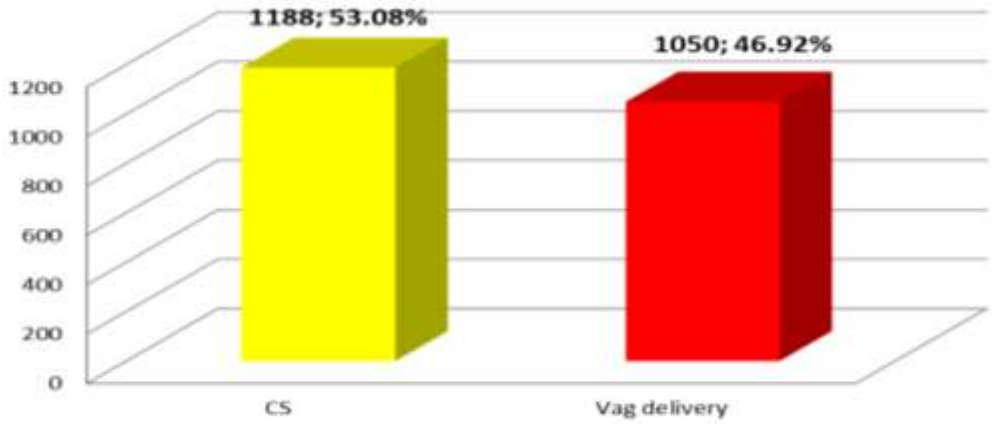

Fig. 3. Types of delivery for primigravida women included in the study

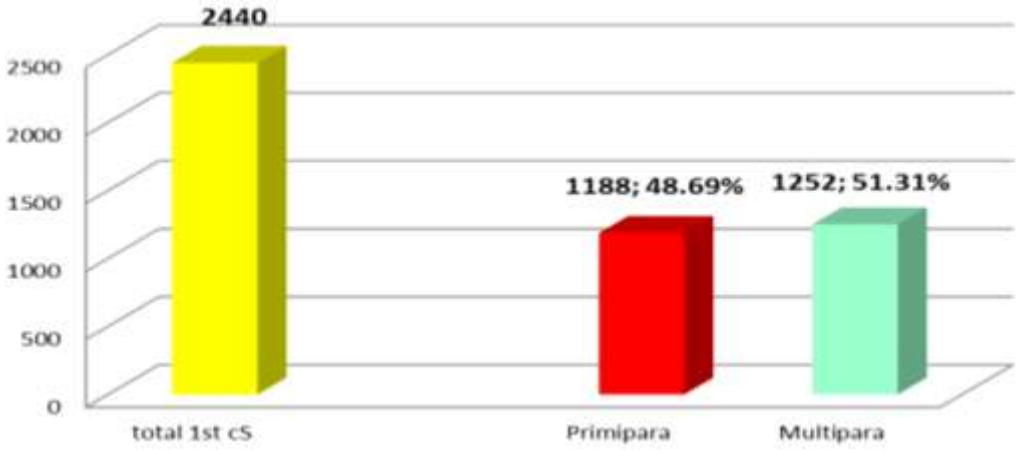

Fig. 4. Distribution according to types of CS& parity

The first CS rate was 41.38%, second CS rate was 28.69% and more than 2 CS rate was 29.93% (Fig. 5), While VBAC (vaginal birth after CS) was low, the rate was 4.6% and percentage of primipgravida among women who were delivered vaginally were 23.56%. (Fig. 6).

The four leading indications were recurrent section (29.93%), labor dystocia (11.04%), hypertensive disorders (8.29%) and fetal distress (8.29%) (Fig. 7), while 20.56% patients had miscellaneous indications for CS (Fig. 8). The most common indications were prelabor rupture of membranes (PROM) (3.91%), intrauterine growth retardation (IUGR) and scanty liquor (3.73%), infertility (3.49%), medical disorders (3.01%) and bad obstetrical history BOH (3.35%).

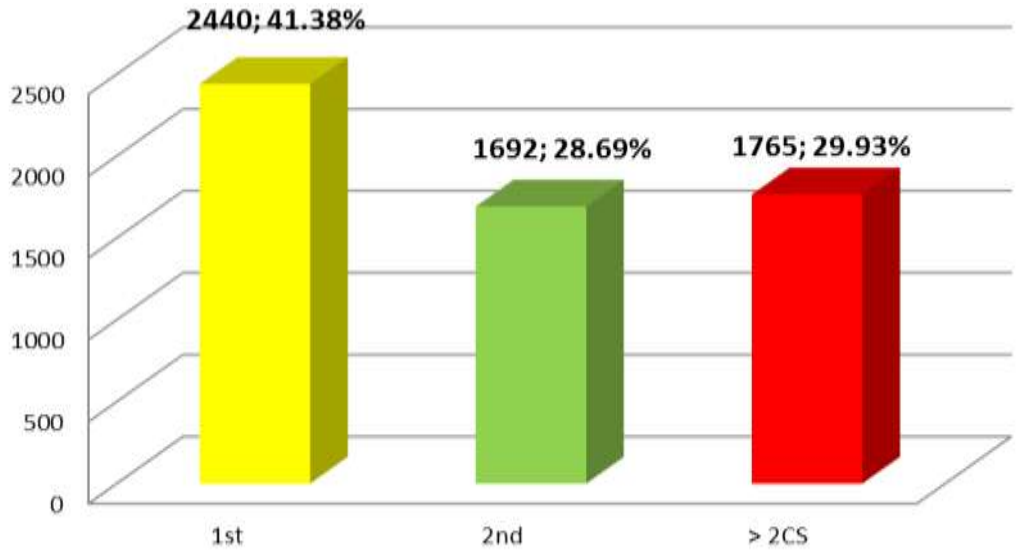

Fig. 5. Number and percentage of recurrence of CS

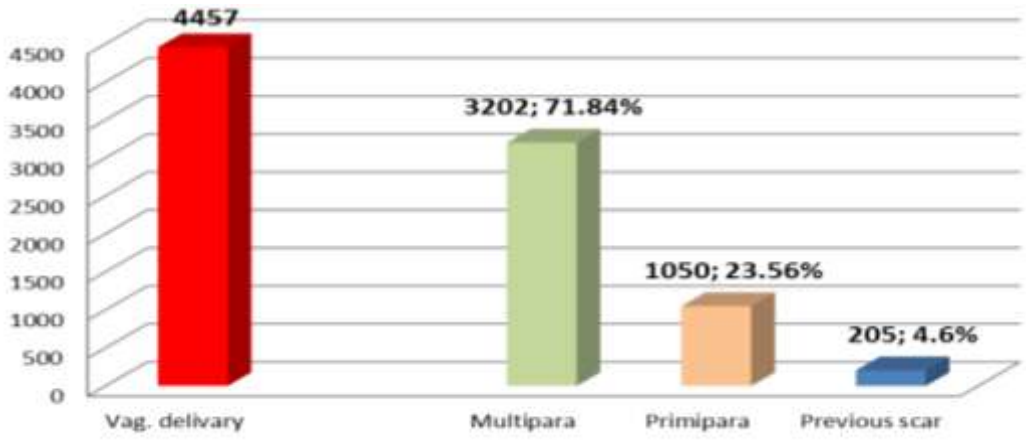

Fig. 6. Distribution of patient who achieved vaginal delivery according to their parity and history of previous one CS

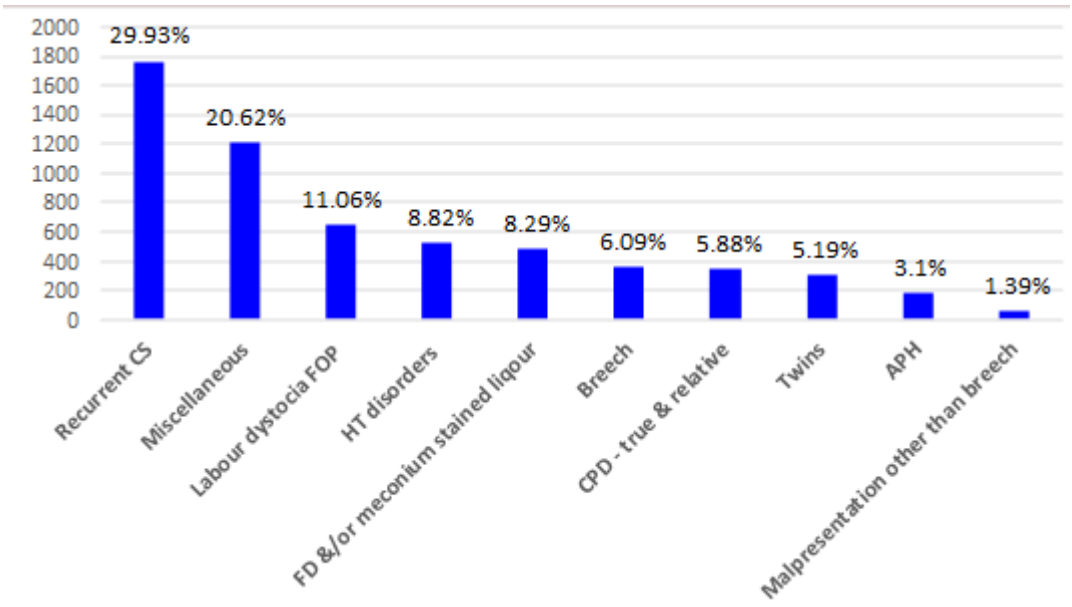

Fig. 7. Major indications for CS. FOP: failure of progress, HT: hypertension, FD: fetal distress, CPD: cephalopelvic disproportion, APH: antepartum hemorrhage

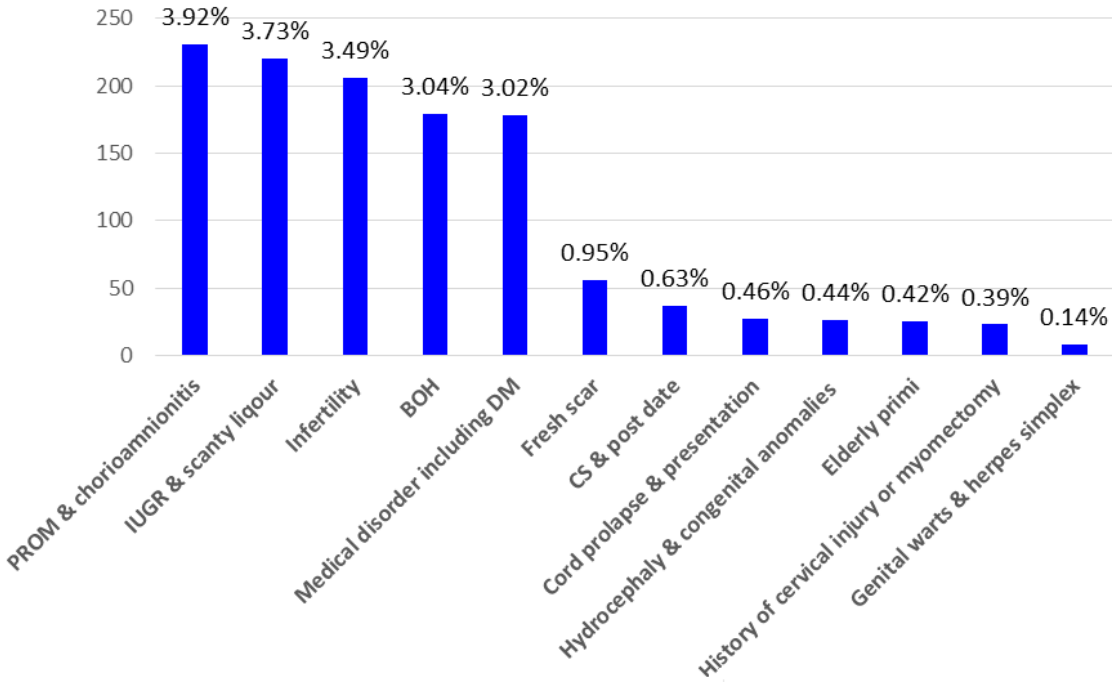

Fig. 8. Miscellaneous indication for CS. PROM: prelabor rupture of membranes, IUGR: intrauterine growth restriction, BOH: bad obstetrical history

Discussion

Almost half of all deliveries performed from the first of Jan. 2012 to the 30th of Apr. 2013 in the Al-Imamein Al-Kadhimein Medical City were by CS. It is important to emphasize that these findings are not meant to be generalized to the city or country.

The rate expected to be high as it is a tertiary referral center and it served high-risk, referred patients in addition to low risk group, but usually

those with low-risk are delivered at maternity hospitals or at home by traditional birth attendants.

In comparing studies and statistics, the rates also high, it was 64.7% in Isra university Hospital Hyderabad [(2)], 33.7% in a tertiary referral center in Eastern Nepal in 2007 [(26)], 34.5% in a tertiary hospital in the Niger Delta, Nigeria [(27)], and in USA, Arkansas State hospitals in 2012, the rate was ranging between 40.4-50.9% [(28)].

Specific child bearing patterns of our women, large families, flaws in antenatal surveillance, absence of a referral system and departmental polices regarding management of cases with labor dystocia, previous CS and fetal distress seem to be the major underlying causes of the high CS rate causing a marked spike in the number of scheduled CS performed in our unit.

The reasons for the dramatic increase in CS rates though not obvious are somewhat complex. The indications for performing CS have changed a lot in recent years and keep on changing for varied circumstances. Most CS are currently performed to benefit the fetus, not the mother.

It is sad that CS are frequently and arbitrarily performed for fetal distress and prolonged labor without due respect to correct diagnosis and unbiased decision [(29)].

In this study fetal distress was based on pathological and non-assuring cardiotocography, which based on continuous electronic fetal monitoring (CEFM) although this not reduce the overall perinatal mortality and the incidence of cerebral palsy [(30)].

Many options have been tried to replace CEFM or to improve its predictability for fetal distress, such as fetal blood sampling, fetal pulse oximetry and analysis of fetal electrocardiogram. However, all are still being used in clinical trials and further studies are needed [(31)].

Recurrent sections are now frequently performed for various reasons. A trial for vaginal birth after a previous CS (VBAC) is considered safer than a routine repeat CS. But, it is unfortunate that there is currently less enthusiasm for VBAC by trial of scar or of labor. It is evident that whereas CS is doctor friendly, VBAC is not.

It was notices that VBAC was low (4.6%) but all over the world the rate decline and keep on declining; the rate of VBAC in USA is down from 17% in 1996 to 11% in 1999 [(32)] and furthermore decrease to 10.2% in 2012 [(33)].

The term fresh scar (related to pregnancy happened within 6 months following CS is over used with no scientific bases. (0.95% of cases); Royal College of Obstetrics and Gynecology (RCOG) recommended that all women previously delivered by one lower segment CS should be offered an opportunity to labor during their next pregnancy by promoting a trial of scar or of labor [(29)]. In addition, cases of previous one CS and postdate (0.63% of cases), no induction of labor was done due to fear of uterine rupture although augmentation is not contraindicated it should only be preceded by careful obstetric assessment, maternal counselling and by a consultant-led decision [(34)]. Induction of labor and failed induction contribute to increase CS rates also. Other reasons included; lack of adherence to standard guidelines and protocols for managing labor and non-availability of system of audit for CS rates .

Other indications such as precious pregnancy, poor Bishop Scores in postterm pregnancies and CS decision in primigravida and previous scar not always made by senior obstetrician, were also contributed causes for increase CS rate.

Defensive obstetrics is another common reason for high rates of CS, as it has been observed that 82% of physicians performed CS to avoid negligence claims [(16)]. Defensive obstetrics violates the fundamental principle of medical practice. In any case it does not work. During the years that defensive obstetrics has grown in numbers, there has been no slowdown in litigation [(35)].

This is closely related to daylight obstetrics for the obstetrician's convenience. Elective and scheduled CS are set in favor of weekdays and daylight. It takes usually 30-45 minutes to perform a CS while conducting a vaginal birth

may need 12 hours or more heavily taxing on the obstetrician's time and patience.

It was concluded from this study, CS rate was high (56.95%) in Al-Imamein Al-Kadhimein Medical City; the majority of patients who underwent CS were scheduled and elective CS. The commonest indication was repeat CS.

Thefollowing are recommendations from this study:

1. In order to turn back the current CS trend, our main target need to be the low-risk primipgravida and previous one CS, in order to avoid unnecessary CS in these groups and to reduce the number of repeat CS in the future.
2. To successfully increase access to VBAC: Iraqi National Guideline and protocol for VBAC should be introduced and applied in all ministry hospitals.
3. Accurate registration of the main indication for CS, a clear and strict CS audit tool should be applied in the maternity unit.
4. Efforts to keep CS rate around certain level is needed, a nation-wide committee that involves policy makers, social leaders and obstetricians is required.

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Conflict of Interest

The author have no conflicts of interest.

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Angiographic Assessment of Exercise Treadmill Test-Detected Occult Coronary Artery Disease in Type 2 Diabetics

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Abstract

Background Myocardial ischemia in type 2 diabetes mellitus may be painless keeping the coronary artery disease occult. Exercise Treadmill Test was one of the modes that have been tried to uncover occult coronary artery disease, yet its results were not assessed.

Objective To show the rate of occult coronary artery disease in asymptomatic type 2 diabetes mellitus subjects using exercise treadmill test, and to assess the exercise treadmill test-detected occult coronary artery disease by conventional coronary angiography , and to inspect the risk factors.

Methods A cross sectional study of 120 randomly enrolled asymptomatic type 2 diabetic subjects who were not labeled to have ischemic heart disease prior to the study. The body mass index, blood pressure, serum total cholesterol, serum triglycerides and glycated hemoglobin A1c were all measured in addition to rating of smoking trend. Exercise treadmill test was, then, conducted to all of the enrolled subjects followed by conventional coronary angiography to subjects whose exercise treadmill test was positive.

Results A proportion of 20.8% of asymptomatic type 2 diabetic subjects showed positive exercise treadmill test results ($p<0.05$). On assessing the exercise treadmill test positive subjects by conventional coronary angiography, 68.4% proved to have true coronary artery stenosis; 69% of them were significant stenosis affecting 1-3 coronary vessels. Smoking trend was the most significant risk factor in type 2 diabetics who proved to have positive exercise treadmill test results, particularly among the males.

Conclusions One fifth of asymptomatic type 2 diabetic subjects have occult coronary artery disease when screened by exercise treadmill test. Two thirds of the exercise treadmill test-diagnosed occult coronary artery disease subjects have significant coronary stenosis when assessed by conventional coronary angiography. Smoker type 2 diabetic subjects, in particular males, are at risk and should be keenly screened for occult coronary artery disease.

Key words Type 2 diabetes, Ischemic, Silent, Occult, Coronary angiography, Exercise treadmill, Smoking.

List of abbreviation: T2D = Type 2 diabetes mellitus, CAD = Coronary artery disease, OCAD = Occult coronary artery disease, ETT = Exercise treadmill test, DIAD = Detection of silent myocardial ischemia in asymptomatic diabetic, CAG = coronary angiography, ECG = Electrocardiography, BMI = Body mass index, HbA1c = Glycated hemoglobin A1c

Introduction

Diabetes mellitus is a strong independent risk factor for the development of atherosclerosis [(1)] and predisposes to the development of other known risk factors, such as hyperlipidemia [(2)] and hypertension [(3)].

Based on the Framingham's cohort study, Kannel and McGee, long ago, had demonstrated that diabetic individuals had a two-to-three folds increased risk of developing atherosclerosis including coronary heart disease [4]. In the report of the Adult Treatment Panel of the National Cholesterol Education Program [5], type 2 diabetes mellitus (T2D) was accorded a coronary artery disease (CAD) risk-equivalent [6]. The extraordinary frequency with which CAD occurs in diabetes lead researchers to inversely describe T2D as "a cardiovascular disease associated with hyperglycemia" [7].

Myocardial ischemia due to CAD, may presents acutely or chronically with variable degrees of myocardial damage and dysfunction. Concerning symptoms, myocardial ischemia is usually associated with pain but it may be painless or silent. Non-perception of myocardial ischemia keeps CAD dormant. The term occult CAD (OCAD) has been, commonly, used to describe silent (painless) myocardial ischemia and vice versa .

According to its severity, OCAD may be recovered coincidentally in a resting electrocardiography (ECG) in the form of ST-T changes, old myocardial infarct changes or left bundle branch block, or detected intentionally by ambulatory ECG (Holter) monitoring during daily activity [8]. Otherwise, provocative tests that stress the heart e.g. exercise treadmill test (ETT) or pharmacologic stress tests can be used to uncover it [8,9].

Recently, there is accumulating evidence that patients with diabetes have a high incidence of silent myocardial infarction and ischemia [9,10,11]. During ambulatory ECG monitoring, diabetic patients were shown to have a high incidence of transient silent ST segment changes [12]. The study entitled "detection of silent myocardial ischemia in asymptomatic diabetic" (DIAD) had shown that silent myocardial ischemia occurs in more than one per five asymptomatic patients with T2D [13].

Since realizing the high rate of silent myocardial ischemia in diabetes, many measures have been tried to uncover the underlying OCAD but controversies exist regarding the optimal screening measure. ETT was one of the modes that had been tried for screening before the emergence of stress echocardiography or nuclear measures. Only, few studies have used conventional coronary angiography (CAG) to examine ETT results in detecting OCAD in diabetic patients.

In this study, we aimed to show the rate of OCAD in asymptomatic T2D patients by using ETT, to assess the positive ETT results by conventional CAG, and to inspect the risk factors among the afflicted subjects.

Methods

This is a cross sectional study that was conducted at As-Salam Teaching Hospital and Ibn-Seena Teaching Hospital in Mosul from January 1, 2011 through January 1, 2013. The subjects were 120 randomly recruited T2D subjects who were not labeled to have ischemic heart disease at any time prior to the study. The subjects' free agreement and consents on the study were taken after discussing the tests and the safety precautions. Exclusion from the study included those who were arthritic and unable to sustain ETT, those who showed abnormal base-line ECG like ST/T changes or bundle branch block, those whose general systemic status was frankly poor, those who aged more than 70 years and those who alleged having claudication on walking. Exclusion from having conventional CAG included those who were atopic or suspected to be allergic to angiography dye.

The contributory data, including age, gender and duration of diabetes, co-morbidities, and drug history were recorded and tabulated. Clinical examination including measurement of body weight, height and estimation of body mass index (BMI) was conducted. Systolic and diastolic blood pressure (Korotkof phases I and V respectively) were measured as the mean of three measurements with a mercury sphygmomanometer.

Biochemical tests for serum lipid profile were measured to all, and glycated haemoglobin A1c

(HbA1c) was determined by spectrophotometer (Cecil CE 1011, Cecil Instrument, Cambridge, UK).

After having a 12-lead resting ECG, ETT was conducted to all of the enrolled patients if, otherwise, not contraindicated or refused by the patient. A maximal symptom-limited exercise was used with a treadmill Xscribe5® TM (Mortara instrument, USA) according to Bruce protocol test format. Twelve ECG leads were recorded continuously and blood pressure was measured throughout the test at rest and at the end of each step .

The ETT was defined as maximal if the patient reached 85% of the predicted heart rate for the age; and the test was regarded as positive if the patient perceived chest pain and/or a horizontal or down-sloping ST segment depression of >1 mm. The test was regarded non-conclusive if it was prematurely ended due to fatigue, arrhythmias, cardiac conducting abnormalities, hypotension, or hypertensive reaction (diastolic blood pressure rise to 115 mmHg and/or systolic blood pressure to 250 mmHg) before the occurrence of any pain or ST-T changes [14]. Cardiovascular drugs including beta-blockers and calcium-channel blockers were stopped 72 hours before the test .

The subjects who showed a positive ETT results were then shifted to have CAG at the cardiology lab of Ibn-Seen Teaching Hospital within 30 days of their ETT. Conventional CAG was performed and evaluated by two independent expert observers. According to Coronary Artery Surgery Study criteria, angiograms were graded as significant if the luminal diameter narrowing of the left main coronary artery was ≥50% or the left anterior descending, left circumflex, right coronary arteries or their major branches was ≥70% [15].

The data were expressed as mean ±SD. An X2 test was used to study the distribution of proportional data (gender and smoking) among positive and negative ETT subjects. Independent two sample structure T-test was used to compare the difference in mean of continuous variables; age, duration, total cholesterol (T. Chol), triglycerides (TG), BMI, systolic and diastolic blood pressures (BP) and HbA1c% between the positive and negative ETT. All the data were processed using statistical package SPSS version (Chicago Inc. ILL). A p-value <0.05 was considered statistically significant.

Results

Out of the 120 asymptomatic diabetics that have been enrolled in this study, the males (n 66) constituted 54% of the sample and the females (n 54) constituted 46%. The subjects' mean age±SD was 49.41±8.9 years, duration of diabetes was 7.5±6 years and HbA1c% was 8.24%±1.5%.

On submitting the subjects (n 120) to ETT, one-fifth (n 25) (=20.8% of the sample) showed positive results (p<0.05), about two thirds (70.83%) showed a negative results and nearly one-tenth (8.33%) were non-conclusive. When the subjects with non-conclusive ETT (n 10) were dropped, the rate of subjects with positive ETT then rose to 22.72% (Table 1). The proportion of positive ETT results among the males was 15/66 (22.7%) and among the females was 10/54 (18.51%). The difference between the two proportions was non-significant (NS). However, the rate of non-conclusive ETT results among the males was significantly lower than among the females (3% vs. 14.8%) respectively (p<0.05) (Table 1).

When the subjects with positive ETT (n 25) were shifted to have conventional CAG, 6 of them (3 males and 3 females) didn't agree to do the angiography. The remaining 19 subjects who accepted sustaining angiography showed that two thirds of them (13/19= 68.4%) have coronary artery stenosis and one third (6/19= 31.5%) have apparently normal coronaries. The stenoses were of variable degrees as follows; 69.24% (9/13) was significant stenosis of 1-3 coronary vessels and 30.76% (4/13) was non-significant stenosis (Table 2). The rate of angiographic stenosis among males was 9/12 (=75%) and among females was 4/7 (=57%)

with NS difference (Table 2). The rate of coronary stenosis on angiography in regard to the total sample of asymptomatic diabetics was 10.8%.

Regarding the risk factors, smoking trend was outstandingly different between positive and negative ETT subjects (32% vs. 16% respectively) and between positive ETT males and females (53% vs. 0% respectively) ($p<0.05$ in both) (Table 1). Other cardiovascular risk factors, including age, duration of diabetes, systolic and diastolic blood pressure, BMI and HbA1c, were shown to be higher among the females, particularly the ETT positive although statistically insignificant (Table 1). In the subgroup that sustained CAG, there were no significant differences in risk factors between the positive and the negative CAG subjects .

Table 1. Positive, negative and non-conclusive ETT among asymptomatic T2D subjects and the risk factors of OCAD including age, duration of diabetes, T. Chol, TG, BMI, blood pressure, HbA1c and smoking

Parameters	Positive ETT (20.8%)			Negative ETT (70.83%)			Non-conclusive (8.33%)		
	Total N=25	Males n=15	Females n=10	Total n=85	Males n=49	Females n=36	Total n=10	Males n=2	Females n=8
Age	50.2 ±7.85	47.4 ±7.30	53 ±7.76	49.21 ± 7.68	50.42 ±7.84	48 ±7.49	p NS		
Duration	7.63 ± 6.09	6.37 ± 4.48	9.02 ±7.9	7.62 ±6.43	7.25 ±5.43	8.13 ±7.64	p NS		
T. Chol	5.42 ±1.33	5.62 ±1.57	5.22 ±0.72	5.32 ±1.09	5.20 ±0.87	5.45 ±1.33	p NS		
TG	1.65 ±0.75	1.72 ±0.18	1.59 ±0.67	1.79 ±0.87	1.85 ±0.95	1.72 ±0.75	p NS		
BMI	27.55 ±1.53	26.5 ±3.68	28.6 ±7.56	27.96 ±1.42	26.37 ±3.17	29.56 ±2.47	p NS		
Systolic BP	148.2 ±19.6	142.6 ±19.4	154 ±18.97	141.92 ±18.7	136.63 ±15.8	147.22 ±20.8	p NS		
Diastolic BP	89 ±9.97	86 ±9.1	92 ±10.59	85 ±9.4	83.36 ±8.5	86.66 ±10.35	p NS		
HbA1c	8.9 ±1.6	8.49 ±1.56	9.5 ±1.7	9 ±2.1	8.8 ±2	9.3 ±2.23	p NS		
Smoking N (%)	8 (32%)*	8 (53%)	0 (0%)	16 (18%)*	13 (26%)	3 (8%)	* p <0.05		

Table 2. : Positive and normal CAG results after exposing positive ETT subjects to conventional CAG

CAG results n=19 (100%)		Males n=12	Females n=7	p value
Positive n=13 (68.4%)	Non-significant stenosis n=4 (30.76%)	2	2	NS
	Significant stenosis n=9 (69.24%)	7	2	NS
Normal n=6 (31.6%)		3	3	NS

Discussion

The high mean±SD of HbA1C% of the enrolled subjects in general (8.24%±1.5%) reflects a bad glycemic control. The burden that hyperglycemia imposes on the arteries is multi-factorial including higher expression of cell-adhesion molecule, monocytes recruitment and migration into the intima [16], LDL oxidation [17], foam cell formation [18] and vascular smooth muscle cell migration [19]. These defects perpetuate the atherogenesis of coronary arteries in diabetes, rendering it earlier in occurrence and more extensive in spread [20].

In addition, there is a growing evidence that diabetes impedes coronary blood flow before the obstructive changes of atherosclerosis by altering the vasodilator function in, both, the epicardial and resistance coronary vessels [21]. Autonomic neuropathy, a major cause of non-perceiving ischemic cardiac pain in diabetes [22, 23], had been found to affect myocardial perfusion [24,25] and to strongly predict silent myocardial ischemia in diabetes [12].

The prevalence of silent ischemia and OCAD in diabetics using different measures of screening widely ranges from 9% to 75% [26-30]. The exercise test has remained at the heart of the work-up of the cardiac patient since its first proposal by Goldhammer and Scherf [31]. The ETT score is a useful and valid tool that can help clinicians to determine prognosis and decide whether to refer outpatients with suspected CAD for cardiac catheterization [32]. Much of the diagnostic power of ETT relies on changes in the ST segment during exercise [33]. However, several studies had shown that chest pain as a cause of termination of the exercise test has discriminating diagnostic values [34]. In diabetic population, the silence of myocardial ischemia calls for a separate assessment of exercise test characteristics [35].

The accuracy of ETT as a screening test of OCAD was reported to be 79%, with a false-negative rate of 18%. For patients who are unable to perform an ETT and for those with a high chance of being 'false negative', alternative screening tests should be performed [36].

The finding, in this study, that 20.8% of asymptomatic T2D subjects were having OCAD (shown as positive ETT), was in confirmation with the result of Zorlu et al using ETT too [10]. The finding was, also, consistent with what Wackerset al had reported in the first step of DIAD study [13] using stress echocardiography for screening the asymptomatic diabetic patients. In stress echocardiography, the patient undergoes pre- and immediately post-exercise ultrasonographic examination of the heart [37]. Comparably, the second step of DIAD, using adenosine technitium-99m-sestamibi single photon emission computed tomography (SPECT) and myocardial perfusion imaging has shown that 60% of asymptomatic diabetics had two or more American Diabetic Association screening risk factors; 50% were capable of performing low-level exercise but only 22% had silent ischemia [13].

Some researchers believe that using stress nuclear testing; Thallium-201 and Technitium-99m-sestamibi, to perform myocardial perfusion imaging increases the diagnostic accuracy over ETT and allows for a more precise assessment of

myocardium at risk even in single vessel disease, which can be associated with false negative ETT results [38].

Bosson et al stated that, both, stress nuclear testing and stress echocardiography have expanded the potential of ETT to screen patients with baseline ECG abnormalities that prevent adequate evaluation through conventional ECG analysis [37].

To assess the diagnose reliability of ETT, the positive ETT subjects, in this study, were submitted to conventional CAG, the gold standard for the diagnosis of CAD. The results disclosed that two thirds (68.4%) of positive ETT (10.8% of the study total sample) were having angiographic coronary stenosis. The discrepancy between OCAD rates as detected by ETT and those detected by CAG in this study (20.8.72% vs. 10.8%), had, also, been seen when other modalities of screening for OCAD were compared to CAG [39]. Using the non-invasive coronary computed tomoangiography (CCTA), for example, had showed that 64% of asymptomatic diabetic subjects had OCAD but only 26% of them had a significant stenosis [28].

In regard to gender, less number of females who sustained CAG (4/7= 57%) showed angiographic coronary stenosis in comparison to 75% of the males. Although statistically non significant, (due to sample number), the apparently lower rate of positive angiography among females than among males (57% vs. 75%), is not merely a false positive ETT result among females. It, rather, reflects a higher rate of small vessel disease among females that was shown as positive ETT but underestimated by conventional CAG. True angina with a negative CAG due to small vessel disease affliction in females is a well known issue [40]. In other words, the apparently higher rate of positive CAG among males means that positive ETT males are more likely to have large (epicardial) coronary disease than females who tend to have small vessel disease. Diabetic males thus deserve a keen coronary imaging.

On comparing the detection rates of significant forms of OCAD by the different measures of screening, it was clear that the rates are nearly comparable, ranging between 18% [41] and 26% [31]. This indicates that ETT that reported an OCAD rate of 20.8% among asymptomatic T2D subjects in this study is a reasonable practical tool for detecting the significant forms of OCAD.

Milder forms of OCAD that are beyond ETT detection can be encompassed in an optimal conservative therapy of diabetes. In addition, having a higher positive angiographic stenosis among males (although statistically non-significant) draws attention towards the genuine requirement of T2D males to a specific care to guard against subtle ischemic myocardial damage. Positive ETT results in females, who were shown to less likely have angiographic stenosis, reflect a higher rate of small vessel disease among females in the study sample.

In spite of the repeatedly documented high OCAD rates among asymptomatic diabetics, some studies have failed to show any difference between diabetic subjects and the general population in regard to the prevalence of silent myocardial ischemia. In the remarkable Danish case-control study, the observed prevalence of silent ischemia on ETT or Holter testing in diabetic patients was 13.5% and was no different in matched controls [41].

Apart from the rate of smoking, the risk factors of the enrolled sample; age, duration of diabetes, BMI, HbA1c, T Chol, TG, systolic and diastolic blood pressures, were not significantly different between the positive and negative ETT subjects. This finding was largely in conformation with Juan et al results except that the latter had reported a significantly older age and longer duration of diabetes among the positive ETT in addition to smoking [29]. Moreover, it was quite congruent with Qais study that showed that the frequency of diabetes and smoking was significantly higher in patients with coronary heart disease in comparison to the control group [42].

Our finding indicates that smoking trend is a strong predictor of significant OCAD among T2D subject. The strongest predictors for abnormal screening tests in DIAD study were abnormal Valsalva, male gender, and diabetes duration but not the traditional cardiac risk factors or inflammatory and prothrombotic markers [13].

In accord with Al-Mukhtar et al [43], the current study found that the females, particularly the ETT positive females, have a higher rate of cardiovascular risk factors. In spite of that the females have less rate of OCAD. This dictates that females have some protective factors against CAD. Coronary protection, in the premenopausal females, is likely to be due to the effect of estrogen hormone that hinders atherogenicity in more than a way [44, 45].

This study concluded that when screened by ETT, one fifth of asymptomatic T2D subjects were shown to be afflicted with OCAD. When the ETT-detected OCAD, were assessed by CAG, two thirds of them were found to have angiographic coronary disease; two third of them were of significant stenosis. Smoking in T2D, especially among males, is highly predictive of the presence of OCAD. The Males were afflicted by OCAD at a lower rate of risk factors than females. Thus, ETT should be highly considered in smoker males with T2D.

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Author contribution

All authors were contributed in collection of patients sample and interchanged the basic interview as well as the base line assessment of patients.

Conflict of interest

There is no conflict of interest to be declared.

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Effect of Nimodipine (0.5%) Eye Drops Against Selentie-Induced Cataract in Rabbits

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Abstract

Background Increase Ca^{2+} level in human lenses found to play a main role in the opacification development.

Objective To investigate the protective effects of nimodipine (0.5%) eye drops against selenite-induced cataract in rabbits.

Methods Twenty-four adult rabbits with body weight in a range of (1.5-2 kg) with no signs of ocular inflammation were divided into three groups (apparently normal group, cataract group, nimodipine (0.5%) eye drops group). Cataract induction was done by a single intravitreal injection of sodium selenite solution in right eye. Lens opacity, pupil diameter, intraocular pressure, pupillary response to light, corneal sensation, conjunctival redness, malondialdehyde (MDA) and reduced glutathione (GSH) levels in aqueous humor and histopathological study of lens were evaluated. Apparently, normal group received distilled water.

Results Nimodipine (0.5%) eye drops resulted in prevention of cataract development, there was no change in pupil diameter, positive pupillary response to light, positive corneal sensation, no conjunctival redness, decreased MDA and increased of GSH levels. Longitudinal lens section showed homogenous appearance and it looked like normal in its feature.

Conclusions Nimodipine (0.5%) eye drops had prophylactic effect against selenite-induced cataract in rabbits.

Key words Cataract, nimodipine, selenite.

List of abbreviation: MDA = malondialdehyde, GSH = reduced glutathione, IOP = intraocular pressure, FFA = free fatty acids

Introduction

Cataract is an opaqueness of the lens which leads to decreased visual acuity [(1)]. Cataract becomes more common with advanced age and it is one of the chief causes of blindness and visual impairment in elderly people throughout the developing world [(2)]. So, detection of factors that might delay or prevent cataract occurrence would be essential both for increasing the well-being of elderly and for decreasing medical care costs. Many factors; such as genetics, cigarette smoking and sunlight exposure are implicated in the progression of lens opacity [(3,4,5)]. Specifically, oxidative stress is thought to have a major role in the etiology of age-related cataract [(6)]. Nimodipine is a member of the dihydropyridine group of calcium channel blockers, which has a high affinity for the cerebral blood vessels and appears to decrease morbidity following a subarachnoid hemorrhage [(7,8)].

The aim of this study was to investigate the protective effects of nimodipine (0.5%) eye drops against selenite-induced cataract in rabbits.

Methods

Twenty-four adult rabbits with body weight in a range of (1.5-2 kg) with no signs of ocular inflammation were divided into three groups (apparently normal group, cataract group and nimodipine (0.5%) eye drops group). The rabbits were housed in Animal House of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. All rabbits were maintained during the study (established at May 2014 to May 2015) with appropriate temperature and good ventilation. Animals were kept on fresh diet and allowed for free access to water. The experiment were approved by Animal Ethical Committee, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Induction of cataract

The rabbits were anesthetized by intramuscular injection of 0.5 ml of (50 mg/ml) ketamine. In addition, lidocaine solution (2%) was applied locally on the eye to obtain additional anesthetization. The induction of cataract in the right eye was done by a single intravitreal injection of 0.1 ml from of sodium selenite solution (0.01% w/v). After injection, the rabbits were monitored every day for caractogenesis [9,10,11].

Preparation of nimodipine eye drops (0.5% w/v)

A 500mg of nimodipine powder (reference standard from Bayer Schering Pharma) and 1% (w /v) of benzalkonium chloride were dissolved in 100 ml of isotonic buffer solution [12].

The right eye of rabbit were received nimodipine (0.5%) eye drops twice daily for five days before cataract induction and 21 days after cataract induction, and left eyes were received distilled water for same time and frequency

Lens opacity

The score of lens opacity (by the use of ophthalmoscope grading criteria) was done in accordance with the classification of Kador [13] and Chylack [14].

Pupil diameter

By using the pupil gauge, measuring of pupil diameter was done and the results would be presented in millimeter units [15].

Pupillary response to light

The light reflex was examined by swinging flashlight to investigate a relative afferent papillary defect [16,17].

Corneal sensation

Corneal sensation could be examined with wisp of cotton wool, which applied and moved from side to side [15].

Conjunctival redness

It could be detected by examination of conjunctiva of both eyes [16].

Intraocular pressure (IOP measurement)

IOP measurement were done by anesthetization the cornea with a local anesthetic (2% lidocaine hydrochloride), and "the foot plate of the tonometer is placed on the cornea (90^{o} on the pupil), a small force (weight) is applied to a central plunger, causing the part of the cornea beneath the plunger to displace inward. The scale reading taking from the tonometer is converted to the corresponding mmHg of tension by referring to a standard chart" [18].

Measurement of glutathione (GSH) and MDA level in aqueous humor of rabbit eyes

Glutathione was measured in accordance with the method of Godin [17] regarding to the reaction of glutathione with 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) at PH of 8, the result was a colored complex which absorbed light at 412 nm. The technique to find out the MDA level is based on the reality that, in acid medium, MDA reacts with thiobarbituric acid (TBA) to form a pink-colored MDA-TBA

complex that exhibits an absorption maximum at 532 nm [20,21].

Histopathological study

The rabbit lens samples fixed by gluteraldehyde (3%) solution for 48 hours. Following washing, treatment with osmium tetra oxide (1%) for 20 minute, washing, dehydration at 4 ºC and embedding, tissues capsules sectioned at (1 micron), these sections stained with solution A (0.4% basic fucshin in 25% methanol) and B (Prepared by mixing the same volumes of (azure II, methylene blue, Na2 CO3, absolute methyl alcohol) and examined microscopically [22].

Statistical methods

The obtained quantitative data was introduced as mean ± Standard error of mean (S.E.M.). In graphic presentation, only the means of these data (i.e. without S.E.M.) were presented. The significance of the differences between mean values was estimated by using paired and unpaired student's t- test accordingly. The obtained difference was considered to be not significant if p value > 0.05, significant if ($0.05 \geq p > 0.01$) and highly significant if $p \leq 0.01$ [23].

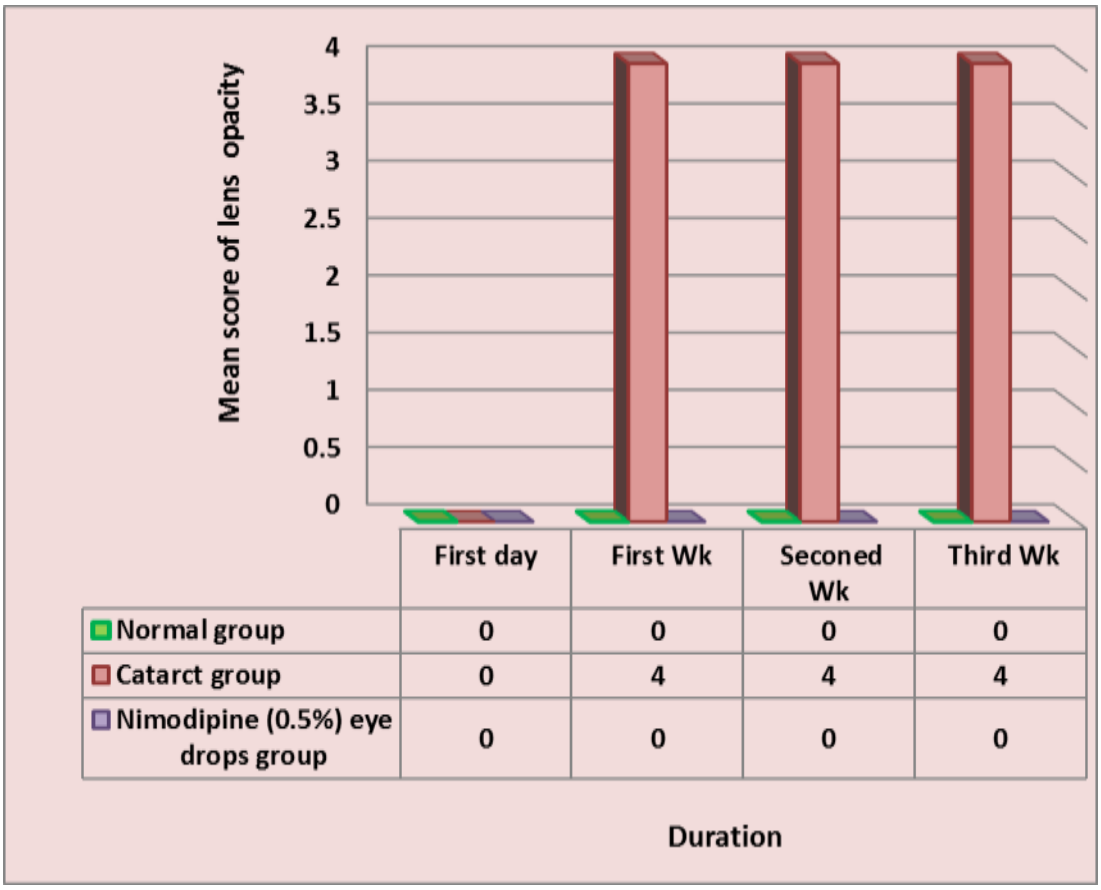
Results

Nimodipine (0.5%) eye drops resulted in complete prevention of cataract development (Figure 1). There was no alteration in pupil diameter, positive response to light, no change in corneal sensation, no conjunctival redness. The mean±SEM of IOP of eyes of rabbits was represented in figure (2) and its associated table. Before administration of nimodipine eye drops (0.5%) to right eyes of rabbits, the IOP of right and left eyes were comparable. After one day of nimodipine eye drops (0.5%) administration to right eyes of rabbits, there was high significant decrease in the IOP of right eyes when compared to left eyes (P value = 0.005). There was high significant decrease in the IOP of right eyes when compared to left eyes at first day of cataract induction (after five days of nimodipine eye drops (0.5%) administration), (P=0.009) and there was high significant difference after one week (P=0.004), after two weeks (P=0.002) and after three weeks of cataract induction (P=0.002).

The levels of GSH and MDA were measured at the end of the study in the aqueous humor of apparently normal, cataract and nimodipine (0.5%) eye drops groups were shown in the figure (3) and figure (4). There was high significant difference between the level of GSH in aqueous humor of cataract group and in the aqueous humor of nimodipine eye drops group (P=0.0001). There was highly significant difference between the level of MDA in aqueous humor of cataract group and in the aqueous humor of nimodipine eye drops group (P=0.0001)

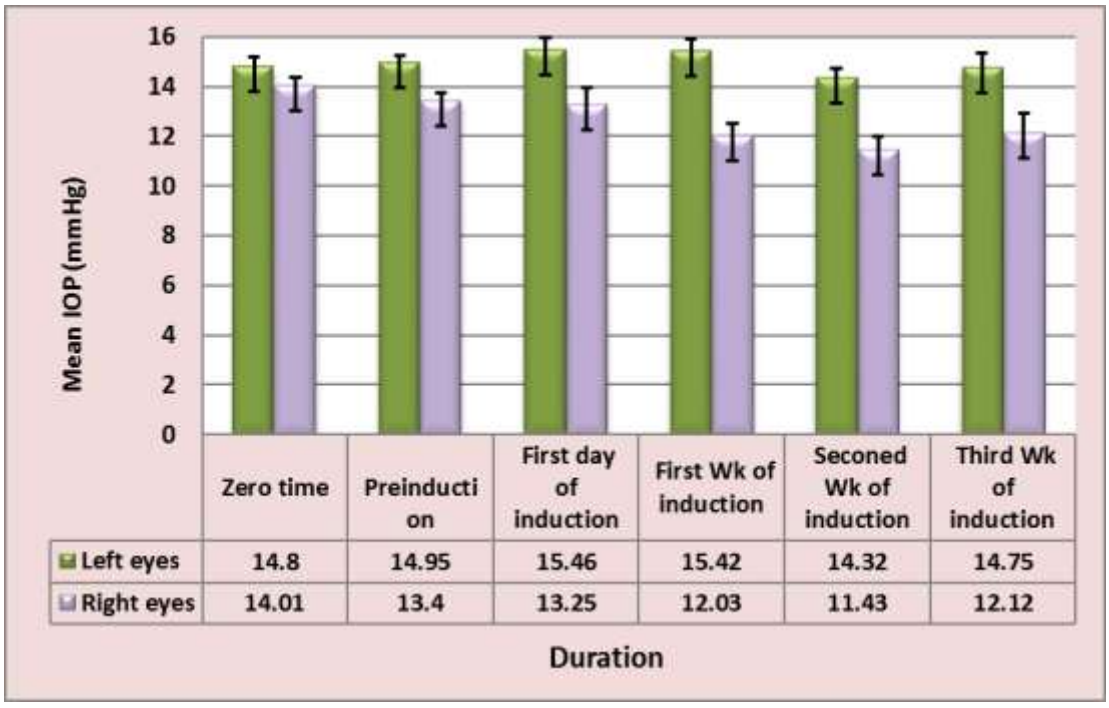
Histopathological study of rabbit lens

The cytoplasm of normal eye was uniform, featureless, and it was stained homogenously as shown in figure (5A). In the lens of cataract group, there was thick darkly stained collectives inside the fiber which extended along the lens fiber, these aggregations characterize the insoluble proteins that build up and aggregate in the lens fiber which caused by the oxidative and sclerotic outcome of selenite on the lens proteins. These aggregations are surrounded by plain or lighter areas produced as a result of losing the cytoplasm its homogenous form as shown by figure (5B). Nimodipine (0.5%) eye drops administration twice daily resulted in prevention of cataract development; and the longitudinal section of lens showed homogenous appearance and it looked like normal in its feature, figure (5C)



	First day	First Wk	Seconed Wk	Third Wk
Normal group	0	0	0	0
Catarct group	0	4	4	4
Nimodipine (0.5%) eye drops group	0	0	0	0

Fig. 1. Mean score of lens opacity in rabbits' right eyes of nimodipine eye drops group



	Zero time	Preinduction	First day of induction	First Wk of induction	Seconed Wk of induction	Third Wk of induction
Left eyes	14.8	14.95	15.46	15.42	14.32	14.75
Right eyes	14.01	13.4	13.25	12.03	11.43	12.12

Fig. 2. Mean IOP of rabbits' eyes of nimodipine (0.5%) eye drops group

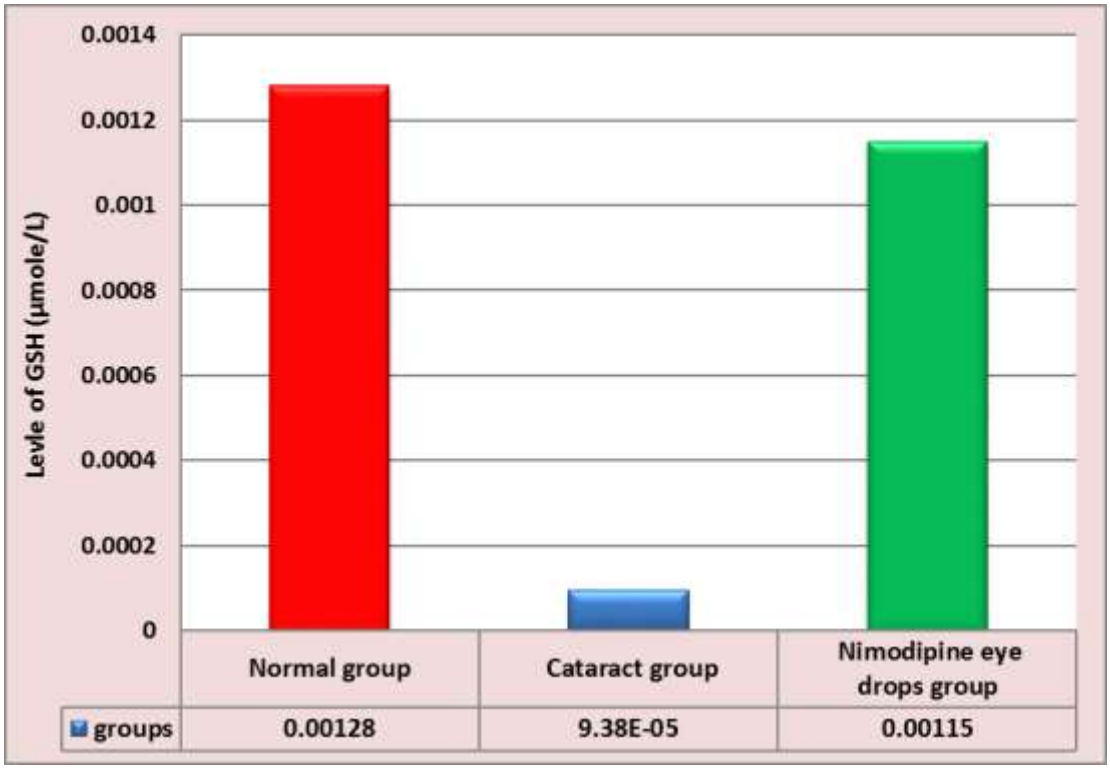


Fig. 3. Levels of GSH (µmol/L) in aqueous humor of groups of study

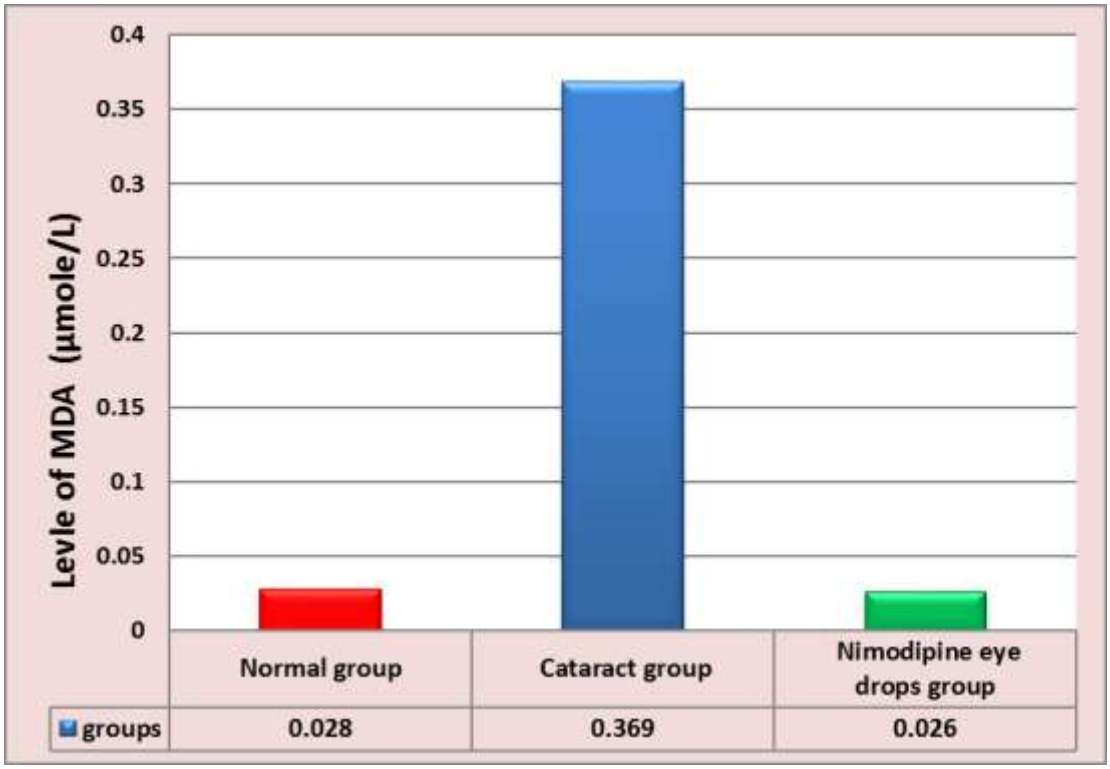


Fig. 4. Levels of MDA (µmol/L) in aqueous humor of groups of study

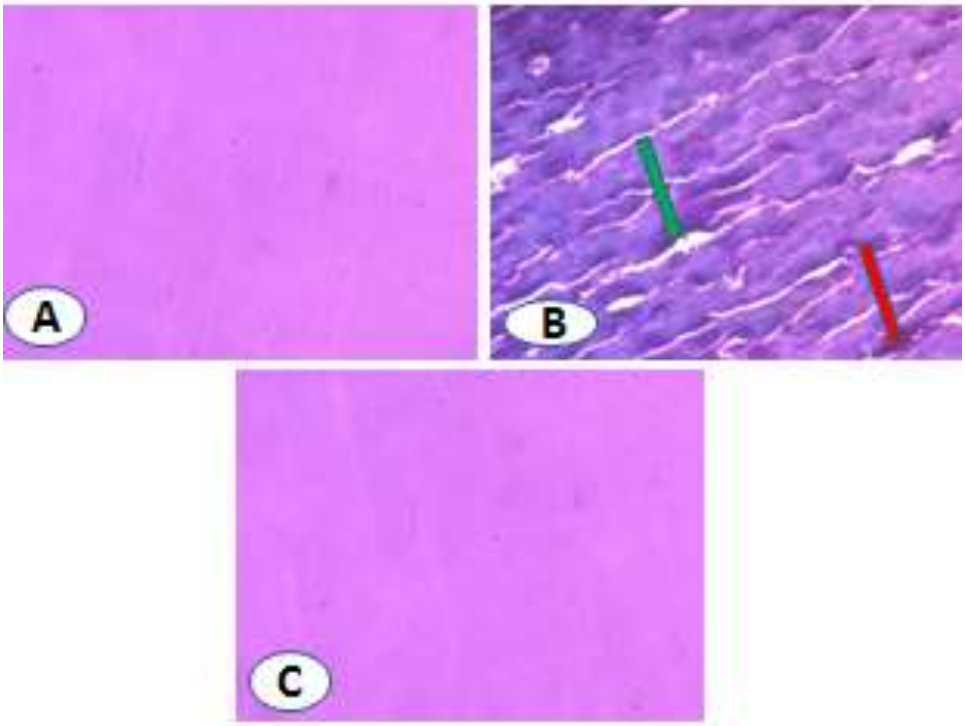


Fig. 5. Longitudinal section of rabbits' lens stained with solution A and B for semithin section (40X). A: Normal group shown homogenous cytoplasm of the normal lens fibers. B: Cataract group shown darkly stained aggregation (red arrows), alternating with clear areas (green arrows) with loss of the homogenous architecture of the cytoplasm of lens fibers. C: Nimodipine eye drops look like normal and shown structureless cytoplasm of lens fibers

Discussion

Nimodipine is a centrally active calcium channel blocker that blocks the voltage-dependent L-type calcium channels [24]. "Intracellular Ca^{2+} overload triggers Ca^{2+} dependent lytic enzymes such as phospholipase-c that induce the release of free fatty acids (FFA) and xanthine oxidase. This leads to the generation of superoxide and the depletion of membrane phospholipids resulting in altered permeability and further Ca^{2+} influx [25]. The increase in the levels of Ca^{2+}in the human lenses with cataract had been found to play a main role in the opacification development [26]. The L-type calcium channel blocker (verapamil) was efficient in attenuating cataract formation in diabetic rats. Lenses extracted from animals, which had been treated with verapamil as well had a greatly decreased Ca^{2+} content. Other researches of diabetic and radiation models of cataract furthermore reported decreased lens opacification as a result of the treatment by L-type calcium channel blockers [27]. Variety of researches have revealed that calcium channel blockers may improve the ocular blood flow in the healthy volunteers and in patients with glaucoma28. Loss of Ca^{2+} homeostasis had been occupied in most types of cataract. Ca^{2+} levels are maintained in the sub-µM range in cytoplasm by the membrane Ca^{2+} pumps, plasma membrane Na+/Ca^{2+} exchangers, and endoplasmic reticulum Ca^{2+} pumps [29]. Increased Ca^{2+} uptake performed in correlation with selenite cataractogenesis, was established to be highest. A main consequence of calcium elevation in the lens is the activation of calpains. Researches on experimental cataract had demonstrated that calpain-induced proteolysis of β-crystallin was a major mechanism in lens maturation as well as cataractogenesis [30]. Alterations of membrane proteins, lipid integrity, and consequent raise of the membrane ion permeability of lens fiber cells had been

established in different pathological conditions. So, selenite-induced oxidative stress and subsequent loss of the Ca^{2+} homeostasis thought to be responsible for the activation of lens calpains, which led to in proteolytic precipitation and aggregation of insoluble proteins [31].

This study concluded that Nimodipine (0.5%) eye drops twice daily had protective effect against selenite induced cataract in rabbits' eyes.

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Author contribution

The study was done by Dr. Shakoor under the supervision of Dr. Al-Zubaidy. Histopathological study was done with the assistance of Dr. Qasim.

Conflict of interest

The authors declare no conflict of interest

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Association of *Porphyromonus gingivalis* with Rheumatoid arthritis

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Abstract

Background Rheumatoid arthritis (RA) is chronic systemic inflammatory disease. *Porphyromonus gingivalis (P. gingivalis)* produce peptidyle arginine enzymes, which lead to citrullination of human protein then lead to formation anticitrullinated peptide antibody (ACPA).

Objective To investigate the role of *P. gingivalis* as environmental factor for RA and association of *P. gingivalis* with development of ACPA.

Methods This study included 31 newly diagnosed RA patients with periodontitis, which included 22 females and 9 men in addition to 30 individual as healthy controls, which included 20 females and 10 men. The exclusion criteria included autoimmune disease (systemic lupus erythematosus, Bachet disease, ankylosing spondylitis, multiple sclerosis), attending Department of Rheumatology in Baghdad Teaching Hospital during period from May 2014 to January 2015. The age range was 20 to 68 years. Disease activity score 28 (DAS28) was calculated for each patient. Five ml of blood sample was taken for detection of ACPA antibody while gingival cervicular fluid was taken by paper point for detection of *P. gingivalis* by polymerase chain reaction (PCR) with specific primer for fimbrial antigen (fimA).

Results The frequency of positive cases with *P. gingivalis* were 13/31(41.90%) while in healthy controls was zero with significant P value (<0.001). The association between anti cyclic citrullinated peptide (ACCP) antibody and frequency of positive cases for *P. gingivalis* was significant (P<0.041). The association of *P. gingivalis* positivity and DAS28 was non-significant (P= 0.003).

Conclusions *P. gingivalis* showed positive association with RA in newly diagnosed patients. The frequency of positive cases for *P. gingivalis* revealed association with positivity of anti-CCP.

Key words RA, ACCP, *P. gingivalis*

List of abbreviation: *P. gingivalis* = Porphyromonus gingivalis, RA = Rheumatoid arthritis, ACPA = anticitrullinated peptide antibody, DAS28 = Disease activity score, PCR = polymerase chain reaction, ELISA = Enzyme linked immune sorbent assay, USA = United States of America, bP = base pair, UV = Ultraviolet

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting 0.2–1% of the population worldwide and is associated with progressive destruction of the joints causes' early mortality and disability. Early diagnosis is challenging because the symptoms of early RA can be non-specific (e.g., malaise, fatigue, weakness, muscle soreness, low down-grade fever, weight loss) and may actually be symptoms of other conditions. Assessment of disease activity and treatment responses is based on measurement of disease activity score28 (DAS28) [(1)].

Although the causes are indefinite, there is accumulating evidence RA is an autoimmune disease characterized by disease-specific antibodies to citrullinated protein antigens (ACPA). Citrullinated proteins are generated

by peptidyl arginine deiminases (PADs), enzymes that catalyze the modification of peptidyl-arginine to peptidyl-citrulline [2].

P. gingivalis belongs to the phylum bacteroidetes and is a non-motile, gram-negative, rod-shaped, anaerobic, pathogenic bacterium. It establishes in the oral cavity, somewhere it is implicated in confident forms of periodontal disease [3]. The Collagen degradation experiential in chronic periodontal disease consequences in part from the collagenase enzymes of this species. It has been revealed in an in vitro study that P. gingivalis can attack human gingival fibroblasts and can stay alive in them in the existence of significant concentrations of antibiotics [4].

The bacterium has concerned interest based on epidemiologic relations between RA and periodontitis and the explanation of a novel bacterial peptidyle arginine deaminase (PAD), suggesting a possible etiologic role of *P. gingivalis* in RA during the creation of citrullinated antigens. In RA, an autoimmune response develops in opposition to citrullinated peptides detected as anti-citrullinated peptide antibodies (ACPA). The existence of anti-CCP are >98% specific for the diagnosis of RA [5].

The mechanisms to citrullination that guide to RA remain unclear. A polymorphism in the PAD4 gene, which may lead to amplified citrullination has been described in populations. *P. gingivalis* is the only prokaryote recognized to have PAD, an enzyme to facilitate catalyzes the posttranslational modification of arginine residues to citrulline [6].

The objectives of this study was to investigate the role of *P. gingivalis* as environmental factor for RA and association of *P. gingivalis* with development of ACPA

Methods

This study included 31 newly diagnosed RA patients with 30 persons as healthy controls. The RA patients attended Rheumatology Department in Baghdad Teaching Hospital were examined by rheumatologist during the period from May 2014 to January 2015 and DAS28 was calculated for each patient according to ACR.

Blood samples

In this study 5 ml of blood were taken from each patients and healthy control by venipuncture, then 3 ml of blood were separated by centrifuge and serum sample was isolated and stored at (-20 °C) until used while 1.6 ml of blood was add to 0.4 of sodium citrate for erythrocyte sedimentation rate (ESR).

ELISA test for anti-CCP:

The anti-CCP was done by ELISA technique (indirect method) according to instructions manual by Human company/Germany .

DNA-extraction from gingival cervicular fluid samples

The wizard genomic purification by promega/USA.

Monoplex PCR for detections fimA of *P. gingivalis* was done according to Nakagawa [7].

The primer set was used in detection of *P. gingivalis* as followed:

Gene	Primer	Product size (bp)	Reference
Type 1b fimA	CAGCAGAGCCAAAAACAAT GCTGTCAGATAATTAGCGTC TGC	250	Nakagawa ,et al [7]
Type 11 fimA	ACAACTATACTTATGACAAT GGAACCCCGCTCCCTGTATT CCGA	200	Amano,et al [8]

Statistical analysis

Prevalence of infection was compared between different variable by Chi-squared test. Significance was attributed to probability $P \leq 0.05$. Computer SPSS and Microsoft were used for determination of probability values.

Results

The mean age in newly diagnosed RA patients was 46.3 years versus 43.6 in controls and the p value non-significant as in table (1).

Table 1. Mean age in newly diagnosed rheumatoid arthritis patients

Age (yr)	Control	Newly diagnosis RA patients
Mean	43.6	46.03
Standard Deviation	11.28	12.35
Median	43	50
Minimum	18	20
Maximum	65	67
P value	>0.05	

Table (2) showed distribution of severity state (high, moderate, mild, remission) in newly diagnosed RA patients.

Table 2. The DAS28 score in treated and newly diagnosed RA patients

		Newly diagnosis RA patients
Remission	Count	3
	%	9.7%
Mild	Count	6
	%	19.4%
Moderate	Count	9
	%	29.0%
High	Count	13
	%	34.5%
Total	Count	29
	%	100%
P value	0.665	

The frequency of positive cases for anti-CCP were 23 out of 31 RA patients while in controls was zero with significant association (P<0.001) as in table (3).

Positivity of *P. gingivalis* in newly diagnosed RA patients was 13 (41.9%) out of 31, while negative in all cases of control with significant difference (P value <0.001) as in table (4) and (Figure 1, 2).

Table 3. Serum anti-CCP positivity in newly diagnosed RA patients versus controls

		Control	Newly diagnosis RA patients
Positive	Count	0	23
	%	0.0%	74.2%
Negative	Count	30	8
	%	100%	25.8%
Total	Count	30	31
	%	100%	100%
P value	<0.001		

Table 4. The frequency of positive cases with P. gingival in newly diagnosed patients and healthy controls

		Control	Newly diagnosis RA patients
Positive	Count	0	13
	%	0.0%	41.9%
Negative	Count	30	18
	%	100%	58.1%
Total	Count	30	31
	%	100%	100%
P value	<0.001		

Table (5) shows significant association between positive cases of *P. gingivalis* and anti-CCP positivity with P value <0.05.

Current study revealed no association between *P. gingivalis* infection and DAS score with P value =0.003 as in table (6).

Table 5. Relationship between *P. gingivalis* positivity and anti-CCP of newly diagnosed RA patients

P. gingivalis	No.	Mean±SD of CCP
Positive	13	168.46 ± 34.33
Negative	18	78.94 ± 25.53
LSD value	----	85.603
P value	----	0.0410

* Significant (P<0.05)

Table 6. The frequency of positive cases with *P. gingivalis* in newly diagnosed patients

DAS28	*P. gingivalis*			
	Negative		Positive	
	No.	%	No.	%
Remission	1	2.8	2	7.7
Mild	1	2.8	5	19.2
Moderate	6	16.7	3	11.5
High	10	27.8	3	11.5
Total	18	51.1	13	49.9
P value	0.003			

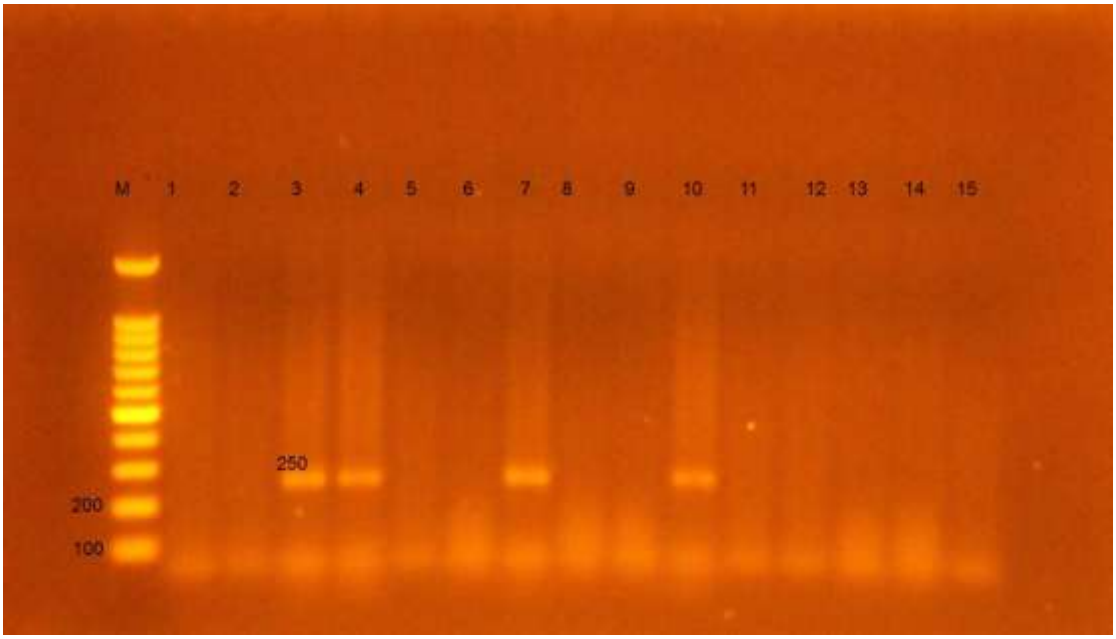

Fig. 1. Gel electrophoresis for PCR product of *P. gingivalis* type Ib fim visualized under UV light.M:1000 bp marker lane (3,4,7,10) were positive for type Ib . The size of product was 250 bp (time 90 mint., 50 volt)

Discussion

Porphyromonus gingivalis genome detection was done for newly diagnosed RA cases by PCR to detect FimA (fimbrillin antigen), which consider highly specific for *P. gingivalis;* two type of FimA were used (FimA type 1b, FimA type II) to increase specificity and to diagnosis bacteria from gingival cervcular fluid [9]. The positive cases were (41.90%) of thirty one newly diagnosed RA patients. Relationship of *P. gingivalis* infection with RA could be illustrated by mechanisms of secreted enzyme peptidyle arginine deaminase, which lead to citrullination and permanent post-translation of arginine to citrulline as a consequence for this event will lead to accumulation of citrullinated peptide with development of anti citrullinated peptide antibody, which concerned in RA and useful diagnostic marker for RA. This agree with Hitchon, *et al* who proposed that citrullination

of human protein by bacterial enzymes will lead to production of ACPA which used as good diagnostic markers for RA [10].

There were 41.90% of newly diagnosed RA patients have infection with P. gingivalis infection as well as there is high titer of ACPA in newly diagnosed RA patients, this finding reflect the role of *P. gingivalis* as one of environmental factor for RA and stimulation for ACPA formations. *P. gingivalis* infection showed a significant association with DAS, so the bacteria may responsible on initiating autoimmune process, which end with development of RA as well as infection with bacteria may exacerbate RA. This finding agree with Al-katma, *et al* (2007), who hypothesized that control of periodontal infections and gingival inflammation by scaling roots planning and plaque control in subjects with periodontal disease reduce the severity of RA [11]. However, this result disagree with Lugli *et al* (2014), who proposed that *P. gingivalis* infection, which causes periodontitis was associated with development of RA not with severity because severity of disease depend on autoimmune process not on infection with *P. gingivalis* [12].

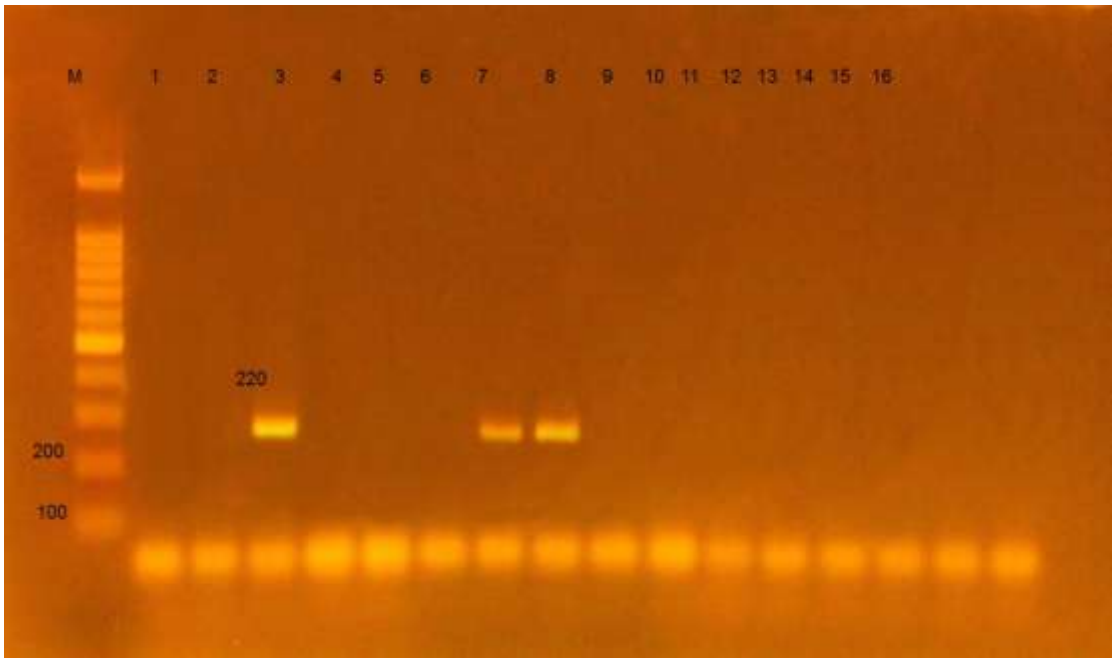

Fig. 2. Gel electrophoresis for PCR product of *P. gingivalis* type II fim visualized under UV light.M:1000bp marker lane (3,7,8) were positive for type II fim . the size of product 220 bp (time 90 mint., 50 volt)

Molecular mimicry between citrullination of protein by bacterial enzyme and human protein may be responsible for production of ACPA [13]. ACPA in newly diagnosed RA patients was high in association with positivity of *P. gingivalis* infections this proved role of bacteria as environmental factor for RA when individual infected with these bacteria will have periodontal disease then there is high tendency to affect with RA [14].

This study concluded that *P. gingivalis* may be consider as risk factor for RA and showed correlation with positivity of anti-CCP.

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Author contribution

Sadeq: Data collection and drafting of the article. Ahmed: Design of the work, data interpretation, drafting and critical revision of the article. Mohammed: samples collection.

Conflict of interest

The authors declare no conflict of interest.

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Causal Beliefs of Schizophrenia among sample of Iraqi Schizophrenic Inpatients' Families in Iraq

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Abstract

Background Schizophrenia is a debilitating mental illness that affects 1% of the population in all cultures.

Objective To investigate causal beliefs regarding schizophrenia among families of Iraqi schizophrenic inpatients, and discuss the differences and similarities across cultures.

Methods Two hundred Iraqi schizophrenic inpatients fulfilling DSM-IV criteria of schizophrenia were included. Causal beliefs of their key relatives were examined. Information list including open question of the causal beliefs of schizophrenia and sociodemographic data was used. The statistical significance of the findings was tested.

Results Key relatives attributed natural causes to schizophrenia more often than supernatural causes. Stress was 44%. Stresses were related to life events, trauma, social problems, war, prison and poverty. Other causes were: 27% organic and 15% personality attributions. Supernatural causal beliefs were only 29%, including witchcraft, envy, possession, devil, karama and wish or punishment of God.

Conclusions The major causal beliefs of schizophrenia amongst Iraqi relatives of the studied sample were stresses. Families' attribution of supernatural causes was similar across cultures. Organicity was more in the European studies.

Key Words Schizophrenia, causes, beliefs, families, Iraq.

Introduction

Schizophrenia is a debilitating mental illness that affects 1% of the population in all cultures. It affects equal numbers of men and women, but the onset is often later in women than in men [1]. Exploring what relatives of patients who have schizophrenia believe about the causes and the psychosocial consequences of the disorder has been claimed to be useful in appraising patients' family environment and planning psychosocial interventions [2,3].

Murdock et al [4] and Minas et al [5] drew together causal beliefs from 139 traditional and contemporary societies from the World Ethnographic Atlas, identifying two broad constructs, natural and supernatural, and a variety of sub-constructs within each of these. These included, within the natural group, causes such as stress, infection, and organic deterioration, and, within the supernatural group, causes such as fate, mystical retribution, and magical causation.

Investigating family members' causal beliefs regarding schizophrenia is an important step in the management of the illness because it may influence the help-seeking pathway of individuals with schizophrenia [6]. It is a widely shared belief that an increase in the public's mental health literacy [7,8] will result in an improvement of attitudes towards people with mental illness. Most of the current anti-stigma programs are based on this rationale [7,9].

In particular, promoting biological concepts as a causal explanation for mental disorders is considered to be a promising strategy. The argument is that if the causes of mental disorders were attributed to factors outside the individuals' control, people's reactions to those with mental illness would be less negative. German study concluded; one has to say that we are facing a dilemma. On the one hand, there are good reasons for improving the public's mental health literacy by informing them about the views shared by mental health professionals on the etiology of schizophrenia, as they may have a positive effect on people's readiness to seek professional help [7,10].

On the other, promulgating biological factors as a cause of the disorder may lead to more instead of less rejection [7] Family members often provide psychosocial support and assistance in seeking treatment for individuals with schizophrenia. It is crucial to understand what family members believe to be the causes of schizophrenia, as this likely influences the family's help-seeking decisions [11].

The aim of this study is to find out the causal beliefs regarding schizophrenia among schizophrenic inpatients' families in Iraq, and compare the results with several other cultural studies.

Methods

Design and setting

This is a cross-sectional study with analytic component. It was conducted in Ibn-Rushd Psychiatric Teaching Hospital, Baghdad, Iraq. The data collection was done during the period 1st Aug. 2009 to 30th Apr. 2010.

Study Population and Sampling

Assessment of 200 schizophrenic inpatients diagnosed by senior psychiatrists as acute schizophrenia either first episode or in relapse, was done

Inclusion criteria

All met the DSM-IV diagnostic criteria for schizophrenia.

Exclusion criteria

Current serious or unstable medical illness history of seizure disorder; history of multiple adverse drug reactions; current substance abuse, and pregnant patients were excluded. All patients assessed and followed up by full mental state examination. Families were assessed for the causes of the illness from their viewpoint and information list for collection of sociodemographic data and the family beliefs about the causation of schizophrenia illness was used. The list include open question about the most likely causes of illness and only the first answer was taken for analysis.

Definition of variables

The independent variables evaluated to explain causal beliefs were socio-demographics (age, gender, marital status, economic status, and level of education), and characteristics of the disease (duration).

Statistical Analysis

Statistical package of social sciences (SPSS) version 12 was used for data entry and analysis.

Ethical Issues

After granting approval from the concerned health authorities in Baghdad, informed consent was obtained from the patients' families after clarifying the objectives of the study. Names were kept anonymous and interviews were conducted with full privacy.

Results

The sample consisted of 200 patients and 200 key relatives. One hundred and twenty two (61%) of the patients were male. The mean ± SD age of the patients was 32.7 ± 9.21 years. One hundred and eleven (57.5%) were single. The mean age at onset 22.9 ± 7.1 years; 95% Muslim, 4% christen and 1% from other religion. About 51.5% satisfied their living, 39% were poor and 9.5% rich economic status.

Educational level; illiterate 8.5%, primary 24.5%, intermediate 29%, high school 17.5%,

university and postgraduates 20.5% (Table 1 and fig. 1).

Table 1. Frequency and percentage of sociodemographic features of schizophrenic patients

Feature		No.	%
Age (years)	17 – 25	54	27
	26 – 35	79	39.5
	36 – 45	43	21.5
	46 – 55	21	10.5
	56 - 65	3	1.5
Gender	Male	122	61
	Female	78	39
Marital Status	Single	115	57.5
	Married	38	19
	Separated	23	11.5
	Divorced	20	10
	Widowed	4	2
Economic Status	Poor	78	39
	Satisfied	103	51.5
	Rich	19	9.5
Educational Level	Illiterate	17	8.5
	PS	49	24.5
	IS	58	28
	HS	35	17.5
	University and PE	41	20.5

PS = Primary School, IS = Intermediate School, HS = High School, PE = Postgraduate Education

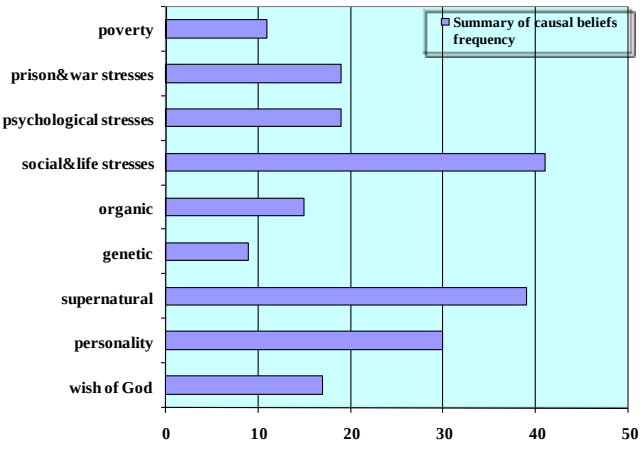

Fig. 1. Summary of the causal beliefs frequency among schizophrenic patients' families in Iraq

The mean duration of illness was 9.76 ± 7.2 years. The etiological beliefs were assessed from the relatives of the patients; mainly those accompanied the patients during the period of admission, it was that stress of various types represents nearly 50% of beliefs as cause of illness.

Social and life stresses (20.5%) include family problems, love affair, study, pregnancy, and military stresses. Psychological trauma and stress (9.5%) of the beliefs; fear, loss of close relative, death of one or both parent, and seeing dead person. War and prisons stress (9.5%). Poverty as believed was reported by (5.5%). Those who were attributed the illness to the organic and genetic reasons were 12%. Organic related causes (7.5%) include trauma, road traffic accidents, infection and early childhood illnesses, brain lesion, head injuries, and drug abuse. genetic causes were 4%. Supernatural power attributed as the causative agent of schizophrenic process founded in 19.5% including; devil, envy, witchcraft, and *karama* (locally known as *sharah*). Personality related causes; they marked it as him / herself, were 15%. Wish or punishments of God were represented in 8.5% (Table 2).

Table 2. Causal beliefs frequency

Causal Belief		No.	%
Wish of God	Positive	17	8.5
	Negative	183	91.5
Personality	Positive	30	15
	Negative	170	85
Supernatural	Positive	30	19
	Negative	170	81
Genetic	Positive	9	4.5
	Negative	191	95.5
Organic	Positive	15	7.5
	Negative	185	92.5
Social and life stresses	Positive	41	20.5
	Negative	159	79.5
Psychological stress	Positive	19	9.5
	Negative	181	90.5
Prison and war stress	Positive	19	9.5
	Negative	181	90.5
Poverty	Positive	11	5.5
	Negative	189	94.5
Total		200	100

Discussion

Illness causal beliefs shape illness experience, and are important in decisions about treatment choice and treatment adherence, and in the success of the therapeutic relationship. The 9 most commonly reported causes by the relatives of the patients in the present study were social and life stress (20.5%), supernatural power (19.5%), personality (15%), psychological stress (9.5%), war and prison stress (9.5%), wish of God (8.5%), organic (7.5%), poverty (5.5%) and genetic (4.5%). . Findings were significant when compare the presence or absence of each cause among the total causes (Table2).

These beliefs were categorized to 3 main groups; stress 44%, supernatural 29% and organic 27%. The Indian study show the majority of patients (70%) considered spiritual and mystical factors as the cause of their predicament; 22% held multiple models of illness [(12)]. Nigerian study show the single most important etiological factors were that "it is Satan's work" (35.8 %) and "it is a natural illness" (23.2 %). Other factors were "genetic" (9.5 %), "witchcraft" (10.5 %) and "curse by enemies" (10.5 %) [(13)].

Causative models are influenced by available knowledge and practices in the culture [(13)]. The African American study show the 5 most commonly reported causes were disturbance of brain biochemistry (49.6%), drug/alcohol abuse (42.5%), hereditary factors (40.9%), brain injury (40.2%), and avoidance of problems in life (37.8%). The mean number of likely or very likely causes endorsed by participants was 7.5 +/- 5.7 [(14)]. Some 47.9% reported one or more esoteric factors as a cause. Of the 6 esoteric factors, possession by evil spirits (28.3%), radiation (20.2%), and punishment by God (19.7%) were most common. Esoteric causes were more commonly chosen by male participants, those with 12 years of education or less, and participants who reported never having known someone with schizophrenia [(14)].

Bali study explore that the key relatives attributed supernatural causes to schizophrenia more often than natural causes. Compared with relatives who listed a natural cause as most important (14 relatives, or 36 percent), relatives who considered a supernatural cause as being the most important (25 relatives, or 64 percent) had a significantly higher mean age and less education and were more likely to have family members with schizophrenia who had never received psychiatric medical treatment [(15)]. Italian study show only 24 percent of the relatives (156 of 652) believed that both biological and psychosocial factors had been involved in the development of their loved ones' schizophrenia. Seventy percent of the relatives (457 of 652) maintained that the disorder was due exclusively to psychosocial factors, such as stress, psychological traumas, or the breakdown of a romantic relationship; 6 percent (39 of 652) thought that the disorder had been caused exclusively by biological factors. Patients' intentional behaviors, such as using drugs or keeping bad company, were mentioned as factors by 28 percent of the relatives (182 of 652) [(16)].

Another Indian study show supernatural cause was named by only 12% of the families and as the only cause by 5%. Psychosocial stress was most commonly cited cause, followed by personality defect and heredity. A small number of families (14%) could not name any cause and 39% named more than one cause [(17)]. German study to examine how the German public's causal attributions of schizophrenia and their desire for social distance from people with schizophrenia found at 2001; Brain disease 70.0%, Heredity 60.2%, Life event 72.3%, Stress at work 57.7%, Broken home 39.3%, Lack of parental affection 30.4%, Lack of willpower 36.4%,and Immoral lifestyle 20.9% [(7)].

This study shows that Iraqi people explore the dilemma of stress in its various components as the major causative factor of schizophrenia (44%). Majority of stresses were related to life events, trauma, social problems, war, prison and poverty. Beliefs of stress were not related

to the current critical situation in Iraq since the mean duration of illness is 9.76± 7.2 years, but may be related to that before 2003. Kaplan and Sadock [19], Barlow and Durand [20], and Sue et al [18] explain the etiology of schizophrenia as; Stress Diathesis Model.

This model postulates that a person may have a specific vulnerability that when acted on by some stressful environmental influence, allows the symptoms of schizophrenia to develop. In the most general stress-diathesis model, the diathesis or the stress can be biological or environmental or both. Supernatural factors were not deferred widely from our study in comparison with the above studies, indicates that Iraqis were of similar thinking about the causal beliefs of schizophrenia with Asian, European, African and American people. Across cultural similarity of traditional beliefs were present like witchcraft, envy, possession, devil, *karama* and wish or punishment of God. Organic beliefs of schizophrenic etiology founded less in our study and Asian and African studies in comparison with the European and American studies.

In conclusion, beliefs about stress, societal, family, personality and esoteric causes in this clinical sample indicate the need for improved psychoeducation of the community at large, there is a need for public mental health literacy and welfare support to actualize the potential of families to play useful community psychosocial roles. Future research should seek to better understand how esoteric beliefs about causation affect attitudes toward people with mental illnesses and acceptance of mental health treatment by those individuals.

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Conflict of interest

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A Comparative Study between Atropine and Tropicamide as Cycloplegic Agents for a Sample of Iraqi Children

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Abstract

Background The ideal cycloplegic drug that is effective, safe, and convenient allowing accurate measurement of the refractive errors by both subjective and objective means is not yet available.

Objective This study was designed to compare the cycloplegic activity and adverse effects of two cycloplegic agents (atropine vs. tropicamide) for children with hyperopia. The response to cycloplegia in different age groups, with or without strabismus, was also compared.

Methods Tropicamide 1% eye drops (Regimen 1) and atropine 1% eye drops (Regimen 2) was evaluated in thirty children with different ages. Cycloplegic refractions and adverse effects were assessed. The results expressed refractions and presented as mean ± SD. A *P*-value of less than 0.05 was considered statistically significant.

Results Tropicamide refraction mean value (+3.60 ± 2.25 D) didn't differ significantly in comparison with that of atropine (+3.92 ± 2.50 D); ($P > 0.05$). Children during regimen 2 (atropine drops) suffered from more frequent and statistically significant side effects ($P < 0.05$), represented by blurred vision, fever; flushing and tachycardia, compared with regimen 1 (tropicamide drops).

Conclusion Tropicamide applied to younger or older children is sufficient to produce good cycloplegia, with an effect approach to and safer than atropine, even in children with a high degree of hypermetropia, and with or without strabismus.

Keywords Cycloplegia, hyperopia, atropine, tropicamide.

List of abbreviation: D = diopter, RT = right, LT = left, SD = standard deviation.

Introduction

As a definition, the accommodative power of the eye is the variable force of accommodation that alters the path of light rays by causing the ciliary body to change the curvature of the lens [(1)].

Muscarinic receptors of the parasympathetically innervated smooth muscle fibers are present in the ciliary body. Cycloplegia inhibits the accommodative power of the eye by blocking the action of the ciliary muscle, allowing the static or objective refractive error of the eye to be measured. This anticholinergic action inhibits cholinergic stimulation (muscarinic receptors) of the iris sphincter and ciliary muscle, which results in mydriasis and cycloplegia [(2)].

Cycloplegic examinations not only allow refractive error to be determined, they also dilate the pupil, preparing the patient for an ophthalmoscopic examination. Cycloplegic refraction is necessary for the evaluation of patients with decreased vision or ocular deviation. It also helps detection of full hyperopia in patients with accommodative esotropia and prevents overcorrection in myopic patients [(3)].

After the discovery of modern laser refractive surgery, cycloplegic refraction has become a valuable preoperative test for accurately determining the refractive error. In older children and young adults, cycloplegic refraction can confirm the diagnosis of accommodative spasm, which is a constant or intermittent involuntary increase in ciliary contraction. Patients with low hyperopia may be presented as myopic during the examination; this so-called pseudomyopia can be identified by cycloplegic evaluation [4,5].

Cycloplegia can be used in pharmacologic occlusion therapy when the nonamblyopic eye is sufficiently hypermetropic where the effective blurring of vision can be obtained by instilling a cycloplegic drug in that eye alone. If under test conditions the patient switches from using the good eye to the amblyopic one, chances are excellent that he or she will also do so during treatment and that penalization will force the amblyopic eye to be used [6].

Medical uses for cycloplegic refraction are limited in adults. As the amplitude of accommodation gradually decreases with age, a closer agreement between cycloplegic and manifest refraction findings takes place [7].

The three most commonly used cycloplegic drugs, atropine, tropicamide and cyclopentolate, act by competing with the physiological muscarinic agent "acetylcholine", resulting in inhibition of ciliary muscle contraction [8]. Numerous studies have documented the efficacy of these cycloplegic drugs. Some authors have shown a significant and others showed no significant cycloplegic effect for some of these agents [9,10].

Most clinicians agree that cycloplegia is necessary when performing refraction in young children, high hyperopia, and patients with strabismus [11]. Fogging and other techniques cannot replace cycloplegic method for preciseness in determining refractive errors in early childhood because it does not depend on patient cooperation or fixation distance [12,13].

The unpleasant nature of instilling eye drops especially in children can prevent completion of the examination and is so important that sprays have been suggested instead of eye drops by some authors. Inadequate cycloplegia can cause inaccurate refraction and lead to inappropriate diagnostic and therapeutic approaches. On the opposite side, over dosage of cycloplegics may cause drug reactions or lead to patient discomfortable feeling [14].

Atropine, the strongest cycloplegic known used frequently in children, has its own advantages, and disadvantages in the form of prolonged action. Several investigators have shown atropine to be more effective in blocking accommodation in young esotropic children. In most cases of esotropia with hypermetropia in children less than 5 years of age, atropine or scopolamine preferably is used to ensure that no residual accommodation goes unrecognized [15,16].

Atropine is the gold standard for complete cycloplegia but it needs at least 3 hours to reach peak effect and must be used for 3 days to produce full cycloplegia. It takes 8-14 days for its effect to wash out from the pupil and ciliary body. Tropicamide, on the other hand, has a faster onset of action and reaches peak effect after 30-45 minutes; its cycloplegic effect washes out after 6-8 hours and has fewer complications [17].

Almost all ocular drugs have undesirable side effects, dividing into those of an ocular and systemic in nature. However, the complications from mydriatic and cycloplegic drugs are rare, compared with their extensive uses [18].

The toxic effects of atropine may be summarized by saying: "blind as a bat, dry as a bone, red as a beetroot and mad as a hatter". Atropine users are "blind" owing to the induced cycloplegia; "dry" due to the inhibition of the sweat and salivary glands; "red" because of peripheral vasodilation to lose heat and overcome the lack of function of the sweat glands; and "mad" owing to the effects on the CNS [19]. Atropine may lead to complications such as fever, tachycardia, convulsions, and even death [20].

The ideal cycloplegic drug that is effective, safe, and convenient allowing accurate measurement of the dioptric error by both subjective and objective means is not yet available. This study was designed to compare the cycloplegic activity and adverse effects between 1% eye drops of atropine versus 1% eye drops of tropicamide for a sample of Iraqi children with hyperopia.

Methods

This study involved 30 children (16 males/ 14 females) their ages were (2-9 years), with hyperopia of more than 1.0 Diopter (unit of refraction power) in at least one eye, enrolled in a prospective fashion among patients attending Ibn AL-Haitham Teaching Hospital and private eye clinics, during January to April of 2014 under supervision of professional ophthalmologists and approval of ethical committee in the College of Pharmacy, Al-Mustansiriyah University, after taking an oral consent from the parents of the participated children.

The primary goal of this study involved a comparison between tropicamide and atropine in cycloplegic effect, onset and duration of action, and their safety. The influence of age, sex, and iris color on the completeness of cycloplegia was considered as a secondary goal.

Because of the expected atropine pharmacokinetic profiles and long half-life, its onset and duration of action were detected by an accurate follow up for mydriatic and cycloplegic effects with the traditional slit lamp by giving a restricted appointment of the enrolled children for ophthalmic investigation depending on the doctor instructions.

The selection of the sample was based on the inclusion criteria which involved children below ten years, with approximate male/female ratio, suffering from hypermetropia with or without strabismus, and having different iris colors. Children excluded from this study involve those with a known cardiovascular disease, ophthalmic disease other than refractive error and/or strabismus, history of allergy to atropine or tropicamide, and inability to comply with the treatment regimen. All children underwent a routine ophthalmic evaluation.

Each child was given two regimens of eye drops, regimen 1 (tropicamide as mydriacyl® / Alcon Lab.), and then after one week, regimen 2 (atropine as isopto® atropine/ Alcon Lab.). Tropicamide 1% eye drops instilled at the hospital or eye clinic in the conjunctival sac two times at intervals of 5 minutes on the day of examination; refraction was performed 30 min after the last drop by mean of retinoscopy. Atropine 1% eye drop instilled at the home twice daily for 3 days prior to and on the day of examination. Post-instillation of drops, the lacrimal puncti was closed by pressure on the medial canthus for 3 minutes, to minimize systemic absorption. Parents were informed about the signs of local and systemic toxicity due to the drugs used and they were instructed to return the children to the clinic if adverse events were noted.

The results of refractive error were expressed as mean ± SD. Student t-test and chi- square test were used for statistical analysis, applying the Microsoft Office Excel Program-2007. A *P*-value of less than 0.05 was considered statistically significant.

Results

A total of 30 children (60 eyes) with age mean of (5.47 ± 1.44 years), range (2-9 years), were enrolled prospectively. Whole refractions were recorded after cycloplegia with tropicamide 1% (regimen 1) and atropine 1% (regimen 2) (Table 1). The mean of tropicamide refraction values (+3.60 ± 2.25 D) did not differ significantly in comparison with that of atropine (+3.92 ± 2.50 D); ($P > 0.05$) (Table 2).

Table 1. Cycloplegic refractions and their difference values

Pt. No.	Tropicamide drops Rt/Lt eye	Atropine drops Rt/Lt eye	Difference Values	Age (years)
1	+2.5/+2.5	+2.5/+2.5	0.0/0.0	2
2	+4.75/+4.75	+5.25/+5.25	0.5/0.5	4.5
3	+6.0/+6.0	+7.0/+7.0	1.0/1.0	3
4	+3.25/+3.25	+4.0/+4.0	0.75/0.75	7
5	+8.0/+8.5	+8.5/+8.5	0.5/0.0	5
6	+1.5/+1.5	+1.5/+1.5	0.0/0.0	9
7	+4.0/+4.0	+4.0/+4.0	0.0/0.0	6
8	+2.75/+2.75	+3.0/+3.0	0.25/0.25	5.5
9	+2.5/+2.5	+2.5/+2.5	0.0/0.0	5
10	+4.25/+5.0	+4.50/+5.50	0.25/0.50	9
11	+1.0/+1.0	+1.0/+1.0	0.0/0.0	5
12	+3.0/+3.0	+3.5/+3.5	0.5/0.5	5
13	+2.75/+2.50	+3.0/+3.0	0.25/0.50	8
14	+4.0/+4.5	+4.5/+5.0	0.5/0.5	6
15	+4.5/+4.5	+5.0/+5.0	0.5/0.5	6
16	+3.0/+3.0	+3.25/+3.25	0.25/0.25	5
17	+3.0/+3.25	+3.0/+3.50	0.0/0.25	7.5
18	+3.25/+3.25	+3.25/+3.25	0.0/0.0	5
19	+3.75/+3.75	+4.0/+4.0	0.25/0.25	8
20	+3.0/+3.0	+3.5/+3.5	0.5/0.5	2.5
21	+1.0/+2.0	+1.0/+2.0	0.0/0.0	5.5
22	+7.0/+7.0	+8.0/+8.0	1.0/1.0	7
23	+3.5/+3.5	+4.0/+4.0	0.5/0.5	3
24	+3.25/+3.25	+3.5/+3.5	0.25/0.25	4
25	+3.75/+4.25	+4.0/+4.5	0.25/0.25	8.5
26	+5.0/+5.0	+6.0/+6.0	1.0/1.0	4
27	+3.5/+3.5	+4.0/+4.0	0.5/0.5	6.5
28	+4.5/+4.5	+5.0/+5.0	0.5/0.5	4
29	+2.5/+2.5	+2.5/+2.5	0.0/0.0	3.5
30	+6.0/+6.0	+6.0/+6.0	0.0/0.0	9

Data represented by Diopters (D).

Children were divided into two groups according to their age, either ≤ or > 6 years. In younger children, the cycloplegic refraction means after atropine was (+4.15 ± 2.88 D) followed by tropicamide (+3.77 ± 2.68 D); (P > 0.05). In older children, the cycloplegic refraction means after atropine was (+3.70 ± 1.87 D) followed by tropicamide (+3.37 ± 1.56 D); (P > 0.05).

In hypermetropic-strabismic children, the mean cycloplegic refraction after atropine was (+4.0 ± 2.25 D) followed by tropicamide (+3.70 ± 2.87 D); (P > 0.05). In hypermetropic children without strabismus, the mean cycloplegic refraction after atropine was (+3.82 ± 1.97 D) followed by tropicamide (+3.45 ± 1.87 D); (P > 0.05).

Regarding gender, no significant association was found between the sex and refractive error values. The effect of iris color on accommodation after instillation of cycloplegic drops was evaluated by dividing the five known iris categories into two groups: a light-iris group and a dark-iris group. Categories 1, 2,

and 3 were combined as the light-iris group and consisted of irises that were blue, gray, green, or light brown, with or without brown or yellow pigmentation. Categories 4 and 5 were brown or dark brown with minimal yellow pigmentation and were termed the dark-iris group. In this study, no association was seen between residual accommodation and iris color.

The onset of mydriatic and cycloplegic effects for tropicamide was faster than that of atropine, while the duration of mydriatic and cycloplegic effects for atropine was longer than for tropicamide (Table 3).

Adverse effects of these cycloplegic agents were summarized in table (4). Children using regimen 2 (atropine drops) suffered from more frequent and statistically significant side effects ($P < 0.05$), represented by blurred vision, fever; flushing and tachycardia, compared with regimen 1 (tropicamide drops).

Table 2. Means of refractive error for each subgroup

Stratification	No.	Tropicamide drops	Atropine drops	*P* value
All participants	30	+3.60 ± 2.25	+3.92 ± 2.50	0.071
Children ≤ 6 years	20	+3.77 ± 2.68	+4.15 ± 2.88	0.096
Children > 6 years	10	+3.37 ± 1.56	+3.70 ± 1.87	0.085
Hypermetropic with strabismus	18	+3.70 ± 2.87	+4.0 ± 2.25	0.073
Hypermetropic without strabismus	12	+3.45 ± 1.87	+3.82 ± 1.97	0.094

Data represented by mean ± SD of refractive errors (Diopter).
P value > 0.05 was considered statistically non-significant.

Table 3. Onset and duration of action for the studied cycloplegic drugs

	Onset of action		*Duration of action*	
Drug	**Mydriasis**	**Cycloplegia**	**Mydriasis**	**Cycloplegia**
Tropicamide	15-30 min	25-30 min	4-6 hr	5-6 hr
Atropine	30-40 min	1-1.5 day	7-10 day	12-14 day

Discussion

The ideal cycloplegic agent should produce complete cycloplegia with minimal complications or morbidity and allow rapid recovery of accommodation. For children who are at the critical age of visual maturation and have higher amplitudes of accommodation acting as an obstacle against accurate refraction, full cycloplegia is a basic procedure in the diagnosis and treatment of those patients [21].

With atropine, a prolonged cycloplegia during a sensitive period might, in some cases, potentiate stimulus deprivation and contribute to amblyopia. Moreover, the parents often have difficulties in applying the drops in a correct manner and doubts often arise whether a full cycloplegic effect has been achieved [22].

Table 4. Adverse effects for the studied cycloplegic drugs

Adverse effects	Tropicamide drops (1%)	Atropine drops (1%)	*P* value
Stinging	2 (6.6)	3 (10)	0.087
Blurred vision	1 (3.3)	4 (13.3)	0.046
photophobia	3 (10)	2 (6.6)	0.079
Fever	0 (0.0)	3 (10)	0.041
Dryness of skin	0 (0.0)	1 (3.3)	0.092
Flushing	1 (3.3)	5 (16.7)	0.026
Headache	2 (6.6)	2 (6.6)	0.065
Tachycardia	0 (0.0)	3 (10)	0.037

Data represented by numbers (%).
P value < 0.05 was considered statistically significant.

Many ophthalmologists believe that other cycloplegic agents like cyclopentolate, tropicamide, and homatropine alone are not enough in children 2 to 5 years old, especially in esotropic children with hyperopia greater than 2.0 diopters who must be repeatedly refracted with atropine to detect latent hyperopia [(23)]. Others have demonstrated that the cycloplegic effect of these agents is comparable to atropine [(24)].

So many authors consider atropine as the drug of choice for complete cycloplegia and believe that other mydriatic agents cannot be an appropriate substitute. However, due to its complications, the difficult regimen, and prolonged impairment of near vision, atropine gradually may replace by other cycloplegic agents which have fewer complications, easier to administer, and has a shorter duration of action [(25)]. Comparisons of combinations of these drugs have also failed to detect the ideal regimen. Possible causes for the variable results could be the differences in drug combinations, therapeutic regimens, and patient populations [(26)].

In the present study, the mean of tropicamide refraction values (+3.60 ± 2.25 D) didn't differ significantly in comparison with that of atropine (+3.92 ± 2.50 D); ($P > 0.05$). The two drugs in this study appear remarkably similar; even in children with a high degree of hypermetropia. Therefore, it may possible to replace atropinization at home with the instillation of tropicamide in the hospital or eye clinic.

For drugs like tropicamide and cyclopentolate, a precaution should be taken as they tend to be less cycloplegic than atropine in young children with high hypermetropia. However, atropine should be reserved for children (less than 6 years) with a large amount of accommodative esotropia and those with a history of tropicamide allergy [(27)]. Observations of this study revealed that 1% eye drops of tropicamide, 5 minutes apart, provide considerable cycloplegia sufficient for refraction in most children.

Certain study was achieved on esotropic children younger than 5 years with atropine versus other cycloplegic agents, and showed that atropine was probably unnecessary [(28)]. In contrast, another study found that atropine uncovered (0.3-0.4) diopter more hyperopia in children younger than 6 years old [(27)]. For cycloplegic refraction, an allowance (or under correction) has to be made for abolished ciliary tone, this tonus allowance is taken as 1.0 diopter in the case of atropine. Some researchers recommended an equal tonus allowance for both atropine and any other cycloplegic; while others suggested that a tonus allowance for any other cycloplegic, with the exception of atropine, is inappropriate [(29)]. In children with a high degree of hypermetropia, cycloplegic drugs other than atropine did not produce a complete

cycloplegia, where 22% of the children had an additional hyperopia of (+1.0) diopter or more which was uncovered by atropine [30].

In this study and for younger children, the cycloplegic refraction mean after atropine (+4.15 ± 2.88 D) didn't differ statistically from that of tropicamide (+3.77 ± 2.68 D); ($P > 0.05$). Considering older children, the cycloplegic refraction mean after atropine (+3.70 ± 1.87 D) also didn't differ statistically from that of tropicamide (+3.37 ± 1.56 D); ($P > 0.05$). There were only four eyes, all of them younger than 6 years of age, which had 1.0 diopter hyperopia uncovered by atropine but not by tropicamide.

The importance of atropine cycloplegia in the evaluation of strabismic children has been mentioned in earlier reports [4]. The current study showed that, for children with or without strabismus, cycloplegic refraction values after atropine and tropicamide was comparable (+4.0 ± 2.25 D and +3.82 ± 1.97 D vs. +3.70 ± 2.87 D and +3.45 ± 1.87 D, respectively). Thus, tropicamide might be considered as the choice for cycloplegic refraction for children with or without strabismus.

Although investigators have reported an association between residual accommodation and iris color, which is related to the ethnicity, no such association was found in this study. A statistically significant association between residual accommodation and ethnicity was found in the previous studies, where a significant difference in residual accommodation between the white and Hispanic populations was reported [25].

In spite that atropine provides adequate cycloplegia, the adverse effects and persistent duration of action have encourage a search for alternate cycloplegic agents. Patients should be caution about the transient acute psychosis, which may occasionally occur even with few drops of atropine. Other neurological toxicities, including seizure and delirium, can also occur with atropine [31]. The incidence of adverse events for diagnostic ocular agents was reported to be less than 1%, where the researchers included a wide range of dilating eye drops, from mild to strong agents. However, significant side effects have also been reported, including tachycardia, tremor, and mental confusion [32].

In the present study, children who received tropicamide (regimen 1) did not show significant side effects when compared with that of atropine (regimen 2), where 13.3 %, 10%, 16.7 %, and 10% of children during atropine drops suffering from blurred vision, fever, flushing, and tachycardia, respectively. The small sample size used may effect on the incidence of these anticholinergic problems.

To achieve complete cycloplegia and to avoid the complications and morbidity of atropine, different combinations and concentrations of cycloplegic agents have been prepared. The possible reason for using a reduced concentration of atropine in younger children was to reduce the incidence of atropine toxicity in those patients. This appeared paradoxical because younger children have a greater accommodative response [33].

From the pharmacokinetic point of view, and as an advantage over atropine, tropicamide instillation has a rapid onset, short duration of action, and can be given at the time of examination in the eye clinic or hospital. The maximum cycloplegic effect is attained after 30 minutes and remains stable for more than 120 minutes [34]. As disadvantages with atropinization at home, compliance is often unsatisfactory, the drug effects are long acting, and it is often difficult to decide whether a complete cycloplegic effect has been achieved [35]. Results of the current study were consistent with this fact, where tropicamide was faster in onset, while atropine was longer in its duration of action.

From above, and due to its complications, difficult regimen, and prolonged impairment of near vision, atropine should be gradually replaced by other cycloplegic agents which have fewer complications, easier to administer, and has a shorter duration of action.

In conclusion, tropicamide applied to younger or older children is sufficient to produce good

cycloplegia, with an effect approach to and safer than atropine, even in children with a high degree of hypermetropia, and with or without strabismus. In addition, the residual accommodation was not associated with age, gender, and iris color, suggesting that these factors may be of no concern when using tropicamide or atropine as a cycloplegic agent in those children.

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Conflict of interest

The author declares no conflict of interest.

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Analysis of Single Nucleotide Polymorphism rs9939609 in FTO Gene of Obese Males in Iraqi Population

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Abstract

Background Obesity is a serious on public health. It contributes in many serious health conditions including high cholesterol, type 2 diabetes, osteoarthritis, high blood pressure, gallbladder disease, coronary heart disease, stroke, respiratory problems … etc. Polymorphism of TA (rs9939609) in fat mass and obesity associated (FTO) gene was found to be associated with obesity in children and adults according to many studies conducted on populations from Europe, America and Asia. Whether Single nucleotide polymorphism (SNP) rs9939609 is an associated with obesity in Iraqi population remains of concern.

Objective To investigate the polymorphism of rs9939609 SNP in FTO gene and its relationship to the obese males in Iraqi population.

Methods One hundred twenty of males were classified as obese based on body mass index (BMI) with mean age 20-50 year and fifty aged-matched healthy males as a control were included in this study. Lipid profile was estimated by using Spinreact-Cє, and an ELISA kit was used to assess the FTO level.

Results The results showed that there are significant differences $P \leq 0.05$ for AA genotype with all parameters whereas TA genotype showed significant differences with most of parameters in revers to TT genotype, which has showed no significant differences with most of parameters. The percentage of TT, TA, AA, alleles were 27.72%, 49.86%, 22.42% respectively, also an elevated of TT genotype frequency was observed in healthy compared to obese. On the other hand, the percentage of T and A allele frequency were 52.65% and 47.35% respectively. Also, an elevated in serum FTO enzyme level was observed in obese.

Conclusion The presence of A risk allele in the Iraqi population is the cause in the incidence of obesity, which reflected its impact on the BMI and central obesity through the disturbances in lipid profile and FTO enzymes value.

Keywords Single Nucleotide Polymorphism, rs9939609, Obesity, FTO gene.

List of abbreviation: FTO = Fat mass and obesity associated gene, SNP = Single nucleotide polymorphism, BMI= Body mass index, T2D = Type 2 Diabetes, CO= central obesity

Introduction

Obesity can be defined as the biological case that resulted from accumulation of excessive fat or these fats, which little used as a source of bio-energy due to the weakness of human activity or tendency of

individual to avoid movement, work or environmental interaction [1,2]. rs9939609 is a Single Nucleotide Polymorphism (SNP), which is located in the first intron of Fat mass and obesity associated (FTO) gene. The AA-rs9939609 genotype is the most serious one [3]. Ten different SNPs were identified in the first intron of FTO gene and they have related to body mass index (BMI) and Type 2 Diabetes (T2D) [4]. It was found that the increasing in BMI is associated with rs9939609 (A) carriers in patients with Type 1 Diabetes (T1D) but not those with diabetic nephropathy [5]. Also, Kilpeläinen et al [6] showed that the polymorphism of rs9939609 was related to T2D in east and south Asia similar to that observed in Europe. The studies by Luczynski et al [7] and Liu et al [8] revealed that the polymorphism of this SNP associated with obesity and cardiovascular risk factors. Variant in the FTO gene is predisposes to cause the T2D through an effect on BMI. Furthermore, 16% of the adults with homozygous for the risk allele had 3 kilograms more and 1.67 fold increased likelihood of obesity as a compared with those not having a risk allele. This correlation was noticed from the age of 7 years onwards and reflects an effect on increasing of fat mass [9]. The polymorphism of rs9939609 resulted in an overexpression of FTO gene in Pakistani obese female [10]. Many studies showed that the risk allele A of rs9939609 in FTO gene was associated with increase of the BMI and incidence of obesity [11-13]. The presence of both risk alleles A and C from rs9939609 in FTO gene and rs17782313 in MC4R gene respectively caused 4-fold increase of obesity risk in childhood and adolescents from Greece origin [14]. Luczyoski et al [15] showed that the main factors that caused an increase of BMI in children with Type 1 Diabetes, especially in female gender, are poor metabolic control and carrying the A allele of the rs9939609. Patients with co-infected of human immunodeficiency virus plus Hepatitis C virus (HIV/ HCV) who carry AT/AA genotype of rs9939609 had higher quota of metabolic disturbances and lower probability of response for successful virologic Hepatitis C virus therapy [16]. It was found that there is a strong correlation between AA genotype of rs9939609 FTO gene and BMI increasing in deep venous thrombosis patients with or without pulmonary embolism [17]. Feng et al [18] observed a significant association in Chinese individuals carrying AA genotype of rs9939609 and increase tuberculosis risk as a compared with genotype TT individuals. In addition, an inverse association was noticed between completed suicide and the rs9939609 A allele which is independent to the correlation between the obesity related A allele of rs9939609 and alcohol addiction [19]. Certain Polymorphisms of FTO gene have an effect on regulation of genetic expression related to the susceptibility for cancer [20].

Methods

One hundred seventy Blood samples (one sample included 4 ml of blood for biochemical analysis and 4 ml of blood for genetic analysis) were collected from men who their ages range from 20-50 years, 120 of them are obese who were classified based on BMI into three groups. Obesity group I (BMI= 30-34.9), obesity group II (BMI= 35-39.9) and obesity group III (BMI= 40 onwards). Other men (50) are apparently healthy and they were grouped as a control (BMI= 18-24.9).

Body measurements

BMI was calculated by dividing weight by height square (Kilogram/meter^2), while the central obesity (CO) represented by the ratio of the waist circumference to the Hip circumference.

DNA extraction

The DNA was extracted from blood samples by using DNA isolation kit and according to the procedure provided by manufacturer (Geneaid).

Genotyping of FTO SNP rs9939609

Polymerase chain reaction (PCR)-touch time technique was used to amplify the FTO SNP rs9939609. The forward (5-AACTGGCTCTTGAATGAAATAGGATTCAGA -3) and revers (5-AGAGTAACAGAGACTATCCAAGTGCAGTAC) primers were used, the primers were provided by manufacturer (Integrated DNA Technologies, USA). The PCR cycling condition was shown in table (1). For genotyping rs9939609 SNP, Restriction fragment length polymorphism (RFLP) technique by using Sac I restriction enzyme (Biolab, New England) was conducted. The enzyme cuts in sites showed in figure (1), the T allele of the FTO SNP rs9939609 has no site for Sac I whereas the A allele has one site for the enzyme. Therefore, one and two fragments will resulted from treatment of T and A alleles by Sac I respectively. Ten µl of reaction mixture contained 5.5 µl free nuclease water, 1 µl NE Buffer 1.1, 1 µl Sac I and 2.5 µl (0.3 µg) of amplified FTO SNP rs9939609. The reaction mixture was then incubated in water bath at 37°C for 1 hour. After that, the polyacrylamide gel (12%) was run for the product of the cleavage mixtures.

Table 1. PCR cycling condition

Cycle step	Temperature	Time	Cycle No.
Initial denaturation	94 °C	5 min	1
Denaturation	94 °C	45 sec	20
Annealing	61 °C (-0.5 °C per Cycle)	45 sec	
Extension	72 °C	45 sec	
The PCR mixture was then applied for cycles below			
Cycle step	Temp.	Time	Cycle No.
Denaturation	94 °C	45 sec	15
Annealing	51 °C	45 sec	
Extension	72 °C	45 sec	
Final extension	72 °C	10 min	1

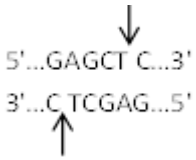


Fig. 1. Restriction sites of Sac I enzyme Parameters estimate

Lipid profile including triglyceride (TG), total cholesterol (TC), high density lipoprotein-Cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were estimated by using kits from Spinreact-Ce. Human Alpha-ketoglutarate-dependent dioxygenase ELISA kit (Cusabio, China) was used to assessment FTO levels.

Statistics Analysis

The statistical analysis Chi-Square (X2 test) was used to assess the significant differences ($P \leq 0.001$) between different factors in parameters.

Results

The present study shows that the CO was increased by increasing in BMI (figure 2 and 3). On the other hand, figure (4) showed the polyacrylamide gel resulted from digestion of

the FTO SNP rs9939609 by Sac I. The effect of the FTO SNP rs9939609 on other parameters included in this study is demonstrated in table (2).

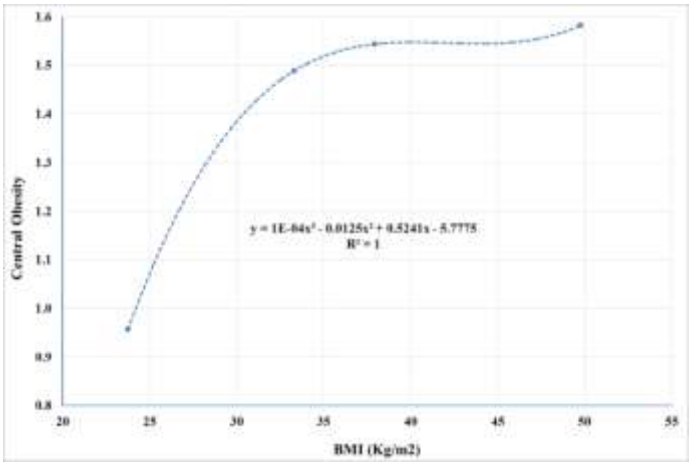
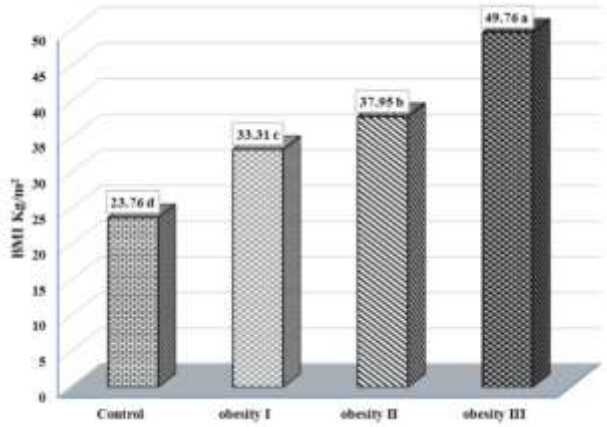
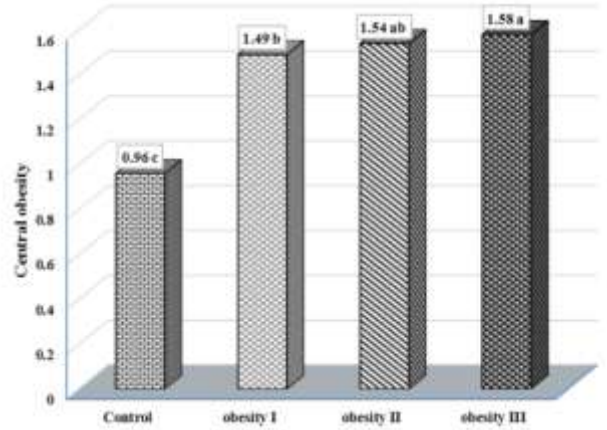


Fig. 2. Relationship between BMI and central obesity



(A)



(B)

Fig. 3. BMI (A) and CO (B) among obesity and control groups

Fig. 4. Analysis of the digested FTO SNP rs9939609 by Sac I on 12 % polyacrylamide. The gel was run for approximately 60 minutes at 80 volts. M: 50 bp DNA ladder; Lanes 1-3: TT (Homozygous-Wild Type); Lanes 4-6: TA (Heterozygous); Lanes 7-9: AA (Heterozygous).

Table 2. The genetic effects of the FTO SNP rs9939609 on investigated parameters

Parameter	TT mean±SD	TA mean±SD	AA mean±SD
BMI Kg/m^2	24.43±1.62 c	35.64±1.57 b	41.54±1.49 a
CO. cm	0.98±0.12 b	1.11±0.18 b	1.55±0.07 a
TG mg/dl	97.96±1.69 c	204.26±0.98 b	236.50±1.40 a
TC mg/dl	147.52±1.78 c	212.09±1.67 b	234.07±1.77 a
HDL mg/dl	51.29±1.01 b	45.03±1.14 b	33.97±1.08 a
LDL mg/dl	69.04±1.44 c	129.68±1.17 b	147.38±1.29 a
VLDL mg/dl	18.94±1.69 c	39.71±1.49 b	45.21±1.41 a
enzyme *FTO*	186.4±1.7 c	333.1±1.9 b	390.7±1.55 a

a, b and c are letters used to indicate the significant differences at P ≤ 0.05. Different letters mean a statistical significant difference between the 3 different groups for the same parameter. Same letters indicate there is no statistical significant different within the same group for different parameters.

Discussion

Individuals with normal homozygous (TT) revealed one band of 182 bp, while the individuals with heterozygous (TA) and homozygous (AA) revealed three (182, 154 and 28 bp) and two (154 and 28 bp) bands respectively (fig. 4). The results in table (2) showed no significant differences between the genotype TT and the majority of parameters. By contrast, the genotype TA showed significant differences ($P \leq 0.05$) with the majority of parameters while the genotype AA has significant differences with all parameters which suggest that the rs9939609 A allele is strongly associated with obesity or in other words, most of the obese are located under the genotype AA. Similarly for genotypes frequency, the results revealed that there are significant differences ($P \leq 0.05$) among the three genotypes, 27.72, 49.86 and 22.42 % for

TT, TA and AA respectively. Also, it has been found that the frequency of the genotype TT was elevated in the control as a compared to obesity groups. The frequency of T and A alleles were 52.65 and 47.35 % respectively. Furthermore, it can be noticed from the table (2) that the FTO level increased in genotypes TA and AA suggesting that the risk A allele has an effect on FTO expression causing an overexpression which reflected on the incidence of obesity. This result is coordinated with a study by Berulava and Horsthemke [21] who observed that the A- rs9939609 allele is associated with an overexpression of FTO gene. The current study was also agreed with a study by Chang et al. who showed that the rs9939609 A allele was strongly associated with obesity and BMI in the Chinese population whereas the allele was most common in the European than in Chinese populations [22]. Li et al also showed that the FTO-rs9939609 allele increased BMI by 0.26 kg/m^2 allele, Waist to Hip Ratio (WHR) by 0.003/allele, body fat percentage by 0.31%/ allele and T2D by 1.15 fold/ allele [6]. Likewise, other studies have pointed to the association of rs9939609 polymorphism with fat cell lipolysis, obesity, inflammation and cardiovascular diseases [3,7,23]. Yang et al revealed that the BMI, waist and hip circumference, systolic and diastolic pressure, TG and LDL-C were higher, whereas the HDL-C was lower in Chinese children and adolescents with a TA or AA genotype compared to those with TT genotype [13].

The current study has concluded that the obesity in men of Iraqi population were associated with the presence of A risk allele of the rs9939609 *FTO* gene that resulted in a turbulence in lipid profile and FTO enzyme value.

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Author Contribution

Authors (1) and (2) made substantial contributions to conception and design, and/or acquisition of data, and/or analysis and interpretation of data.

Authors (3), (4) and (5) participated in drafting the article or revising it critically for important intellectual content. Also, they give final approval of the version to be submitted and any revised version.

Conflict of interest

The authors declare no conflict of interest.

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Genetic Polymorphism of the Glutathione S-transferase M1 and T1 Genes in Baghdad Population

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Abstract

Background Glutathione S-transferases (GSTs) are enzymes of Phase II, which play an important role in cellular detoxification by conjugation of reduced glutathione with a wide variety of potentially toxic and carcinogenic. Polymorphisms of genes coding for the glutathione S-transferase (GST) enzymes were known to be associated with susceptibility to various forms of cancer, or outcome of multichemotherapeutic regimens.

Objective To analyze of the frequencies of the major polymorphisms of the GSTM1 and GSTT1 in Baghdad population to provide a basic database for future genetic and clinical studies.

Methods Peripheral blood was obtained from 142 healthy individuals randomly selected from two districts in Baghdad. The age of studied participants ranges from 15-46 yeares at time of inclusion. Genomic DNA was isolated from leukocytes using Wizerd genomic DNA purification kit. The GSTT1 and GSTM1 gene polymorphism were evaluated using multiplex polymerease chain reaction in which albumen gene was used as internal controls.

Results Twenty nine individuals (20.4%) revealed no amplification of the GSTT1 gene (null type). The null GSTM1 genotype was found in 66.2% (94 individuals). Twelve percent (17/142) had null genotype for both genes. The most frequently observed genotype was GSTT1 positive/GSTM1 null genotype (54.2%) while the GSTT1 null/GSTM1 positive genotype observed in 12 individuals (8.5%). GSTT1postive/GSTM1 positive genotype was found in 36 (25.4%) of the sample study.

Conclusion Data of the present study showed that the frequency of GSTT1 and GSTM1null genotype of the sample study is in concordance with those documented for Caucasian, Asians, and Arabs population. This study provides information for frequency distribution of GSTT1and GSTM1 null genotypes in the Baghdad population and information about genetic difference between Iraqi individuals and other population that provide basis for future genetics and clinical studies.

Keywords glutathione S-transferase, polymorphisms

List of abbreviation: GSTs = glutathione S-transferases, PCR = polymerase chain reaction

Introduction

Glutathione S-transferases (GSTs) represent a major superfamily of phase II drug-metabolizing enzymes that catalyze the conjugation of a large variety of endogenous and exogenous compounds, including environmental carcinogens and anticancer drugs and their metabolites with reduced glutathione [(1)] and thereby protect cellular macrolecules against toxic foreign chemicals and oxidative stress

Moreover, GSTs are involved in the metabolism of isothiocyanates naturally occurring molecules that were recently shown to inhibit the development of tumors in many

experimental models [2]. The GSTs include three major families of proteins: Cytosolic; mitochondrial and microsomal; the cytosolic GSTs constitute the largest family (1). In mammalian eight classes of cytosolic GSTs have been recognized: alpha (GSTA), mu (GSTM) theta (GSTT), pi (GSTP), zeta (GSTZ), sigma (GSTS), kappa (GSTK) and omega class (GSTO) [3,4].

Among the numerous GST genes, GSTM1 and GSTT1 genes have been extensively studied because of the high prevalence of homozygous deletions of these genes, which have been associated with the loss of enzyme activity with a decreased ability to detoxify and increase vulnerability to cytogenetic damage, placing null individuals at increase cancer risk [5-7].

A mu class genes of GST family involve three alleles, two of them are active [GSTM1*A, GSTM1*B] and the third is a null allele [GSTM1*0]. GSTM1*0 allele is a deletion. Null phenotype (express no protein) produces by homozygotes for this allele (GSTM1 null genotype). GSTM1*A and GSTM1*B that differ by C to G substitution at base position 534, and the catalytic effectiveness of the enzymes encoded by these alleles is similar [8]. GSTT1 is represented by two alleles: a functional or wild allele (GSTT1*1), and a nonfunctional or null allele (GSTT1*0). Studies have shown that the total or partial deletion of the gene (GSTT1*0 allele) causing a deficiency in its enzymatic activity [9].

The frequency of different polymorphic variants' varies with the ethnicity of a population; a number of studies from many countries on different geographic and racial groups have shown varied results on the incidence of null genotype frequency [10,11]. The frequencies of homozygous deletions of GSTT1 gene are higher in Asians and Africans than in Caucasians], whereas homozygous deletions of GSTM1 gene are higher in Caucasians and Asians than in Africans [12,13].

Moreover, numerous studies have evaluated the association between polymorphisms of GSTs gene and the susceptibility to develop various types of cancer, such as gastric cancer, oral cancer, bladder cancer, and chronic myeloid leukemia CML in different ethnical groups worldwide [14-17]. Furthermore, other studies have found an association between GSTM1 and GSTT1 deletions and disease susceptibility, drug response and resistance to chemotherapy treatment [18-20].

This study was done to analyze the frequencies of the major polymorphisms of GSTM1 and GSTT1 in a Baghdad population to provide basic information for future genetic and clinical studies.

Methods

The GSTT1 and GSTM1 gene polymorphism were evaluated using multiplex polymerase chain reaction (PCR) in which, albumen gene was used as internal controls. The PCR was carried out in mixture containing 5 µl of genomic DNA, 25 µl of Go Taq green Master Mix (1X) and 2 µl of each primer (20 PMol for each one) completed to 50 µl with molecular grade water.

The sequences for the forward and reverse primers were 5-GAA CTC CCT GAA AAG CTA AAG C-3 and 5-GTT GGG CTC AAA TAT ACG GTG G-3 respectively for GSTM1. For the GSTT1, the primer sequences were F-5-TTC CTT ACT GGT CCT CAC ATC TC-3 and R-5-TCA CCG GAT CAT GGC CAG CA-3 respectively for GSTT1. Forward and reverse primers for albumin gene were 5-GCC CTC TGC TAA CAA GTC CTA C-3 and 5-GCC CTA AAA AGA AAA TCG CCA ATC-3 respectively [21].

PCR amplification was performed with an initial denaturation at 94 ˚C for 3 minutes, followed by 35 cycles at 95 °C for 1 minute, 59 °C for 1 minute, 72 °C for 1 minute and a last step was extension at 72°C for 5 minutes. PCR products were analyzed on a 2% agarose gel stained with 0.5 µg/mL ethidium bromide. The GSTT1, GSTM1 and albumin produce 480 bp, 215 bp and 350 bp respectively. The presence of Albumin without GSTT1 or GSTM1 band on agarose gel reflects their deletion [21].

Results

Genotyping analysis

Genomic DNA was isolated from blood cells; all samples yielded intact genomic DNA. The GST genes (GSTT1, GSTM1) were successfully amplified in 142 samples (70 females and 72 males).

Gel electrophoresis of the amplified products revealed the presence of bands of GSTM1 gene at level of 215bp and GSTT1 gene at the level of 480 pb bands. Null genotypes show absence of PCR product. In each reaction, co-amplification of the albumin gene revealed a band with a product size of350bp (Figure 1).

The presence or absence of GSTM1 and GSTT1 genes was detected by the presence of a band at 215 bp (corresponding to GSTM1) and a band at 480 bp (corresponding to GSTT1). A band at 350 bp (Corresponding to albumine gene) was always present and was used as an internal control to document successful PCR amplification. Lanes 1, a negative control. Lane 2,8 individuals with null alleles for both GSTM1 and GSTT1 genes showing only one band at 350 bp corresponding to the internal control (albumine gene fragment). Lanes 3,4,5,9,10 individuals with GSTT1 present (480 bp) and GSTM1 null alleles. Lanes 6 and 7: individuals harboring GSTT1 null and GSTM1 present (215 bp) alleles. Lanes 11, 12 individuals with both GSTT1 and GSTM1 alleles present. M is a DNA molecular marker (100bp).

Figure (2) summarize the frequency distribution of GSTT1 gene in the study sample. Postive amplification of GSTT1 was found in 113/142 (79.6%) individuals. Twenty nine individuals (20.4%) revealed no amplification of GSTT1 gene (null type).

The null GSTM1 genotype was found in 66.2% (94 individuals) as shown in (figure 3). The distribution and frequencies of the combined genotypes of GSTT1 andGSTM1We was examined (Figure 4), it revealed that the most frequently observed combinnant was GSTT1 positive /GSTM1 null genotype (54.2%) while the GSTT1 null/GSTM1 postive genotye observed in 12 individuals (8.5%). The GSTT1 positive/GSTM1 positive genotype was found in 25.4% (36) of the sample study and twelve percent (17/142) had null genotype for both genes.

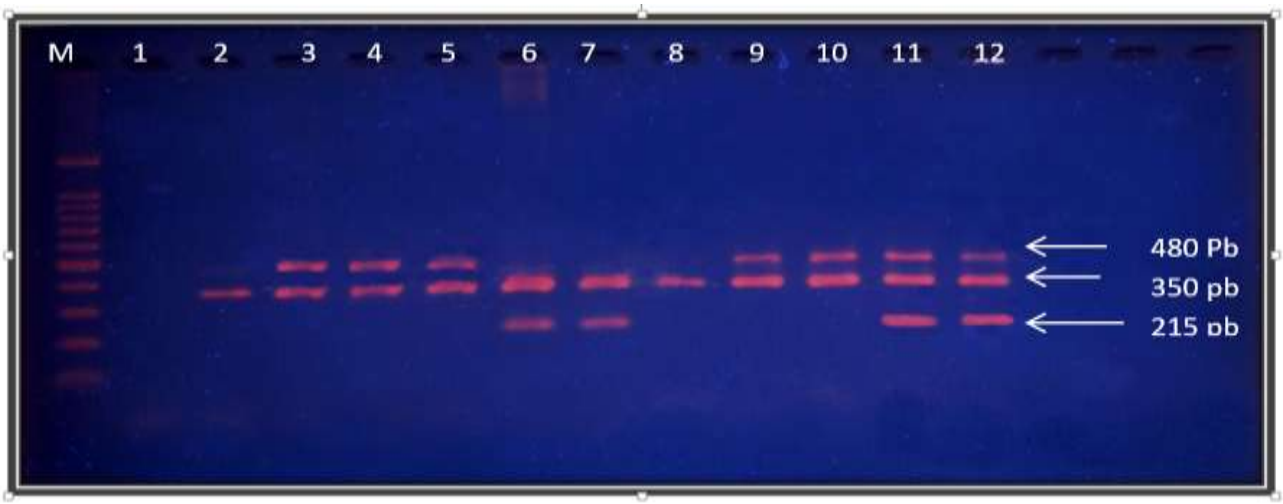

Fig. 1. Multiplex PCR products analyzed on 2% agarose gel. The presence or absence of GSTM1 and GSTT1 genes was detected by the presence of a band at 215 bp (corresponding to GSTM1) and a band at 480 bp (corresponding to GSTT1). A band at 350 bp (Corresponding to albumine gene) was always present and was used as an internal control to document successful PCR amplification. Lanes 1, a negative control. Lane 2,8 individuals with null alleles for both GSTM1 and GSTT1 genes showing only one band at 350 bp corresponding to the internal control (albumine gene fragment). Lanes 3,4,5,9,10 individuals with GSTT1 present (480 bp) and GSTM1 null alleles. Lanes 6 and 7: individuals harboring GSTT1 null and GSTM1 present (215 bp) alleles. Lanes 11, 12 individuals with both GSTT1 and GSTM1 alleles present. M is a DNA molecular marker (100bp).

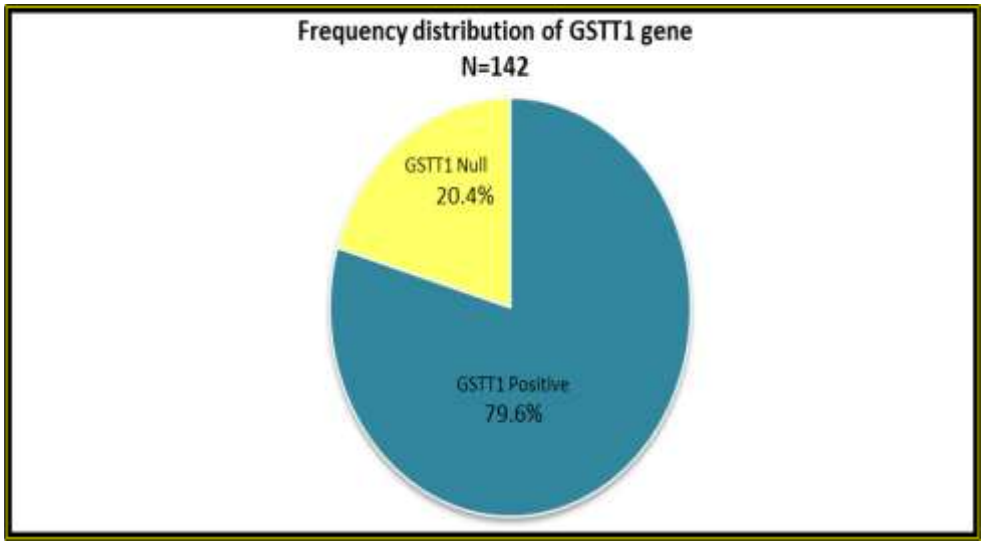

Fig. 2. Frequency distribution of GSTT1 gene in the total sample

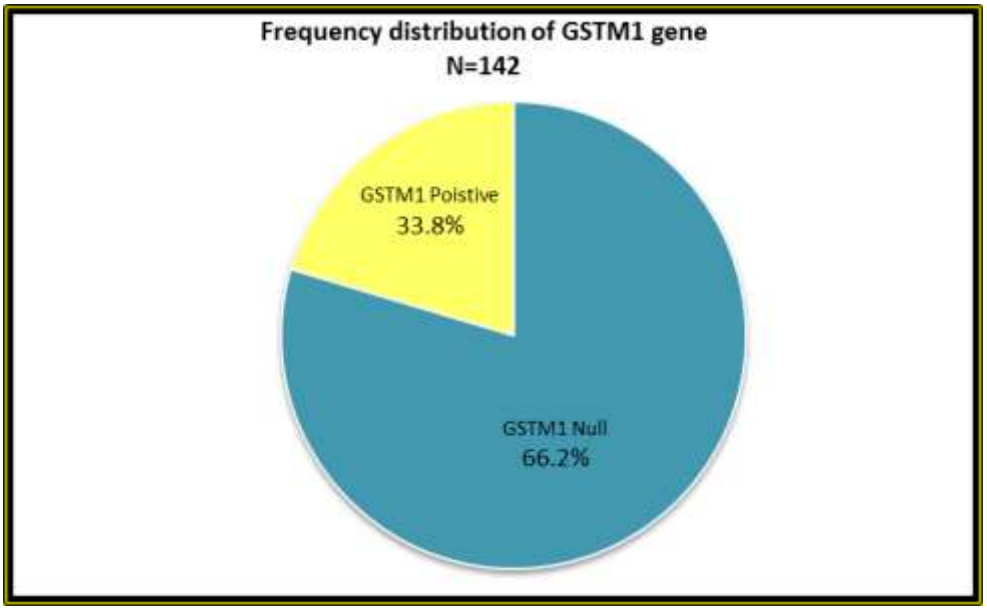

Fig. 3. Frequency distribution of GSTM1 gene in the total sample study

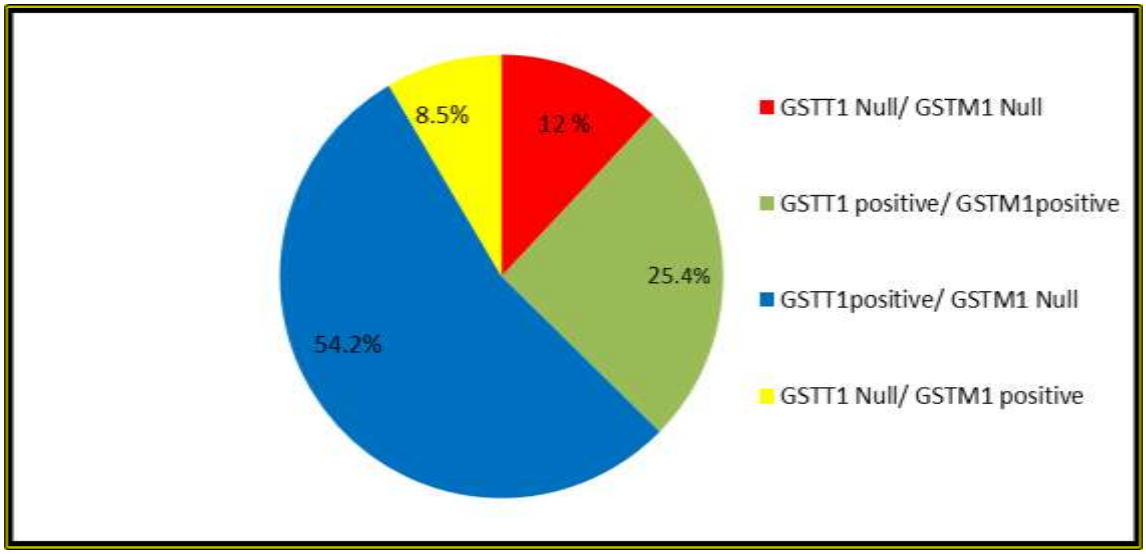

Fig. 4. The frequency distribution of four types of gene combination in the total sample (n=142)

Discussion

The present study revealed that the frequency of GSTT1 null genotype is (20.4%), this frequency is higher than those reported in a previous case control study that was performed by Al-Awadiy et al [22] in the Iraqi population, which was (6.9%). The GSTT1 null genotype frequency was similar to Caucasian (19.7%) and concordant with those documented for Turkish (23%) population and was lower than that reported in Arab (37.1%) and African (26%) population [25,30] (table 1).

The GSTM1 null genotype frequency (66.2%) reported in the current study was comparable to that observed in Asians and Caucasians (40-62%) [23] but higher than that observed in African (26.7%) The proportion of double nulls (GSTM1 and GSTT1 null genotypes), synonymous of absence of enzyme activity in the sample study (12%) was concordant to that previously reported by Al-Awadi et al [22] for the Iraqi population(10%) as well as for other Arab populations, including Bahraini (14.4%); Lebanese (16.3%) population; and it is nearly similar to that of the Caucasians and African population and less than that observed in Arabs (Saudi) population [24,25].

Worldwide variation in GSTT1 and GSTM1 frequency were previously reported. Abdel Halim et al [24] demonstrated that the difference of GTSM1 and GSTT1 alleles' frequency among different population are attributed to their evolutionary histories, and to selection arising from varied exposures to toxic substances.

The (GST) genes are crucially involved in the detoxification of a variety of exogenous carcinogens and mutagens. Individuals with GSTM1 and GSTT1 null genotypes being less efficient at processing carcinogens and radicals oxygen species [7].

The polymorphisms in both GSTT1 and GSTM1 genes were widely studied in relation to genotoxic damage in peripheral blood cells of the general population and of groups occupationally exposed to known or suspected genotoxic materials. Many studies focused on the possible association between the GSTT1 and GSTM1 polymorphisms and various diseases but the results are variables.Several studies have reported a relationship between combination of the GST genotype and risk of various diseases and some of them had suggested a possible synergistic effect between GST genotype [26,27].

Table 1. Comparison between the results of the present study with the frequency of GSTM1 and GSTT1 In the other regions of the world

Population	n	GSTM1 null %	GSTT1 null%	Double null%	Reference
Baghdad	142	66.2	20.4	12	Present study
Bahrainis		49.7	28.7	14.4	
Lebanese	167	52.5	37.6	16.3	24
Tunisians		20.7	16.7	3.97	
	141				
Syria		54.6	25.9	17.2	28
Arabs	172				
(95% Saudi)		63.4	37.1	21	25
	513				
Iranian		46	72	16	29
	235				
Turkish		52	23	NA	30
	50				
Caucasian		53.1	19.7	10.4	25
	486				
African		26.7	26.8	12.6	25

n = number of subjects, NA = data not available

In conclusion, this study provides information for GSTT1 and GSTM1 postive/null genotype in the Baghdad population this data will help the future clinical and genetic studies relating to the differences in the response or toxicity to xenobiotics or drugs known to be substrates of glutathione-S-transferases.

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Author contribution

Research proposal was done by Dr. Nada and Prof. Dr. Ban. While collection of samples, individuals' interview and interpretation of results were done by Dr. Farha.

Conflict of interest

The author declared no conflict of interest for the present research outcome.

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Expression of CD41 (GPIIb) and CD61 (GPIIIa) in Patients with Glanzmann Thrombasthenia Using Flow Cytometry

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Abstract

Background Many genetic mutations causing severe reduction or defect in GPIIb (CD41) and/or GPIIIa (CD61) receptors in Glanzmann Thrombasthenia (G.T.). Flow cytometry can be used for quantification of these two receptors for diagnosis and further identification of G.T. types which are type I, II and III.

Objective To detect the occurrence of CD41 (GPIIb) and CD61 (GPIIIa) in patients diagnosed with Glanzmann thrombasthenia and to classify them accordingly by flow cytometry.

Methods A descriptive cross sectional study was conducted on 28 patients with G.T. collected from the National Center of Hematology and Children Welfare Teaching Hospital over 5 months from Dec. 2014 to Apr. 2015. Those patients were subjected to all hematological investigations required for the diagnosis of G.T. Glanzmann Thrombasthnia Italian Team Protocol (GLATIT) was used to assess the severity of bleeding in those cases at time of collecting samples after taking clinical information either directly with their full agreement or from their files.

Results Majority of cases were below 20 years of age with male to female ratio 1:1. According to the results obtained by flow cytometry. Majority of cases (86%) were classified as type I. A single case (3%) was found to be of type II whereas type III constituted 11% of the total no. of cases. Family history and consanguinity were found in 79% and 93% of the affected families respectively.

Conclusion Type I is the most common followed by type III then type II. Most cases were mild bleeders followed by moderate then severe bleeders. Platelet function test is essential to diagnose the variant form of G.T. (type III) which reveal a normal or near normal expression of both receptors as detected by flow cytometry. More than one type of G.T. had been found in one family with variable bleeding severity suggesting that more than one mutation can occur in the same family.

Keywords CD41, CD61, Glanzmann thrombosthenia.

List of abbreviation: PT = prothrombin time, PTT = partial thrombopastin time, TT= thrombin time G.T. = Glanzmann thrombasthenia, PRP = Platelet rich plasma, CD = Cluster of differentiation, GP = Glycoprotein, GIT = Gastrointestinal tract

Introduction

Glanzmann thrombasthenia (G.T.) is a rare genetic autosomal recessive hemorrhagic disease caused by a defect in platelet function leading to the development of bleeding manifestations as epistaxis, gum bleeding, menorrhagia, petechiae, bruises. It is found mainly in regions where consanguineous marriage is common as in Iraq, Iran, India and among Jews. It affects males and females equally [(1)]. Historically, the disease was first discovered in 1918 by Edward Glanzmann, a Swiss pediatrician, who noticed that one of his cases presented with spontaneous bleeding symptoms with normal platelet count and morphology, prolonged bleeding time, normal

prothrombin time (PT) and partial thrombopastin time (PTT), normal level of coagulation factors [2]. At the molecular level, two important platelet surface glycoprotein are implicated in the disease, GPIIb (CD41) and GPIIIa (CD61). These two glycoproteins form a complex that functions as a receptor for fibrinogen and other molecules included in platelet functions, leading eventually to platelet aggregation and formation of platelet plug [3-5]. Genetically, two genes responsible for the biosynthesis of GPIIb, IIIa complex, both are located on chromosome no. 17, which are ITGB3, ITGA2B. Various types of mutation can affect them leading to the development of G.T. [2,3]. In this disease, where either one or both glycoproteins are absent or have defective function, this will render the other glycoprotein also either absent or defective in its function causing no binding between platelet and fibrinogen, no platelet aggregation, leading to a defect in primary hemostasis and hemorrhagic manifestations will appear [4,6]. Patients with Glanzmann thrombosthenia presents with normal platelet count and morphology, normal PT, PTT, TT, normal level of clotting factors and VWF. Platelet function test by aggregometry is used for diagnosis of those patients where it reveals absent aggregation of platelets in response to all physiological agonists as ADP, epinephrine, collagen except for high dose of ristocetin, which is the characteristic pattern of G.T. [5]. However, flow cytometry is another technique that has an increasing application in this field, the corresponding antibodies (anti CD41 and CD61) is directed by flow cytometry against CD41 (GPIIb) and CD61 (GPIIIa) respectively, so the percentage of these receptors expression can be determined and hence the types and sub types of G.T. can be identified [7,8]. Severe reduction (< 5% for both receptors) in the expression was consistent with type I, moderate reduction (5-20%) goes with type II whereas > 20% expression is regarded as type III [7].

Glnazmann thrombosthenia is divided into three types:[8]

Type I: severe reduction in expression of both receptors.

Type II: weak expression of both receptors.

Type III: normal or near normal expression of both receptors (a qualitative defect).

The objectives of this study was to detect the occurrence of CD41 (GPIIb) and CD61 (GPIIIa) in patients diagnosed with Glanzmann thrombasthenia and to classify them into types accordingly by flow cytometry.

Methods

This descriptive cross sectional study was conducted on 28 patients who attended the National Center of Hematology and Children Welfare Teaching Hospital as highly suspected cases of Glanzmann thrombasthenia; collected over a period of time from December 2014 till April 2015.

Those patients were selected randomly regardless their age and gender (there was no specific age or gender to be included neither excluded from the study; suspected cases of all age groups and both sexes were taken). They presented with clinical and laboratory features of G.T. as mucocutaneous bleeding as epistaxis, bruises, gum bleeding, petechia, gastrointestinal bleeding; either spontaneously or after trauma, family history, normal PT, PTT, TT and platelet count and morphology.

Ethical aspects was taken in consideration while carrying out this research. Assuring that no physical and psychological impacts would be gained to patients who subjected to the tests, no mention of their names while announcing the results. As the majority of cases were in the pediatric age group, the full agreement of their parents was taken prior to sampling. The same agreement was taken from the adult ones. However, the leftover of the blood sample was taken for the tests of this research for the majority of cases who were already referred from other centers to evaluate their platelet by platelet function test and flow cytometric analysis. The blood samples were collected with EDTA anticoagulated tube for flow cytometric analysis. Trisodium citrate was used

for samples to be analyzed by light transmission aggregometry. The samples centrifuged to obtain.

Those patients were subjected to platelet function test to diagnose G.T. at the National Center of Haematology. Various platelet agonists were used in platelet function test, which include ADP, collagen, epinephrine and ristocetin; all with the light transmission aggregometer were manufactured by BIODATA CORP. USA.

The bleeding severity of those patients were assessed according to Glanzmann thrombasthenia Italian Team protocol (GLATIT) [9] as follows:

- Mild: minor bleeding symptoms or patients who bleed only after trauma or surgery.
- Moderate: spontaneous and life threatening bleeding as GIT bleeding.
- Severe: repeated bleeding episodes requiring blood or platelet transfusion.

The leftover of the blood samples of some patients were used in the tests of the study whereas other patients were referred from other hospitals and specialists specifically for performing the tests, which are included in the study.

Flow cytometric analysis was done at private lab. Anti CD41 manufactured by Abcam. Cambrige and anti CD61 manufactured by Partec. USA; both are anti human primary conjugated antibodies of mouse origin, were used for immunophenotyping of CD41 and CD61 markers respectively by flow cytometry.

A sample of 2 ml of peripheral venous blood were collected in EDTA containing tube from patients; who already submitted to platelet function test, for immunophenotyping. Whereas additional 5 ml of blood were collected in tube containing trisodium citrate from newly diagnosed cases for performing platelet function test.

The blood samples were centrifuged either by PDQ platelet function centrifuge manufactured by BIODATA, USA, double centrifugation protocol or by single centrifugation at 1000 rpm for 10 min. the platelet rich plasma which were transferred to glass tubes and transported to the lab at room temperature by hand to be analyzed within 8 hrs in maximum for flow cytometry and 4 hrs for aggregometry.

100 µl of PRP was taken for flow cytometry. 0.45 ml for each agonist was added in platelet function test. The result of flow cytometry is represented in a dot blot while the result platelet function test is recorded as waves or traces for each agonist.

Statistical analysis

The statistical analysis was performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013.

Results

The three types of G.T. has been identified in the studied sample when flow cytometry is applied as shown in table 1. Males and females were equally affected 1:1. 86% of patients were of type I with severe reduction in receptor expression; 92% of them were classical, in which, there was < 5% expression of both receptors and 8% were heterogeneous, in which, one receptor has < 5% expression while the other one has > 5% expression. Type II constituted only 3% (1 out of 28 patients) and the remaining 11% were of type III.

More than one type of G.T. had been identified in members of the same family. The bleeding severity was assessed in the studied cases and revealed that 43% of the patients were mild bleeders, 39% were moderate and 18% were severe bleeders as shown in figure 1. There was no correlation between the severity of bleeding and the level of receptor expression as well as with the types of G.T. as illustrated in table 2. Majority of cases 82% were below 20 years of age. Mean age was 12 year ± 11.78 SD, median was 11 year and the range was (3-58 year). A single case was at the sixth decade of life.no cases were found in the 4^{th} and 5^{th} decade of life and age had no impact on bleeding severity as shown in table 3. Family history was found in 79% and consanguinity

reached 93% of the cases. Flow cytometry results had shown reduction of expression of CD 41 and CD 61 receptors in comparison with normal levels as in figures (3, 4). Platelet function test showed some variation in the trace of high dose of ristocetin as shown in figure 5 and figure 6. In some patients, there was persistent agglutination while other case showed no response at all; with no statistical significant correlation between it and the percentage of receptor expression. Epistaxis was the most common bleeding manifestation 75% followed by petechiae and bruises 64%, gum bleeding 46% and the least frequent was GIT bleeding that occurred in 18% of the patients as illustrated in figure 2.

Moreover, age and gender had no effect on type of G.T. neither severity of bleeding as in table 4.

Table 1. Types of Glanzmann thrombasthenia

Type of Glanzmann thrombasthenia	No. of patients	Percentage of types	Median of CD41 expression	25-75 Percentile of CD41 expression	Median of CD61 expression	25-75 Percentile of expression
Type I	24	86%	2.18	1.2- 2.47	3.22	1.72-3.99
Type II	1	3%	5.93	5.93	5.66	5.66
Type III	3	11%	92.85	45.89-95.82	92.76	44.25-94.66
Total	28	100%	---------	---------	---------	---------

Table 2. Correlation between severity of bleeding and percentage of receptor expression showing no statistically significant correlation between them

Receptor expression	Median & percentile	Severity of bleeding Mild (n=12)	Moderate (n=11)	Severe (n=5)	P value
CD41	Median	2.58%	2.28%	2.13%	0.737 NS
	25-75 Percentile	(1.46-3.98)%	(1.22-2.64)%	(1.56-4.03)%	
CD61	Median	3.85%	3.35%	3.58%	0.840 NS
	25-75 Percentile	(2.15-5.19)%	(1.64-4)%	(2.85-4.98)%	

Chi-square test was used to describe the association between data, α =0.95

Table 3: Correlation between age and bleeding severity

Age groups	Mild	Moderate	Severe	Total	P value
≤ 10 years	6	3	4	13	0.745
11-20 years	4	6	0	10	
21-30 years	1	2	1	4	
31-40 years	0	0	0	0	
41-50 years	0	0	0	0	
> 50 years	1	0	0	1	
Total	12	11	5	28	

Chi-square test was used to describe the association between data, α =0.95

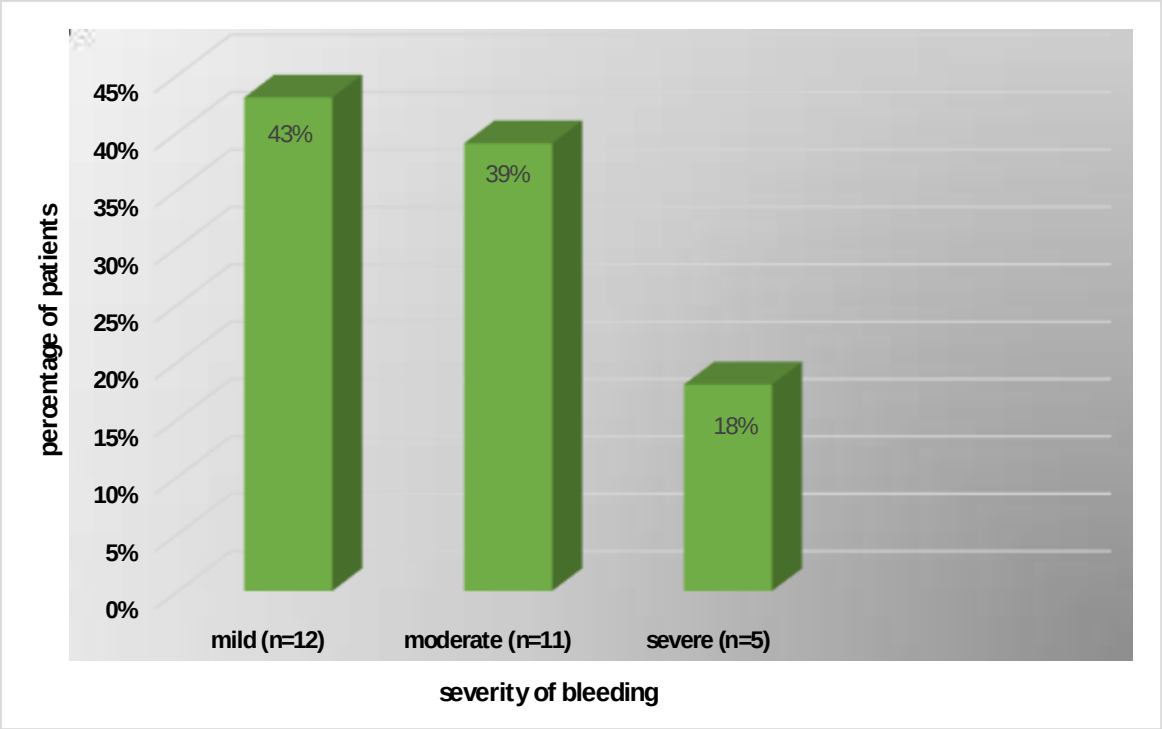

Fig. 1. Distribution of patients according to severity of bleeding

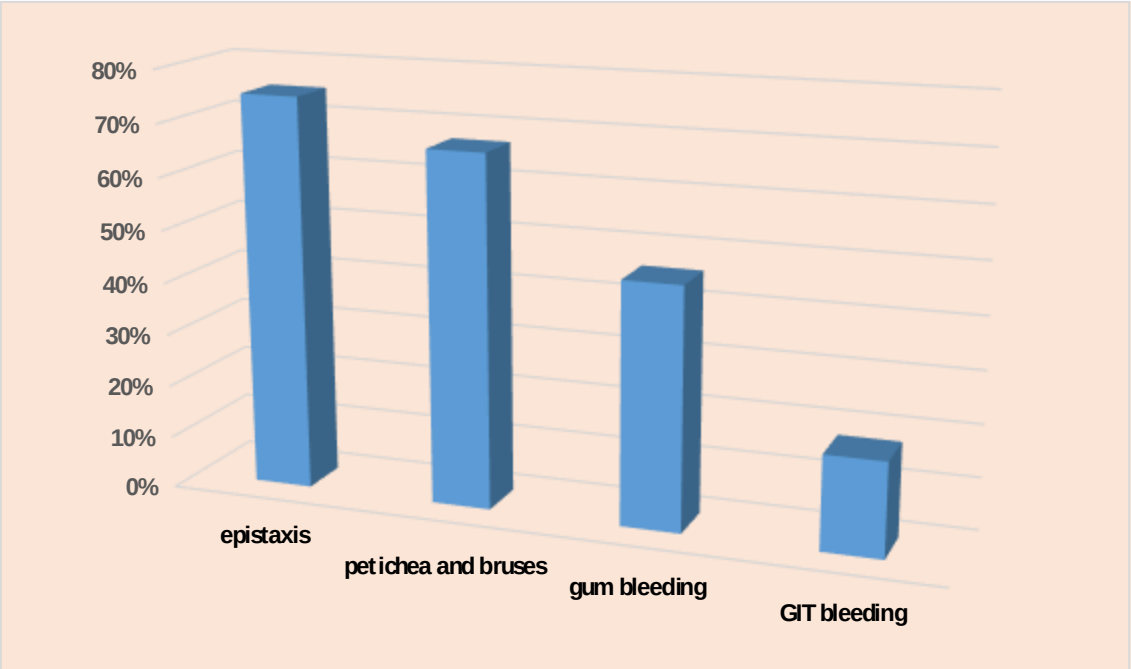

Fig. 2. Percentage of bleeding manifestations

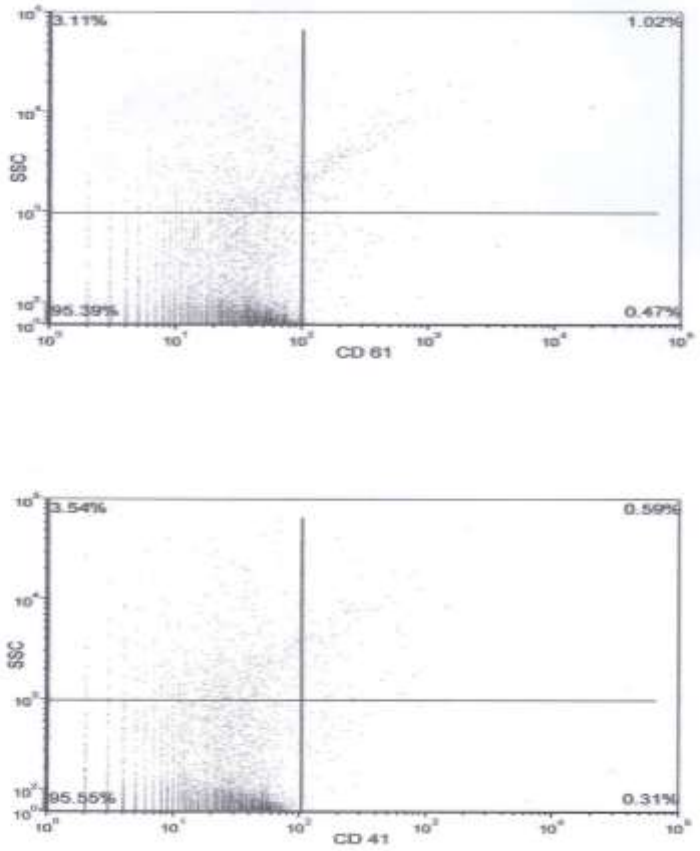

Fig. 3. Dot blot showing reduced expression of both CD41 and CD61 receptors

Discussion

When flow cytometry was applied on platelet rich plasma samples of 28 patients with G.T, it revealed that type I G.T. was the most common type (86%), followed by type III (11%), then type II (3%). This result was in agreement with the result of Kannan et al study (India 2003) that revealed highest frequency in type I (64%) and higher frequency in type III (24%) rather in type II (12%) (10). However, other studies as Alireza Farsinejad (Iran 2010) (11) and Layla Beshwari (kingdom of Saudi Arabia 2005) (12) showed similar result regarding type I but type III was more common than type II. More than one type of G.T. was found in the same family (two sisters, one of them was of type I while the other was of type II). Such finding suggests than more than one type of mutations can affect the genes leading to the occurrence of G.T. In the current study, most cases were below 20 years with absence of cases within the 4th and 5th decade of life. This can be related to chance or the small studied sample or racial factors that may affect the type of the causative mutations. Consanguinity was found in 93% of the patients and it goes with the fact that G.T. as other autosomal recessive disorders can be a common finding in areas where consanguineous marriage is common as in Iraq.

Seventy nine percent of the cases had a positive family history of the disease while the remaining 21% had no such history. This suggest that despite that G.T. is an inherited disease; de novo cases can occur in some individuals due to an acquired mutation affecting the genes. These results approached the results of other studies. When Glanzmann

thrombasthenia Italian Team Protocol was used to assess the severity of bleeding in the studied sample; majority of cases were mild and moderate bleeders and severe ones were of least frequency. However, they gave a history of severe bleeding manifestations when they were at a younger age, but such finding was not proved statistically when the correlation between the severity of bleeding with age and expression was tested and that goes well with the fact that the type of genetic mutation and other unidentified factors can affect the bleeding severity rather than the level of expressed receptors.

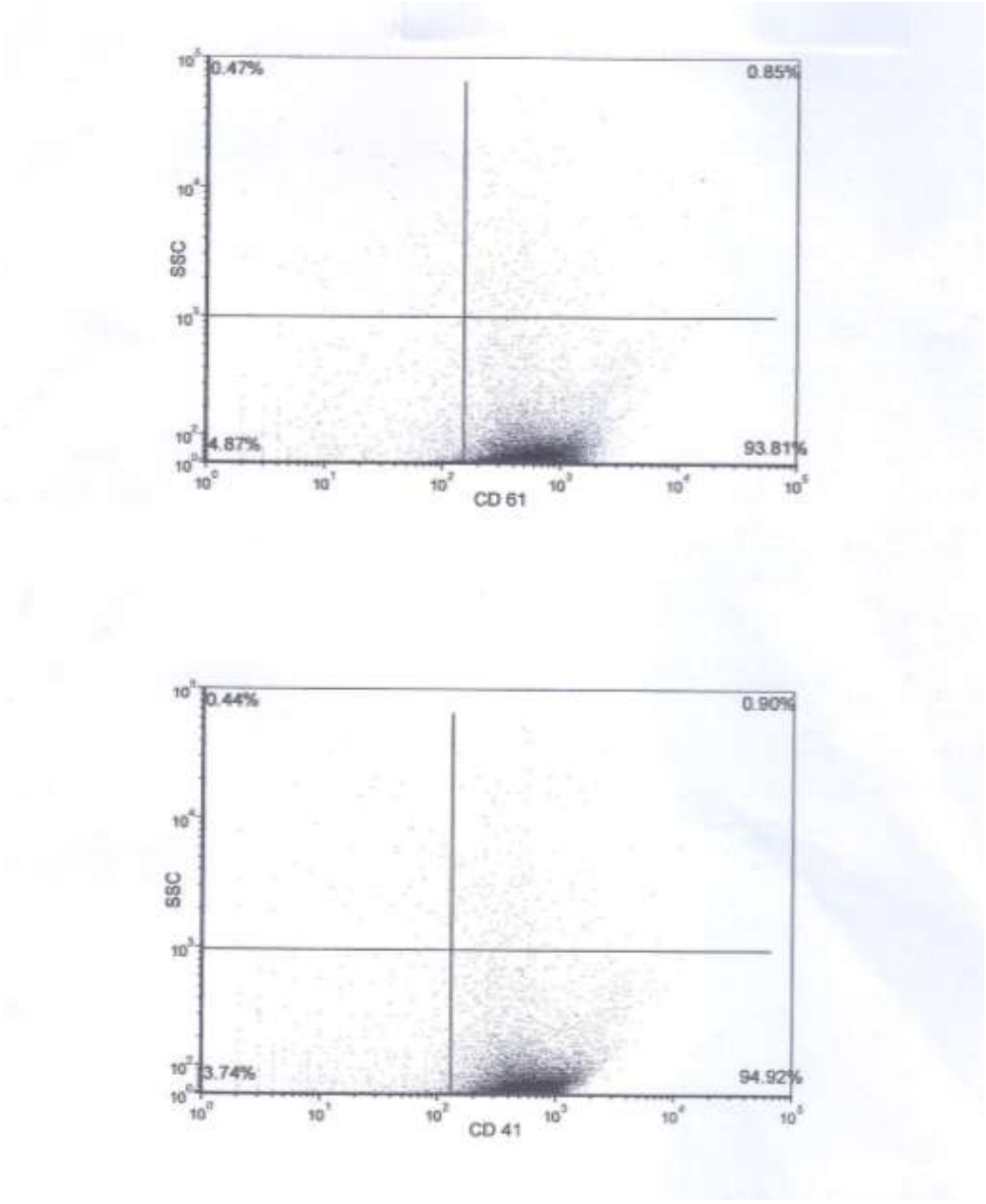

Fig. 4. Dot blot showing normal expression of both CD41 and CD61

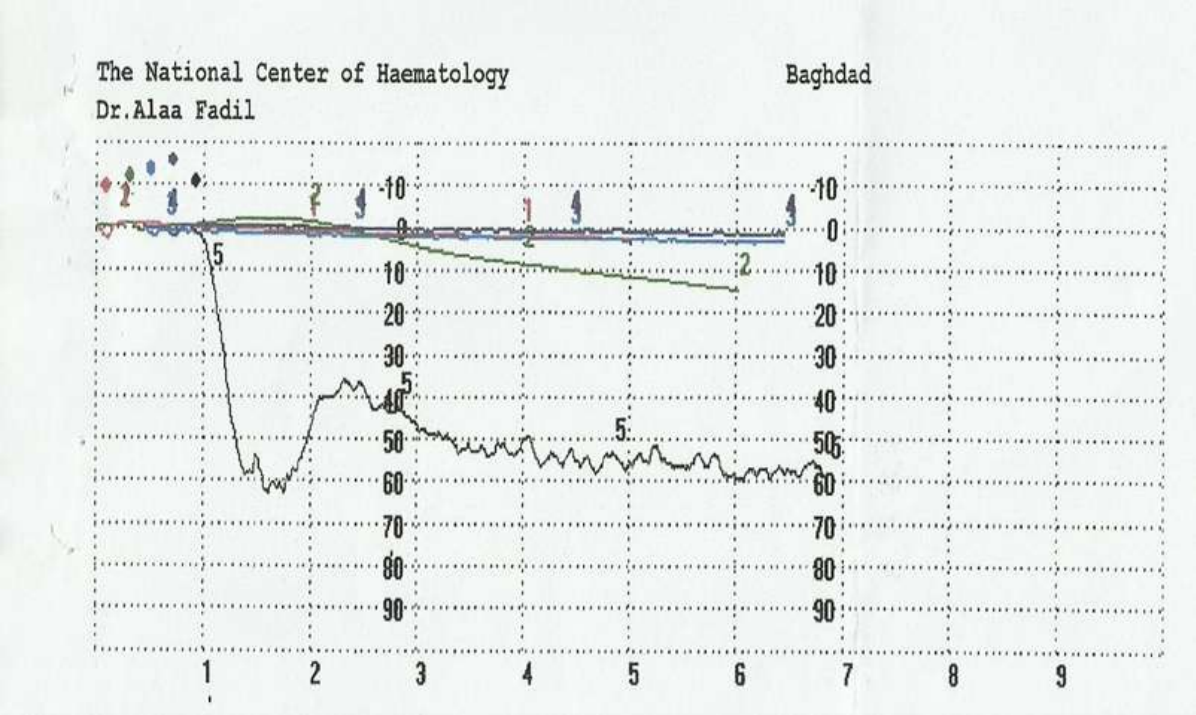

Fig. 5. Platelet function trace with persistent aggregation in response to high dose of ristocetin 1.2 mg/ml. 1- Red: ADP. 2- Green: collagen. 3- Blue: epinephrine. 4- Black: 0.5 mg/ml ristocetin. 5- Purple: 1.2 mg/ml ristocetin. X-axis represents time in minutes. Y-axis represents transmission of light.

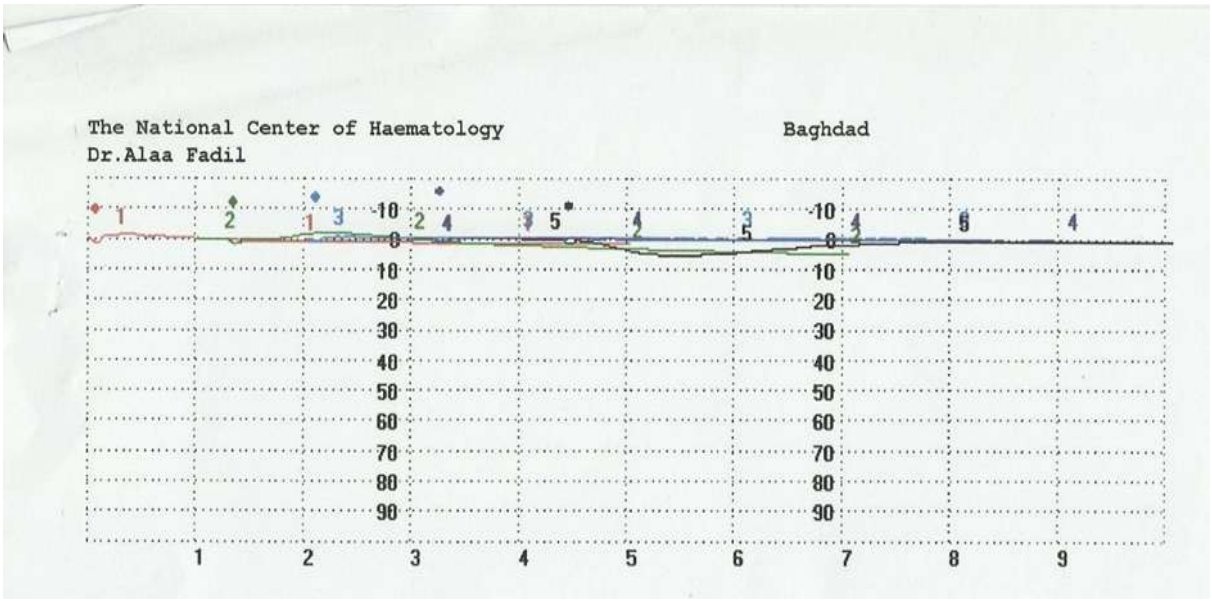

Fig. 6. Platelet aggregation trace with no aggregation in response to high dose of ristocetin 1.2 mg/ml. 1- Red: ADP. 2- Green: collagen. 3- Blue: epinephrine. 4- Black: 0.5 mg/ml ristocetin. 5- Purple: 1.2 mg/ml ristocetin, X-axis represents time in minutes. Y-axis represents transmission of light.

Table 4. Correlation between age, gender and types of Glanzmann thrombasthenia

		Scoring system						p value
		Type I	%	Type II	%	Type III	%	
Age groups	≤ 10 years	11	45.8%	0	0.0%	2	66.7%	0.186 NS
	11-20 years	8	33.3%	1	100.0%	1	33.3%	
	21-30 years	4	16.7%	0	0.0%	0	0.0%	
	58 years	1	4.2%	0	0.0%	0	0.0%	
	Total	24	100.0%	1	100.0%	3	100.0%	
Gender	Female	13	54.2%	1	100.0%	0	0.0%	0.502 NS
	Male	11	45.8%	0	0.0%	3	100.0%	
	Total	24	100.0%	1	100.0%	3	100.0%	

This study concluded that all types of Glanzmann thrombasthenia had been found in Iraq. Majority of them were of type I. More than one type had been identified in one family. The expression of both receptors had no impact on the severity of bleeding. Consanguinity can be considered as risk factor for G.T.

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Author Contribution
Hala made the contact with patients, gathered information regarding their clinical history and results of the hematological investigations, performed the centrifugation of majority of blood samples taken for flow cytometry as well as assisted in performing the immunophenotyping by flow cytometry, attending the platelet function test procedure, sorting and organization of data and results, organization of some figures and tables, writing thesis. Dr. Subh was the supervisor and guidance throughout the whole work. Yusra provided data of some cases, performed platelet function test for the vast majority of the patients. Dr Nidal provided data of some patients.

Conflict of interest
None.

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Combined Effect of Fractional CO_2 Laser and Topical Application of Growth Factor Complex Solution on Old Facial Acne Scar

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Abstract

Background Acne is one of the most common skin conditions. Acne scars are usually leading to disfigurement and psychosocial problem.

Objective To evaluate the efficacy and safety of carbon dioxide CO_2 fractional laser and to evaluate the efficacy and safety of growth factor solution when is used in constant with CO_2 fractional laser in the treatment of old severe acne scarring.

Methods Twenty seven patients were divided in to three groups according to their ages and scarring ages. Group I & II were treated by CO_2 fractional laser only. Group III were treated by CO_2 fractional laser plus growth factor. A period of six-month follow-up was done after the last session. Responses to the treatment were graded on quartile grading scale.

Results Near total improvement was observed in group I age (18-25 years), only (3) of them had marked improvement > 50% after 3 laser sessions. Group II, age (32-50 years), after 6 laser sessions (6) patient had (grade II) moderate improvement, (2) of them minimal improvement. Group III, age (32-45 years) after 6 laser sessions, two patients had (grade IV) > 75%, (5) of them (grade III) 51- 75% marked improvement.

Conclusion Newly formed acne scarring in young patient responds and improved well to CO_2 fractional laser. Growth factor complex increased the improvement in old acne scarring.

Keywords Acne, scars, fractional CO_2, laser, growth factor complex solution

List of abbreviation: CO_2 = Carbon dioxide laser, AFR = Ablative fractional resurfacing, MTZ = Microscopic treatment zone, GF = growth factor, PIH = Hyperpigmentation, mm= millimeter, nm= nanometer, mj = Millijoule, ms = Millisecond, cm= Centimeter

Introduction

Acne is one of the most common skin conditions [(1)]. Acne scars are usually leading to psychosocial distress due to disfigurement and social stigma problem [(2-4)].

Scars originate in the site of tissue injury and may be atrophic or hypertrophic [(5)]. The wound healing process progresses through 3 stages as follow; inflammation, granulation tissue formation and matrix remodeling [(6,7)]. In matrix remodeling, if the healing response is too exuberant, a raised nodule of fibrotic tissue forms hypertrophic scars, but if the response is inadequate, it will result in diminished deposition of collagen factors and formation of an atrophic scar [(8)]. In addition, genetic factors and the capacity to respond to trauma are the main factors that influence scar formation [(9)].

About 80-90% of people with acne scars have atrophic scars [(5,10)]. Atrophic scars are sub classified into: ice pick, boxcar, and rolling scars.

- Icepick scars are narrow (< 2 mm), deep, sharply emarginated epithelial tracts that extend vertically to deep dermis or

subcutaneous tissue (V-shape). They rarely respond to laser treatment [11,12].

• Rolling scars are wider than (4-5 mm), (M-shaped), dermal tethering of the dermis to the subcuticular fat [5].

• Boxcar scars maybe shallow (0.1 – 0.5 mm) or deep (≥ 0.5 mm) and are most often (1.5 – 4.0 mm) in diameter (U- shape), shallow boxcar scars and most deep boxcar scars are amenable to fractional laser [13].

Several modalities have been implicated to cure acne scarring including chemical peel, surgical excision, punch grafting, dermabrasion and tissue augmentation with a variety of dermal fillers, have been used to improve atrophic acne scars with varying degrees of success [3,14].

Different types of laser, including the nonablative and ablative lasers are very useful in treating acne scars. Carbon dioxide laser and Erbium YAG laser are the most commonly used ablative lasers for the treatment of acne scars. These abrade the surface and help tighten the collagen fibers beneath [5]. All ablative lasers showed high risk of complications [5], the high risk of complications following traditional CO_2 resurfacing has warranted the development of new treatment modalities [4,15]. This modality was the fractional photo thermolysis [16,17]. The use of ablative laser in a fractional mode was introduced in 2006 [18]. Fractional laser is the delivery of energy in a manner sufficient to cause a thermal or ablated defect that extends into the dermis and is deeper than its width [4].

Ablative fractional resurfacing (AFR) creates microscopic treatment zone (MTZ) to stimulate a wound healing response [19,20], with this technique the tissue surrounding each column is spared, ultimately resulting in rapid epidermal regeneration. This may offer increased efficacy and decreasing the complications associated with the traditional ablative resurfacing [4].

This study was done to evaluate the efficacy and safety of CO_2 fractional laser in the treatment of patients with moderate to severe acne scars alone or in combination with applying growth factor complex (solution contain multiple growth factor include epidermal and fibroblast growth factor).

Methods

Twenty-seven patients (19 females and 8 males), age (18-50 years), Fitzpatrick skin type lll and lV with mild, moderate to severe acne scaring were included in the study.

The study had done in Laser Medicine Clinic, Institute of Laser for Post-graduate Studies, University of Baghdad and Laser Medicine Clinic, University of Dijlah.

The patients treated with ablative CO_2 fractional laser of 10,600 nm wavelength the laser fluence had delivered with setting of energy (24.2-28.6 mJ) per-pulse, pulse width (1.1 ms), MTZ 90.26/cm^2/pass, spot size 0.1 mm diameter, fluence 364.33 J/cm^2, 4-5 pass as shown in (Table 1).

Table 1: Treatment density

Treatment Energy (mJ)	Treatment parameters (MTZ/cm^2/pass)	Total density (MTZ/cm^2)
≥ 24	≥ 90	≥ 361
≥ 28	≥ 87	≥348
≥ 28	≥ 165	≥ 625

Patients with the following criteria were excluded from the study: active infections, pregnancy, and smoking, those who had any procedures such as chemical peeling or dermabrasion done before and those on oral retinoid drugs within the past 10 months, and photosensitive patient.

Patient with history of herpes simplex virus infection prophylaxis antiviral treatment post laser procedures can give to minimize the incidence and adverse sequelae of these infections.

The patients divide in to three groups as in (Table 2)

• Group l: 12 patients with mild to moderate acne scarring, age (18-25 years), duration of acne scarring 12-18 months, (new acne scars).

• Group ll: 8 patients with severe acne scarring, age (32-50 years), duration of acne scarring > 6 years, (old acne scars).
• Group lll: 7 patients with severe acne scarring, age (32-45 years), duration of acne scarring > 6 years, (old acne scars).

Table 2: Groups of patients included in the study

Patient	Age (yr)	No. of patient	Duration of acne scars
Group I	18-20	12	12-18 months mild to moderate acne scars
Group II	32-50	8	> 6 years old acne scars
Group III	32-45	7	> 6 years old acne scars

Preparation the patients to laser sessions should be done; at first, the acne is cleared before treating scarring, sunscreen cream was advised at the start of therapy and continued throughout the duration of the treatment. Topical anesthesia with Emla (eutectic mixture of lidocaine and prilocaine), 1 hour with occlusive dressing prior to laser irradiation was applied. After an hour of application, the anesthetic cream was gently removed. The face washes thoroughly and let the skin dry before treatment, covers the patient eyes with non-reflecting protective goggle. Immediately after laser session, only Group lll patient have growth factor complex solution (which contain two type of growth factor Epidermal + Fibroblast GF, concentration of 1 ml per liter; pure concentration of 1 mg per liter. FDA-registered manufacture, USA) Put on their face. The others groups put only a thick layer of sun block. Cold compressor used to reduce dis comfort or burning sensation. They were also instructed to limit sun exposure. Moisturizing cream used at night, used sterile water when wash the faces.

Photographic documentation was used before and after each treatment session. We compared improvement rate of scars after every sessions at 3-4 weeks interval then adverse effects and recovery times were recorded in each session and visit.

Improvement in acne scars was recorded on a specially devised pro forma with a quartile grading scale as shown in (Table 3).

Table 3: Scale of clinical improvement

Grade	% of improvement
1	< 25% minimal improvement
2	26-50% moderate improvement
3	51-75% marked improvement
4	> 75% near total improvement

Results

On completion of study, (27) patients were available for evaluation. All of them had improvement in their acne scarring, especially Group I (Fig. 1, Fig. 2) who had new acne scarring treated more easily, and after (2-3) session (9) patient showed grade lV (near total improvement), and only three of them showed grad lll (51-75% marked improvement), (Table 4).

Group ll (Fig. 3, Fig. 4); (8) patients with severe old acne scarring treated by CO_2 fractional laser after six session, (6) patient showed grade ll (26-50% moderate improvement), and only (2) patients showed grade I (<25% minimal improvement).

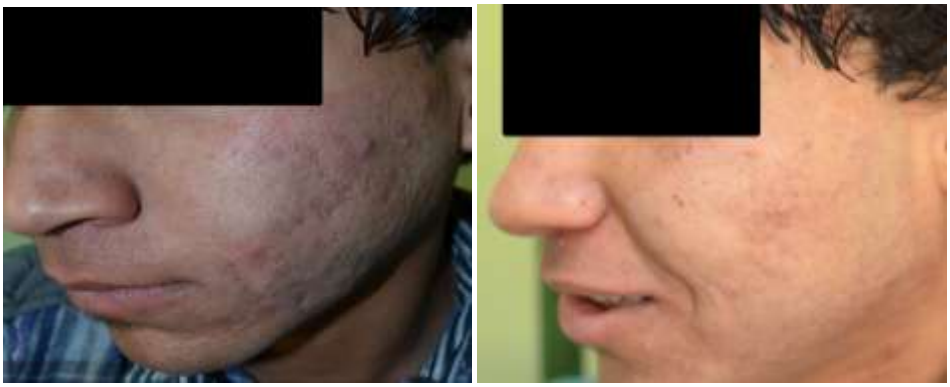

Fig. 1. Group 1: Prelaser (left) and post laser (right) as three session with (grade IV) >75% improvement.

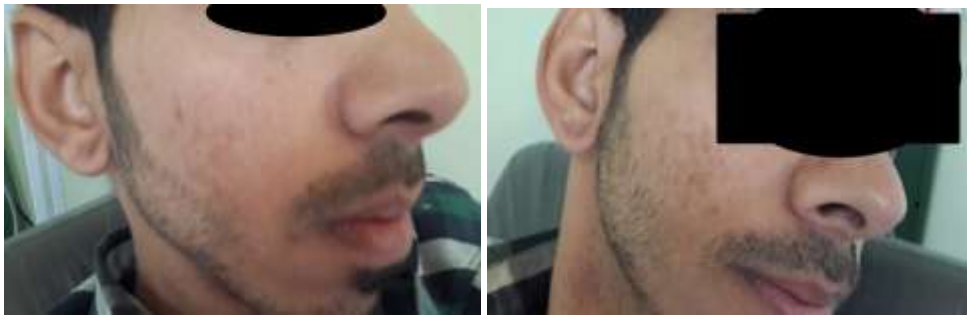

Fig. 2. Group 1: Prelaser (left) and post laser (right) as three session with (grade III) >50% improvement

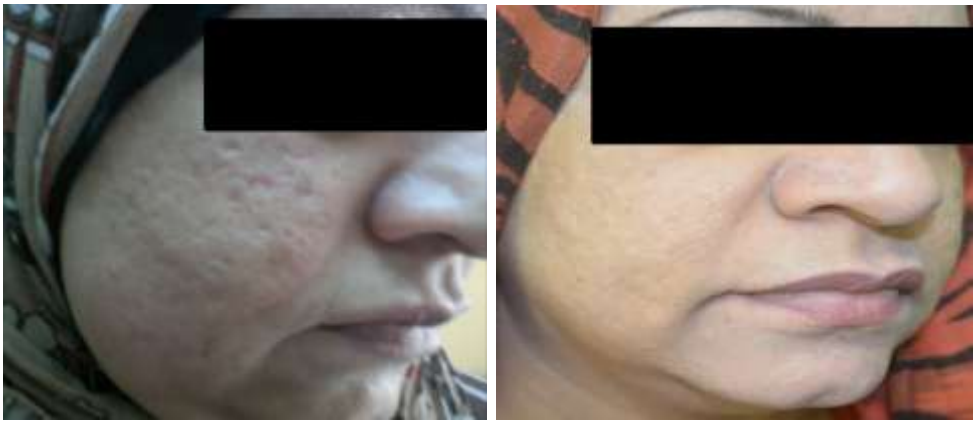

Fig. 3. Group II: prelaser (left) and post laser (right) as six laser sessions with (grade II) moderate improvement

Table 4: The improvement in acne scar in three groups of patients

Patients	Age	No. of patient	Treatment	No. of sessions	Improvement
Group I	18-20	12	CO_2 fractional laser	2-3	(9 patient) > 75% grade 4 (3 patient) 51-75% grade 3
Group II	32-50	8	CO_2 fractional laser	6	(6 patient) 26-50% grade 2 (2 patient) <25% grade 1
Group III	32-45	7	CO_2 fractional laser + growth factor	6	(2 patient) > 75% grade 4 (5 patient) 51-75% grade 3

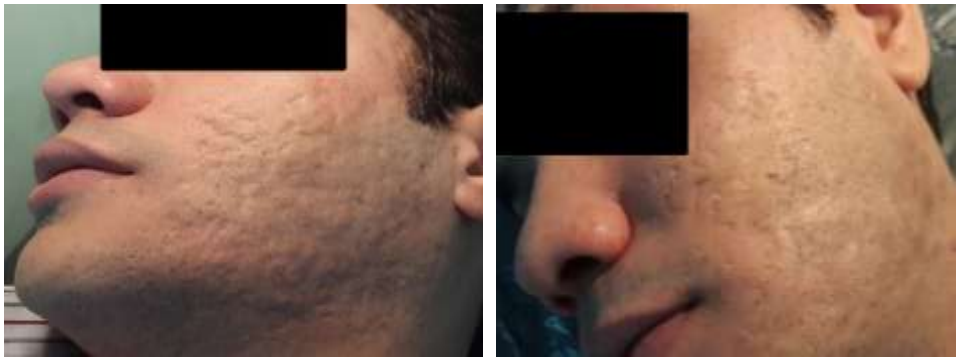

Fig. 4. Group II: Prelaser (left) and post laser (right)as six laser session with (grade I) minimal improvement

Group lll (Fig. 5, Fig. 6); patient with severe old acne scarring treated by fractional CO_2 laser plus applying growth factor complex after six laser session (2) of them showed grad lV (>75% near total improvement), and (5) of them showed grad lll 51-75% marked improvement). Growth factor complex enhance the Results in this group, as showing in (Table 5).

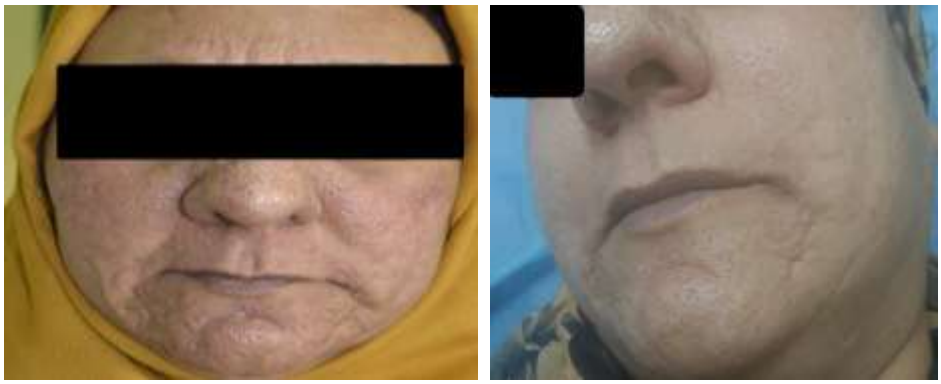

Fig. 5. Group III: Prelaser (left) and post laser (right) as six laser session with (grad IV) >75% near total improvement

Table 5: Comparisons between two groups of patients had old severe acne scars

Group II	Group III
Treated by CO_2 fractional laser	Treated by CO_2 fractional laser + growth factor complex
Down time (redness swelling erythema and edema) seven days	Down time (redness swelling erythema and edema) 2-3 days
Improvement in skin texture and firmness	More Improvement in skin texture and firmness
Improvement in acne scars (5 patient) 26-50%, (2 patient) 1-25%	Improvement in acne scars (2 patient) > 75% (5 patient) 50-75%
No of session 6	No of session 6

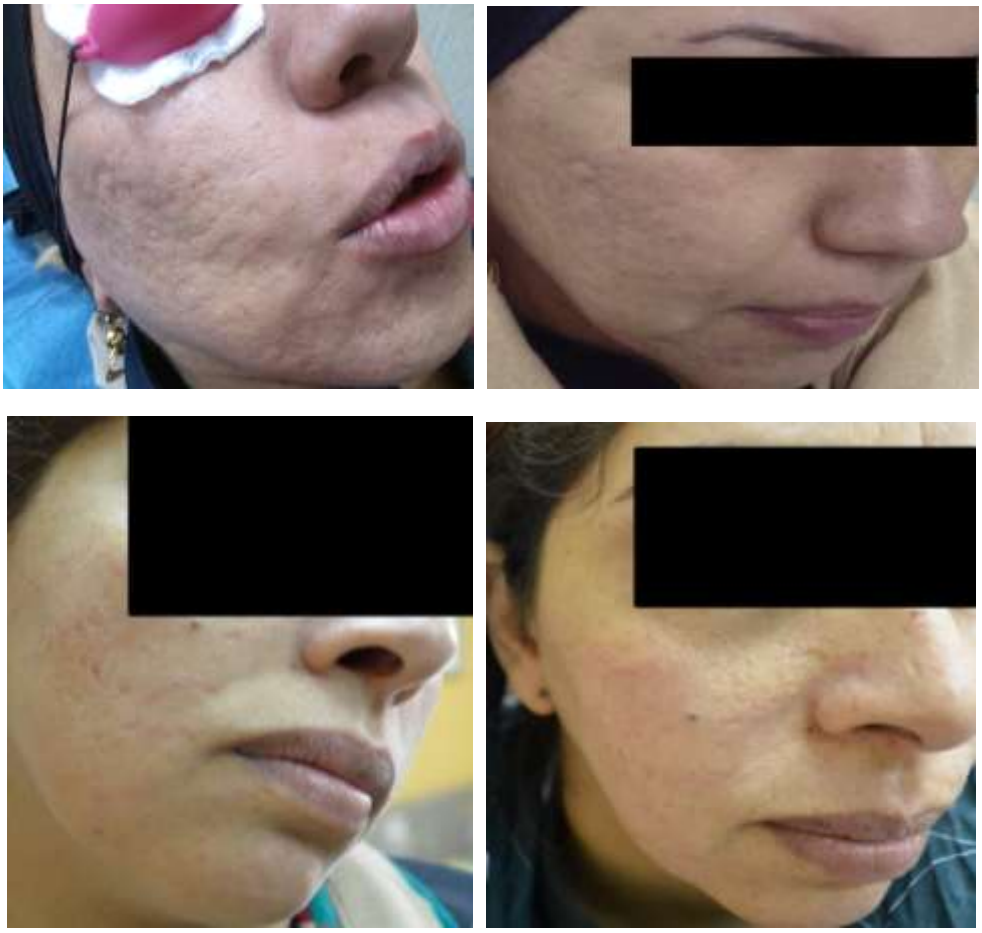

Fig. 6. Group III: Prelaser (left) and post laser (right) as six laser session with (grade III) marked improvement

All subjects reported that any discomfort associated with procedure was only during active intervention and resolved immediately as post procedure. Except the patients with growth factor complex explain burning sensation resolve after 1 hr.

Swelling and mild to moderate erythema resolved after seven day except in patient with growth factor complex resolved after 2-3 days.

Prolonged erythema seen in one patient (group ll), incidence (3.5%), three-month duration, and this patient had previous history of rosacea, treated by metronidazole gel and responded to treatment after two months.

Post inflammatory hyperpigmentation (PIH) was seen in two patient, incidence (7%), one of them (group l) with skin type lV, had previous history of (PIH). Second patient (group ll) skin type lV have multiple laser session, and both of them treated by topical hydroquinone preparations.

A bronzed or tanned appearance seen in one patient (group l), incidence (3.5%) that was evident at six months follows up visit. This may be due to sun exposure without used sunscreen; this patient was treated by sun block and vitamin C cream.

There were no incidences of infections, scarring, hypopigmentation, or other serious complications (Table 6).

Table 6: The complication seen in three groups of patients

Patients	Transient erythema and edema	Prolonged erythema (3 months) duration	Hyper-pigmentation	Hypo-pigmentation	A bronzed or tanned appearance	Burning sensation	Scarring formation	Infection
Group I	All patients	-ve	One patient	-ve	One patient	-ve	-ve	-ve
Group II	All patients	One patient	One patient	-ve	-ve	-ve	-ve	-ve
Group III	All patients	-ve	-ve	-ve	-ve	All patients	-ve	-ve

Discussion

Fractional ablative laser therapy is a relatively new therapeutic modality which will likely be widely used because of its efficacy and limited side-effect profile [21,22].

Acne scarring is a complex problem that is not amenable to a simple, definitive solution. A combination of several treatment procedures over multiple treatment session may be appropriate [10,23-25]. In this study, (group l), which involved young s had newly formed acne scars treated by CO_2 fractional laser, nine of them reached near total (grade lV), after 2-3 laser sessions. This may be due to young age patients, because aging and ultra violet exposure lead to reduction of procollagen synthesis, increase of collagen degradation in dermal extra cellular matrix, increase in irregular elastin deposition [26], and rendered the old acne scar more deep resistance to laser treatment [27].

It has been seen in this study that the acne scarring gave a good result when was treated as soon as possible by fractional CO_2 laser. Because the CO_2 lasers have a double effect: they promote the wound healing process and arouse on amplified production of myofibroblasts and matrix proteins such as hyaluronic acid [28].

The other two groups have old severe acne scarring, (Group ll), (8) Patients treated by fractional CO_2 laser; only (6) of them showed (grade ll) (26-50% improvement). While group lll (7) patients who was treated by CO_2 fractional laser plus applying growth factor complex solution on their acne scarring showed more improvement, (5) patients had (51-75%) improvement (grade lll), and (2) of them had more than 70% improvement (grade lV).

This may be due to the effect of growth factors which stimulate the migration and proliferation of fibroblasts [29], it was also regulating fibroblasts in treated skin, thereby resulting in neo collagensis [30,31].

This study also revealed that there was a synergistic effect of growth factor with CO_2 fractional laser because the second one creates

micropunctures in to stratum corneum and dermis before topical application of growth factor complex, with the assumption that reduced barrier will result in greater efficacy of growth factor [26].

The complication in this study was very few, may be because the study was done in winter fallow up the patient in summer, so that the Hyperpigmentation (PIH) seen in two patients only. Both of them skin (type lV) dark skin [32,33], with history of neglect used sun Block, Both of them treated by topical hydroquinone preparations.

Erythema of longer duration it was found in one patient (group ll), may be due to increase number of laser passes performed [34,35], and increase number of laser session.

One patient (group l) experienced a bronzed or tanned appearance that was evident at the 6 months follow-up visits. May be due to secondary desiccation and/or optical changes in portions of the epidermis and dermis, along with the underlying erythema and wound healing response [4].

Lastly, all participants felt an ascending improvement rate during and after the course of treatment. Also we see significant improvement rate in skin texture and increase firmness after treatment in all patient especially patient with growth factor.

This study concluded that treatment of moderate to severe facial acne scarring by CO_2 fractional laser provides a safe and effective treatment with minimum complication. Additionally, treatment of acne scars in the early stages by CO_2 fractional laser, gives the best results than late treatment.

Also this study concluded that concomitant use of CO_2 fractional laser with growth factor complex solution for the treatment of old severe acne scars give the best results, with minimal down time and no complication.

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Author contribution

The study was done by Dr. Fatima M. Ali and Dr. Ali S. Mahmood supervised the research.

Conflict of interest

There was no conflict of interest.

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The Use of Methanolic extract of *Boswellia serrata* in Combination with Dextrin and Glycerin for Treatment of Experimentally Induced Thermal Injuries in Rabbits

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Abstract

Background Thermal injuries and burns are considered as the major health care problem worldwide. Burns in general are life-threatening condition with high morbidity and mortality. Wound infection and delayed wound healing are the main essential problems in burned patients and represent the major goals for new therapeutic strategies. Frankincense, the gum resin of the *Boswellia serrata* tree have traditionally been used in folk medicine for centuries to manage different chronic inflammatory diseases, the anti-inflammatory potential of lipophilic *Boswellia serrata* extracts were confirmed by experimental data from animal models and clinical studies on humans.

Objective Comparing the wound healing effect of methanol extract of *Boswellia serrata*-dextrin-glycerin combination to that of silver sulfadiazine.

Methods The dried powder of the oleo gum resin was extracted by cold method (maceration) using methanol and then the dried extract was mixed with dextrin and glycerin (30 gm, 40 ml, 60 gm) respectively. Thirty-two domestic male rabbits were divided into four groups, eight animals for each group, the groups were; intact healthy (H), burned without treatment (Gr1), burned and treated with silver sulfadiazine (Gr2) and burned and treated with the methanolic extract of *Boswellia serrata*–dextrin-glycerin combination (Gr3). Thermal injury was induced in all groups (except the intact healthy group) and treated topically on burned area once daily for 28 days. Tissue levels of vascular endothelial cell growth factor (VEGF) and tumor necrosis factor alpha (TNF-α) were assessed in addition to skin histological examination at the end of the study (day 29).

Results Histopathological evaluation showed enhanced inflammatory response, granulation tissue formation, and collagen deposition due to the appropriate regulation of TNF-α and VEGF.

Conclusion Topical use of the methanolic extract of *Boswellia serrata*-dextrin-glycerin combination significantly enhanced wound healing activities.

Keywords *Boswellia serrata*, VEGF, TNF, thermal injury

List of abbreviation: *B. serrata* = *Boswellia serrata*. VEGF = Vascular endothelial cell growth factor, TNF-α = tumor necrosis factor alpha

Introduction

One of the most common universal problems worldwide is burns, which is the leading cause to ugly skin scarring and serious handicapping, the effect of burns extends to the entire body besides the skin [(1)]. The function of the skin is to protect the internal body organs from any hostile external environment of different pollution, temperature, humidity and radiation. Also, skin has important function, such as preserving water and heat regulation [(2)]. A burn is a kind of skin injury that is caused by heat, electricity, chemicals, light radiation, extreme cold or friction [(3)]. The world health organization

reports that more than 90% of burn injuries occur in the developing countries or underdeveloped ones where the death from large burns (more than 40% of total body surface area) reach 100% [4].

Frankincense, the gum resin of the *Boswellia serrata* (*B. serrata*) tree, was well known to ancient civilizations and is still considered as for ritual purposes in the Catholic Church and traditional ceremonies in Northern Africa [5]. High performance liquid chromatography (HPLC) analysis of Indian and African samples of B. serrata gum resin yielded 12 different pentacyclic triterpene acids, the most important is alpha boswellic acid and acetyl boswellic acid. This method provides differentiation and standardization of the resin of different origin and gum resin phytopharmaceuticals [6].

Dextrin is mainly produced by an enzyme called amylase in human that is usually present in saliva mixes with the food in the mouth, and then acts on the starch in a slightly alkaline medium to convert it to dextrin [7].

Glycerin (CAS No. 56-81-5) is a polyhydric alcohol which its molecular formula is $C_3H_8O_3$. Glycerin (also referred to as glycerol) is a simple polyol compound that has three hydroxyl (OH) groups [8].

The objectives of this study was to compare the wound healing effect of methanol extract of Boswellia serrata-dextrin-glycerin combination to that of silver sulfadiazine.

Methods

Thirty-two domestic male rabbits, weighing 1250-1750 grams were divided into four groups each of eight animals, they were housed in animal house of Collage of Medicine, Al-Naharain University. Before starting the study, the animals were left for 48 hours to acclimatize to the animal room conditions of controlled temperature, allowed free access to water and food. Thermal injury was initiated by a metal bar (20*20*100) mm, heated in boiling water and preserved in equilibrium for about 15 min. with the present of thermometer and the animals were anesthetized using ketamine: xylazine (22-50 mg/kg: 2.5-10 mg/kg IM) and the bar was applied for about 45 seconds on their shaved back [9]. The experimental protocols are:

H: intact healthy group

Gr1: induced burn without treatment

Gr2: induced burn and treated with silver sulfadiazine

Gr3: induced burn and treated with methanolic extract of *B. serrata*-dexrin-glycerin combination.

The extraction process was first done on the Oleo gum resin using methanol by maceration (cold method), then the methanolic extract was mixed with dextrin and glycerin with continuous heating and stirring using magnetic stirrer and electric heater, the final combination was left to cool down [10].

At the end of the experiment the animals have been sacrificed by ether on day 29. The skin tissue were cut in two parts; one for tissue homogenization to determine tissue levels of vascular endothelial cell growth factor (VEGF), tumor necrosis factor alpha (TNF-α) and the other part for histological examination to give scoring level of inflammatory response of the wound.

Principle of the assay of TNF-α and VEGF a quantitative sandwich enzyme immunoassay technique (ELISA); where antibodies specific for TNF-α and VEGF have been pre-coated onto a microplate. Samples and standards are pushed into the wells and the TNF-α contents are bound by the immobilized antibody; then removing the unbound substances, adding a biotin conjugated antibody to the wells, washing, adding avidin conjugated Horseradish Peroxidase to the wells, washing again, adding a substrate solution to the wells and color would appear in proportion to the amount of TNF-α and VEGF bound in the first step. The color must be stopped and the intensity of it is measured at 450 nm (This assay was done as directed by the assay layout sheet of the manufacturer company: Cusabio).

Preparation of skin tissue for histological examination by fixation in 10% formalin and processed according to Bancroft and Stevens [11].

Statistical analysis was performed using SPSS-21 and descriptive statistics were formulated as mean and standard error mean (mean ±SEM). One Way Analysis of Variance (ANOVA) and t-test was used to assess and the difference was considered significant when p value was equal to or below 0.0512.

Results

Gr3 group showed a significant reduction in the levels of TNF-α in skin tissue homogenate; in addition to significant reduction of VEGF in skin tissue homogenate compared to other groups ($P < 0.05$) as shown in figure 1 and 2 respectively, while Gr1 and Gr2 animal groups showed non-significant difference on TNF-α and VEGF level in skin tissue homogenate ($P > 0.05$) but significantly different compared with H group, and according to histopathological examination (figure 3,4,5 and 6) of skin, Gr3 showed better inflammatory response, granulation tissue and fibrosis.

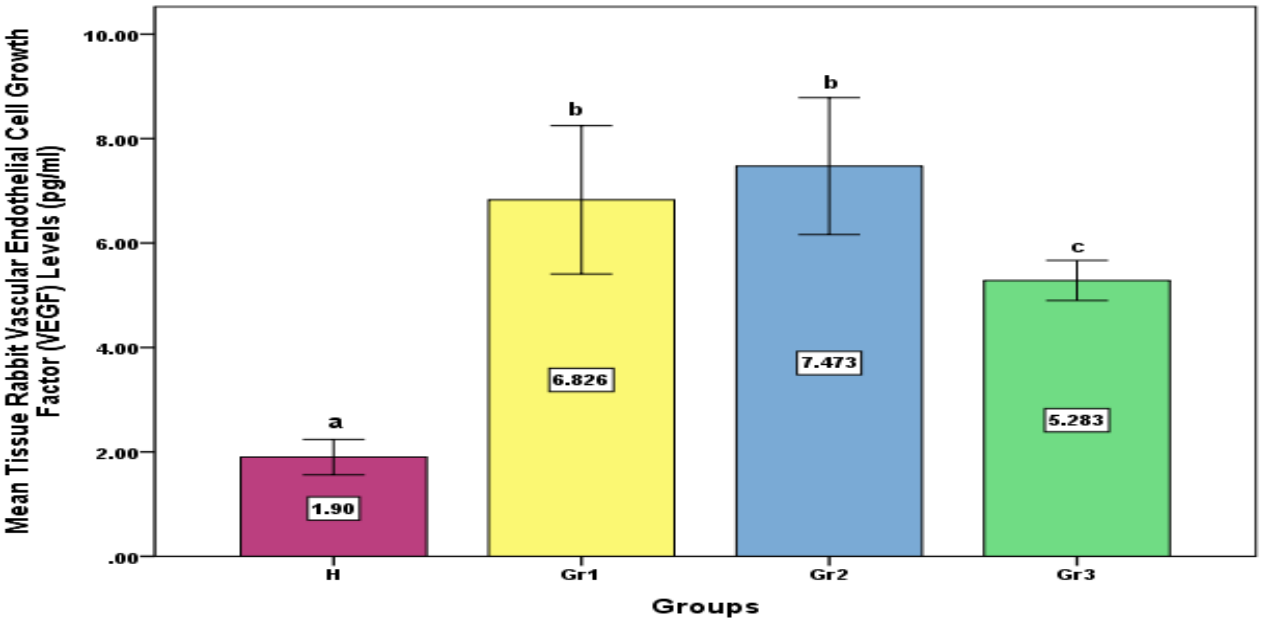

Fig. 1. Tissue VEGF level in the study groups.
H= intact healthy, Gr1= burned without treatment, Gr2= burned and treated with silver sulfadiazine, Gr3= burned and treated with ME of B. serrata-dextrin-glycerin compound

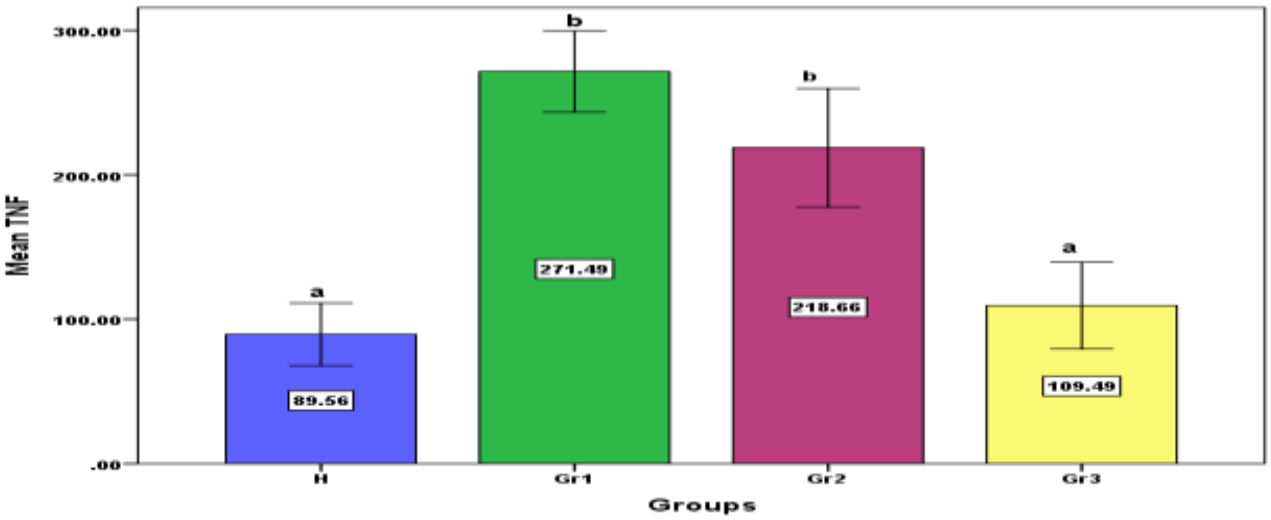

Fig. 2. Tissue TNF-α level in the study groups. H= intact healthy, Gr1= burned without treatment, Gr2= burned and treated with silver sulfadiazine, Gr3= burned and treated with ME of B. serrata-dextrin-glycerin compound

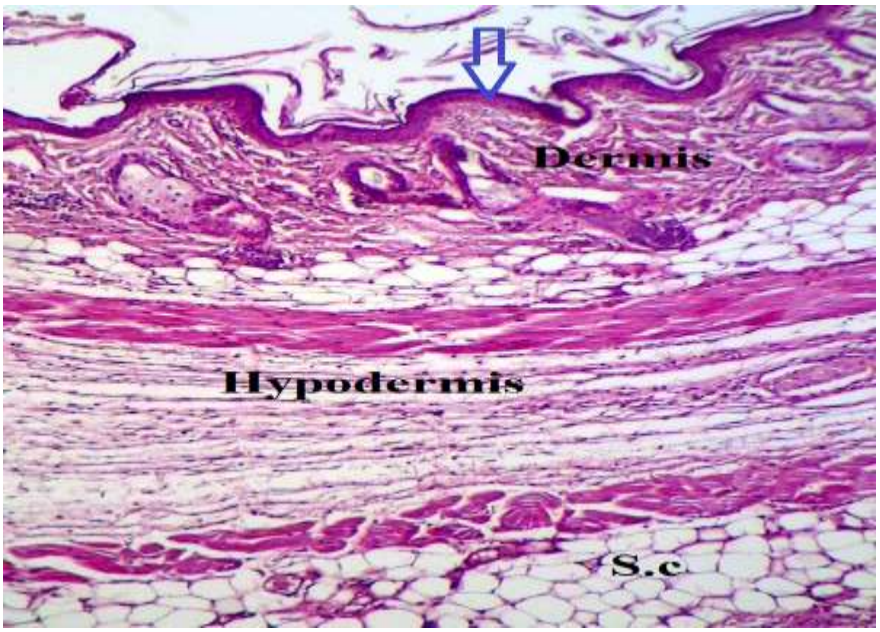

Fig. 3. Light microscopic section of rabbit skin tissue of H group (the intact healthy) showing normal skin tissue: epidermis (blue arrow), dermis, and hypodermis and subcutaneous. H&E (40X), (S.c) subcutaneous

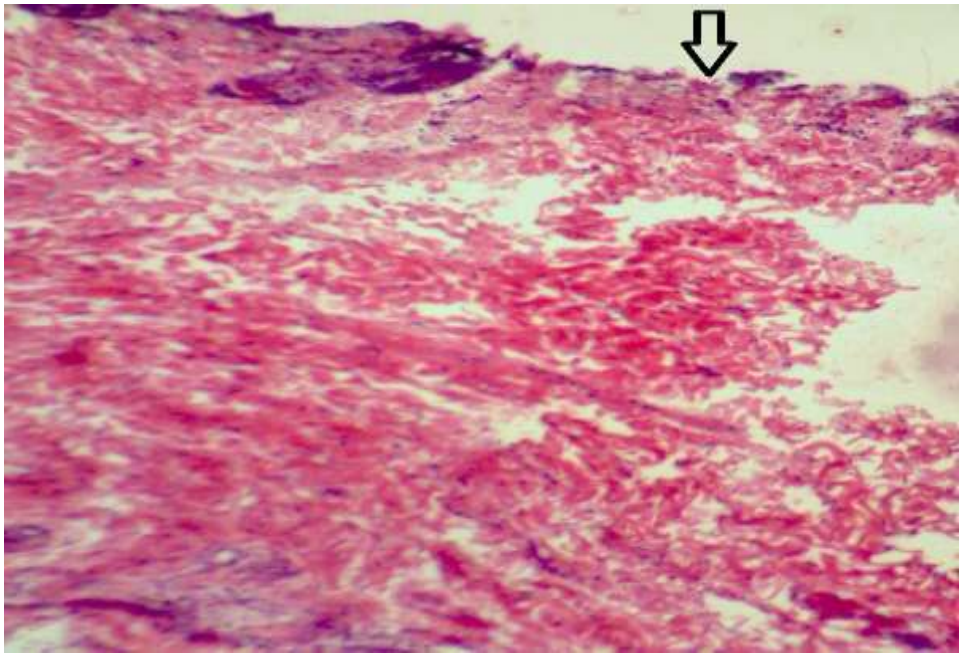

Fig. 4. Light microscopic section of rabbit skin tissue of Gr 1 (burned without treatment) showing burning skin layers and ulceration (black arrow). H&E (40X)

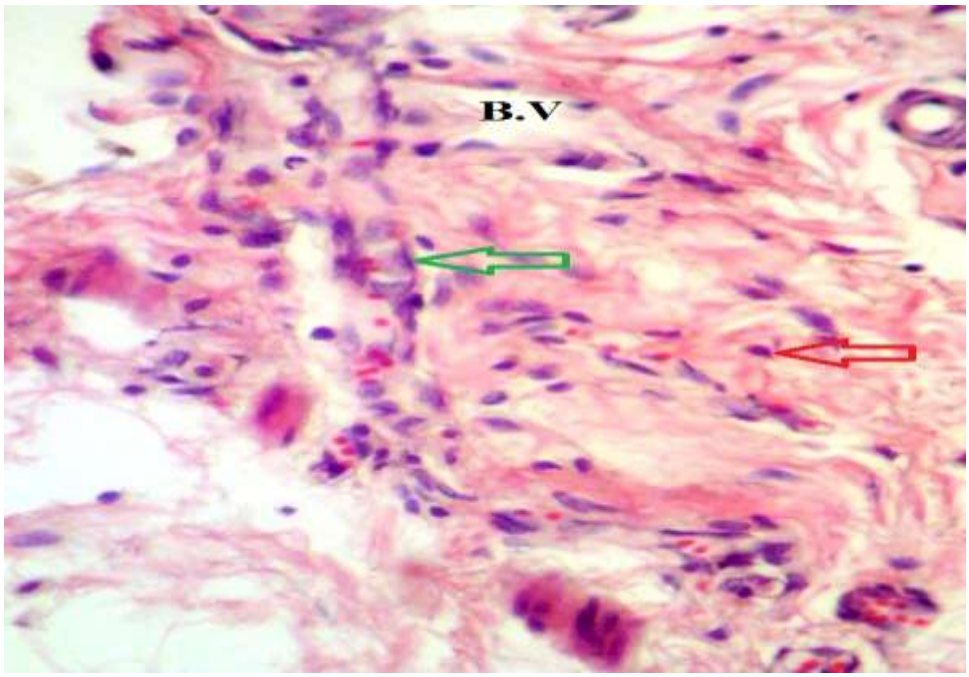

Fig. 5. Light microscopic section of rabbit skin tissue of Gr 2 (Silver sulfadiazine treatment) showing inflammatory (red arrow) with mild reactive fibroblast (green arrow) and collagen fiber. H&E (40X). (B.V) blood vessel

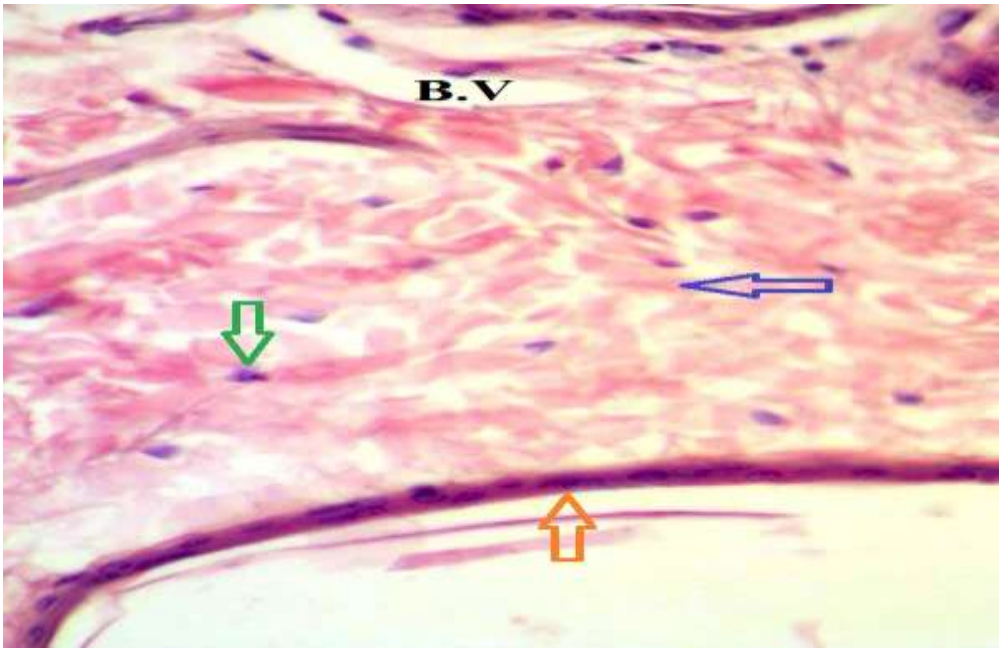

Fig. 6. Light microscopic section of rabbit skin tissue of group3 (Methanolic extract of *Boswellia serrata* -dextrin-glycerin combination) showing reactive fibrous tissue formation with present of fibroblast cells (green arrow), epidermis (orange arrow) and abundant of collagen (blue arrow). H&E (40X). (B.v) blood vessel

Discussion

Burn is the most widespread injury where oxidation process takes place associated metabolic and biological changes. Animal studies is useful because the pathophysiology and histopathology of thermal injury in animals is very similar to in humans [13]. Oleo gum resins from Boswellia species are being used in traditional medicine for the management of a different disease [14], experimental data from animal models as well as clinical studies on humans confirmed the anti-inflammatory potential of lipophilic *B. serrata* extracts [5].

The anti-inflammatory effect of the Methanolic extract (ME) of *B. serrata* may be attributed to the presence of diversity of phytochemicals in the methanolic extract such as α & β pinenes, α, β and γ boswellic acids and other terpenoids [15]. These chemical constituents may exert the direct anti-inflammatory effect by the direct inhibition of the vascular endothelial growth factor by interfering with VEGFR2 activation [16,17].

In this study, the extraction process was done sequentially and using the cold method "maceration", cold method for extraction were used because this method is more suitable for thermosensitive constituents so that it will ensure the essential phytochemical constituents was not subjected to degradation by higher temperatures [18]. It had been noticed that there are several factors seem to affect the variation in the yield and the composition of phytochemicals in the extract, these include: type of extraction method, duration of the extraction process, temperature of the water bath, agitation, solvent type used and its pH, concentration and polarity; particle size of the powdered plant part and solvent to sample ratio [19]. The vast majority of the hot aqueous extracts of the antibacterial active plants exhibited low activity when compared to the methanolic extract. The extraction yield of the current study was 14.2 % w/w, which come to agree with Lin and coworkers [20] in 2013 who showed that the typical yield of frankincense essential oil was 10% (w/w) of gum resins within a range of 8-13%.

Wound healing process is a physical building of molecules for tissue repair and secreted by

fibroblasts and others present at the site of the burned wound [20]. It's well known that wound healing process is affected by tissue levels of VEGF which in turn will affect angiogenesis and the direct and indirect activation of the fibroblast, and there is a link between angiogenesis and scarring and suggest a novel role for VEGF in mediating the quantity and quality of scar tissue generated during wound repair [21]. Johnson and Wilgus [22] in 2014 said that treatment of hypertrophic scar patients with interferon α2b has been linked to a reduction in angiogenesis and VEGF suggesting that reducing VEGF may improve scars, similarly, treatments used to induce keloid regression have been shown to reduce VEGF levels in keloid tissue.

Tumor necrosis factor-α is an important mediators of the acute and severe inflammatory reaction in thermal injury that affect wound healing process [23], it is involved in the early initiation of wound healing process and low levels can promote wound healing indirectly, however, high levels can delay wound healing [24]. Rapala [25] showed in 1996 that after daily applications of TNF-α for 4 days; an inhibitory effect on tissue repair was observed after 4 and 7 days. Collagen formation, indicated by the hydroxyproline content of the sponge, was significantly lower in the group treated with TNF-α than in the controls; this effect could be abolished with indomethacin and Indomethacin alone stimulated collagen production by 40%.

All these findings come to agree with our study results, which showed that VEGF and TNF-alpha tissue levels in the ME of *Boswellia serrata* treated group was significantly less than burn without treatment group (Gr1) and SSD treated group (Gr2).

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Author contribution

Abbas is a researcher who has done the technique of this work and conducted the writing of manuscript. Dr. Abdulkareem and Dr. Bahaa participated in supervision and in scientific review of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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