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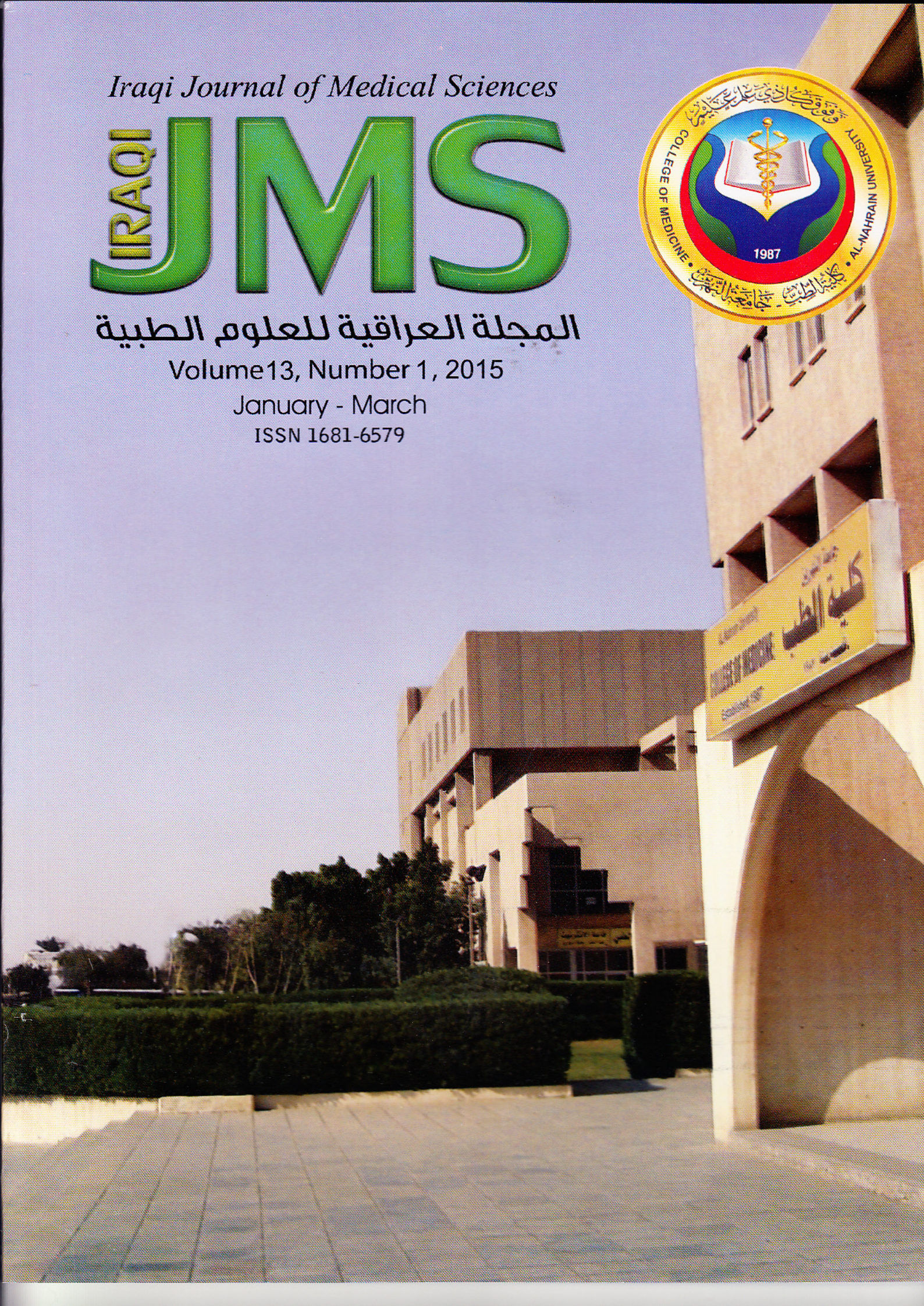

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Iraqi Journal of Medical Sciences

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A Medical Journal Encompassing All Medical Specializations

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Biomechanical Properties of the Lip as an Approach for Tissue Engineered Lip

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Abstract

Background Tissue engineering is a rapidly progressing field of science that provided the surgery with better options for treatment; lip has unique anatomy, shape and functions. Large lip defect is one of the great challenges for plastic surgeons, the gold standard option is autologous tissue replacement with drawbacks of donor site morbidity and suboptimal outcomes, tissue engineering came up with new option for partial thickness defect to address the skin-vermilion loss as a composite graft but not the orbicularis oris muscle, before preceding for tissue engineered orbicularis oris muscle it is wise to study the biomechanical properties of the lip and then matching the measures with tissue engineered product as basic biomechanical properties affect the lip shape and functions.

Keywords Biomechanics, stiffness, tissue engineering, lip, orbicularis oris, vermilion reconstruction, perioral dynamics

Introduction

Reconstruction of large lips defect is one of the major challenges for plastic surgeons due to the complex lip anatomy and biomechanics. Improper reconstruction leads to poor aesthetic and functional outcomes in term of speech, facial animations, stiffness and oral continence which effects eating and drinking. Large lips defects could be attributed to iatrogenic causes after tumor excision, traumatic, burn, congenital anomalies and necrotizing soft tissue infections. The current available options for treatment are autologous tissue transfer whether it is local, regional or free tissue transfer; in addition to the donor site morbidities, they do not precisely mimic the original lost tissue in term of unique morphology and function. However, loss of more than 50% of the lip brings the face transplant as another option of treatment for functional concern but imposing the patient for life-long immune-suppression.

Tissue engineering and regenerative medicine came up with novel alternative option for lost tissue, tissue engineering is" An interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function"[(1)]. Different models now are available: skin [(2)], oral mucosa [(3)] and muscle [(4)].

Histology and Anatomy

Mechanical properties of the engineered tissue are effected by their components especially the scaffold for extracellular matrix, it is wise to be familial with lip histology which effects also the functional outcome also. Basically lip tissue

engineering needs composite tissue from 3 types: skin, oral mucosa (for dry and wet vermillion) and muscle (orbicularis oris). Skin is composed from 3 layers: epidermis, dermis and hypodermis. Epidermis stratified squamous epithelium which contains five strata: Stratum Corneum (Cornified Layer), Lucidum, Granulosum, Spinosum, Germinativum (Basali). There are 3 types of cells encountered which are melanocytes, Langerhans cells and Merkel cells whereas dermis consists of connective tissue with structural elements of collegen and elastic fibers in addition to extracellular matrix. Skin appendages are involved mainly in the dermis as sebaceous glands, sweat glands, apocrine glands and hair follicles with papillary muscle. Blood vessels and nerve ending (Panician corpuscles) are located in the deep reticular layer of the dermis [(5)].

On the other hand, mucosa is composed also from 3 layers: surface epithelium, lamina properia and submucosa, there are few differences from skin histology table 1. Oral epithelium is keratinized stratified squamous which is either wet or dry depending on the amount of minor salivary glands, oral epithelium consists of 4 layers: the keratinized layer , granular layer, spinous layer and the basal layer. There are 3 types of cells as well which are melanocytes, Langerhans cells, and Merkel cells. The lamina properia is a connective tissue made by collagen and elastic fiber with extracellular matrix; mucosal appendages are minor salivary glands which are more abundant in the wet vermillion than dry vermillion. Fordcyte spot or granules are variant which can be found in the oral mucosa which correspond to sebum deposition from displaced sebaceous glands [(6)].

Table 1. Histology comparison between skin and lip mucosa

Skin	Oral mucosa
3 layers: epidermis, dermis, hypodermis	**3 layers**: surface epithelium, lamina properia, submucosa
5 layers of cells: Stratum Corneum (Cornified Layer), Lucidum, Granulosum, Spinosum, Germinativum	**4 layers** of cells: keratinized layer , granular layer, spinous layer, the basal layer
melanocytes, Langerhans cells, and Merkel cells	melanocytes, Langerhans cells, and Merkel cells
Skin appendages: sweat glands, sebaceous glands, apocrine glands, hair follicles	Salivary glands, Fordyce spots

Orbicularis oris muscle is complex striated muscle surround the oral fissure in form of spectrums of full ellipses as sphincter; there are four independent indentified quadrants (left, right, upper and lower) each quadrant consists from larger par peripheralis and smaller pars marginalis. So the lip has eight distinct anatomical segments. *Pars periphralis* attached to the modiolus through it stem fibers which are reinforced by buccinators, levator anguli oris, zygomaticus major and depressor anguli oris, then forms triangular muscular sheet which is thickest at skin-vermillion junction. *Pars marginalis*: is the part closely related to speech consists from fibers lodged within vermilion then meet with the other side fibers before attach to the dermis of vermilion, the anatomical and dynamic orientation between pars periphralis and pars marginalis is complex that maintains the unique lips' shape at both static and dynamic states (Fig. 1 A & B) [(7,8)].

The complexity of the lip action, in part, is related to the number of muscles attached, the upper lip is attached to 6 muscles at least: levator anguli oris, levator labii superioris, levator labii superioris alaeque nasi, zygomaticus major and depressor septi muscles, while the lower lip has attachments with 4 muscle:

depressor anguli oris, depressor labii inferioris, mentalis and orbicularis oris inferioris muscles [9], those direct labial tractors may act in group or individually to produce movement at the level of quadrants, pars or smaller portion.

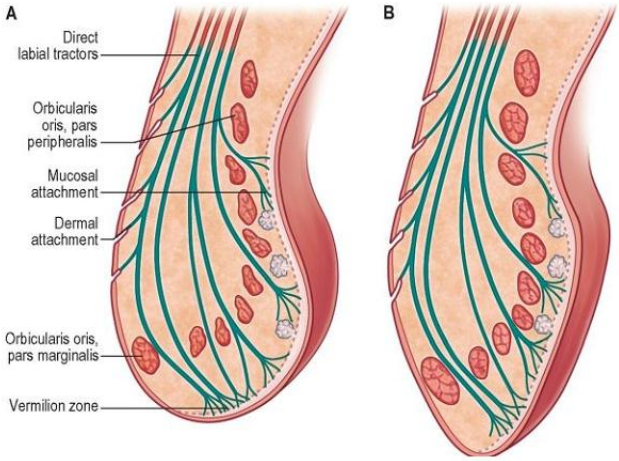

Fig. 1. Sagittal section of the upper lip in repose (A) Slightly contracted (B)

Lip protrusion is passive in its initial stages. It may be suppressed by powerful contraction of the whole of orbicularis oris or enhanced by selective activation of parts of the direct labial tractors However, the action of direct labial tractor will be modified by the oribularis and modiolar muscle, beyond a certain range of mouth opening the movement of the lips is almost dominated by the mandibular movement. Controlled three-dimensional mobility of the modioli enables them to integrate the activities of the cheeks, lips and oral fissure, the oral vestibule and the jaws [3].

Modiolar muscles themselves have anatomical variation that can be reflected on the movement's outcome e.g. risorius muscle found only in about 20% of Australians and 80% to 100% of Chinese and Malays [10], zygomaticus major muscle could be bifid with two distinct insertion points giving the appearance of dimple during smile [11].

Tissue engineering so far revolutionized a composite graft for lip reconstruction in form of muco-cutaneous junction (vermilion border) as continuous human oral mucosa-lip-skin construct, but they didn't address the orbicularis oris [12].

Stepping forward for full tissue engineered lip involving all three components (skin, mucosa and orbicularis oris) needs to know the biomechanical properties of the lip, as it is as intricate structure with different tissue compositions, attachment to different structures in variable directions and planes renders single movement of the lip quite complex in term of biomechanical parameters.

Basic of biomechanics

Biomechanics is the science concerned with the structure and movement of human, plants, organ and cells [13], there main potential parameters to be measured in biomaterials are biomechanical properties, strength (stress, strain and shear), modulus, elasticity, stiffness, viscoelasticity (creep, stress relaxation, and toughness and finally anisotropy, isotropy.

Strength: is ability of resistance of deformation before failure it can be expressed as a *stress* (when axial load is applied as compression force and expressed in pressure units) or *shear* (when the load is applied in multiple different directions and measured in pressure unit as well) or *strain* (when axial load is applied as tension force and it is quantified by division of length change over normal length so it is dimentionless), *Stiffness, elastic modulus or Young's modulus* is the ratio of stress to strain in the also can be calculated as the value of the force required divided by the degree of deformation, *elasticity:* is the ability of materials to return to its original shape after the load had been removed which is a basic requirement for lips, *toughness:* the amount of energy that absorbs by material then deforms before rupture *viscoelasticity:* is the response variability of stress and strain according to the rate of loading (time dependent) thus the lip is viscoelastic structure, *Creep* is the slow increase of the length (increasing strain) of a material over given period when imposed under a constant tensile stress, *Stress relaxation* is the decrease in stress over given period when a material is elongated to a set length, *Hysteresisis*

the property of viscoelastic materials of having a different unloading response than its loading response, *anisotropy:* is the complex mechanical behavior in response to loading that differs according to the direction of the leading force so lips are anisotropic structure, while *isotropy* has similar behavior regardless the direction of the force due to uniform structural unit [14].

Application of those parameters could be in static or dynamic state, practical application is easier for tissue having uniform structural unit with implications in single plane like skin [15]. Lips have more complex measures due to 3D anisotropic structure which involves different consistency of tissues. Even though, through the last four decades, many scholars had studied the perioral biomechanics both passive and active forms.

Eric Muller, a pioneer of biomechanics revealed that the perioral muscular attachments are complex due to interdigitations which interacts with very low inertial load [16], hence, unlike limb muscle, represents a great challenge for biomechanical testing and sampling. The biomechanical properties of the passive perioral tissue like tension, torsion, stress and viscoelasticity have much greater influence on mechanical output comparing to limbs [17].

Muller developed the first device to study perioral stiffness; it was three-dimensional space-frame model to provide baseline biomarkers in order to compare them with disease cases effect the perioral performance [16]. Geometrical and mechanical baseline indices had been identified among children younger than 12 years age, upper lip curvature coefficient showed maximum values in those aged between 2-3 years whereas upper lip elasticity, like other indices, had no significant difference with age [18].

Both active and passive perioral movements had been analyzed in relation to the muscle length (interangle span) in both healthy and disease people, the results were that the active force increased 4 times during maximum voluntary contraction with the increment of length with dramatic increase in male more than female, whereas passive forces showed no significant difference in relation to muscle length [19].

Shadmehr defined the "postural module" when a group of muscles synergize together to achieve a class torque of functions at constant equilibrium position but the stiffness is variable at this stage as part of activation of that postural module [20].

Velocity of lips movement is substantial for voice production specifically the lips move in higher velocity when the oral closure occurs during consonant sound production, i.e. another potential importance is the coordination between upper and lower lip for closure of oral aperture in specific time [21].

The lip shape is highly affected by the orbicularis oris muscle anatomy which is clearly evident on the lip gestures and protrusion with a link to cultural differences, the same effect on the lip gestures and protrusion had been elicited by jaw posture using the 3D model study [22].

Lip stiffness is one of the major parameters had been thoroughly assessed due to its magnificent role in movement, it can been can quantified after exerting specific displacement on the tissue and calculating the ratio of the resultant force over the displacement distance this will be expressed as stiffness quotient [19, 23].

A 3D model with multilayer deformable mesh had been made to assess the facial biomechanics including the perioral tissues for both active and passive muscle state presenting the data by linear approximations and the main target parameters were mass and stiffness for different anatomical layers (epidermal, dermal fatty, fascial and muscular layer) [24]. Another 3D model (3D finite element face model) to study the elastic properties of the face identified the significant effect of muscle stiffness during activation on the lip shape in term of protrusion and rounding [25]. Lip stiffness is potential for proper sound production (especially fricative) throughout the articulation process [21,26].

Lip stiffness measurements can be obtained through automated non invasive technology with real time acquisition and analysis of data during non contraction phase, the nonlinear regression technique revealed significant

relation between muscle stiffness and the provided displacement distance [27]. The same technique was used for both males and females proved no significant difference of gender on stiffness quotient [28].

Recently the (OroSTIFF) device provided measurements on non-participatory perioral stiffness; in addition to stiffness coefficient, the muscle activity pattern during active phase was also determined through the root-mean-square of both lips individually [29].

In conclusion, lips are intricate structure, mechanical properties had been studied because they affect the lip shape, gestures and functions in both static and active states, many properties are not related solely to the lip structure itself only but also to the attached surrounding tissues, also some measurements variations are attributed to the gender, age and race but baseline indices had been identified.

Limitations

Study of the tissue engineered product is usually done in vitro before application in vivo or clinical field, it is difficult to mimic the biomechanical environment with precise matching of tissue attachments and innervations, but, to some extent, is possible to identify some parameters in static or passive state and match accordingly. Another profound issue is the skeletal muscle tissue engineering, although the problem of vascularisation of tissue constructs had been resolved, the engineering of composite graft for 3 elements (skin, mucosa, muscle) simultaneously is still in challenge.

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Nocturnal Enuresis and its Relation to Child's Behavior in a Sample of Children from Baghdad, Iraq

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Abstract

Background Nocturnal enuresis is one of common clinical problems in pediatric population that frequently diagnosed among school age children.

Objectives To determine the prevalence of nocturnal enuresis, to detect the socio-demographic factors that may correlates with nocturnal enuresis and to assess the emotional and behavioral disorder in children with enuresis.

Methods A cross sectional study was performed among children (5-15 years old) visited the general pediatric out patient in Al-Imamain Al-Kadhimain Medical City in the capital Baghdad during the period from the first of December 2013 to the first of April 2014, a special questionnaire was prepared for this study with assessment of Parents' Rutter Behavioral Questionnaire.

Results Out of 623 children studied the overall prevalence of nocturnal enuresis was 29.5% (n=184). Male gender (60.3% of enuretic children), young age (37% of enuretic children at age 5-6 years old), positive family history of nocturnal enuresis (founded in 71.7% of enuretic children), large family size and increased number of household children (half of enuretic children living in extended family and household children more than four), these were significantly associated with the prevalence of nocturnal enuresis. Among the enuretic children, about half of them (45.1%) had moderate school performance, 50.5% had positive history of recurrent urinary tract infection and 63% had behavioral disturbances, so these factors were significantly associated with the prevalence of nocturnal enuresis.

Conclusion Nocturnal enuresis is a common problem among school children, with four-fold risk among children with disturbed behavior. Most of the families do not have adequate attention about enuresis and most of the enuretic children don't receive professional treatment.

Key words Nocturnal enuresis, prevalence, behavioral disturbance, and Rutter Behavioral Questionnaire.

List of abbreviation: % = percentage with total column, C.I = confidence interval, DSM = diagnostic and statistical manual of mental disorders.

Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V); nocturnal enuresis (also called bedwetting) is the status that a child must meet four criteria in order to be diagnosed with clinical enuresis: (a) repeated voiding of urine into clothing or bedding (whether involuntary or intentional); (b) the behavior is clinically significant as manifested by either a frequency of twice per week over the course of at least three months, or clinically significant impairment or distress in academic (occupational), social, or other important areas of functioning, (c) chronological age is at least 5 years of age, or have attained the equivalent developmental level, and (d) voiding behavior that is not exclusively due to the physiological effect of a substance (e.g. a diuretic or antipsychotic

medication) or another medical condition (e.g., spina bifida, diabetes, or a seizure disorder) [1].
Enuresis may be classified as primary in a child who has never established urinary continence for more than six months or secondary if resumption of enuresis occurs after at least six months of urinary continence. Alternatively, enuresis is said to be mono-symptomatic if it is uncomplicated or non-mono-symptomatic if concomitant lower urinary tract symptoms exist [2].

By the age of 3 years, nearly 75% of children attain nighttime dryness. Boys tend to be slower than girls in acquiring dryness. The prevalence of bedwetting decreases with increasing age. It is estimated that around 10-20% of 5-year-old children wet the bed at night. However, by adolescence only 1% continues to have this problem.

Genetic predisposition is the most frequently supported etiologic variable; the risk of nocturnal enuresis is 15% if neither parent was affected, 40-44% if one parent was affected and 75-77% [3].

Nocturnal enuresis was once thought to be a psychological condition; it now appears that psychological problems are the result of enuresis and not the cause [4]. For most children, bed-wetting is not an act of rebellion; on the other hand, stress is a cause of people who return to wetting the bed. Researchers find that moving to a new town, parent conflict or divorce, arrival of a new baby, or loss of a loved one or pet can cause insecurity and contributing to returning bedwetting [5,6].

Iraq has passed through several conflicts and Baghdad –the capital had never been secured since 10 years that contributed for abnormal behaviors among children, for which this study was conducted to determine the prevalence of nocturnal enuresis in a sample of children from Baghdad and to assess the emotional and behavioral disorders in children with enuresis.

Methods

This cross-sectional study was carried out from the first of December 2013 to the first of April 2014 at the pediatric out-patient in Al-Imamain Al- Kadhimain Medical City/ Baghdad-Iraq. The study involved all children aged 5-15 years taken consecutively during the study period. Children with cerebral palsy, spina bifida or diabetes mellitus were excluded from the sample.

The parents' or the care giver of the children were interviewed directly using a questionnaire specially prepared for this study that consisted of three parts:

The first part included socio-demographic characteristics of the sample.

The second part was designed to enquire about the presence of nocturnal enuresis and its relevant characteristics. Children without nocturnal enuresis were asked about the age of dryness.

The third part consisted of Rutter Behavioral Questionnaire [7] as reported by the parents (preferably the mother); that was translated to Arabic language by the researcher and approved by three psychiatrists.

Approval of Al-Nahrain College of Medicine Institution Review Board to conduct this study was obtained and a written informed consent from the parents to participate in this study before data collection was obtained also. Data collected were analyzed using SPSS-16 software for windows, chi-square test, t-test, ANOVA and multivariate logistic regression tests were used for the statistical analysis whatever applicable and *P* value of less than 0.05 was considered statistically significant.

Results

A total of 623 children with mean age of 8.00 ± 2.49 years (ranged 5-15 years) were included, of them 53.3% (n=332) were males.

According to DSM-V [1], the overall prevalence of nocturnal enuresis was 29.5% (n=184), 84.8% (n=156) were of primary type and 15.2% (n=28) had secondary nocturnal enuresis. Enuresis was every night in frequency in 58.7% (n=108) of the children, 5.4% (n=10) had 4-6 times per week, and 35.9% (n=66) had 1-3 times enuresis per week. Males were significantly more enuretic than females (60.3% versus 39.7% and P= 0.02).

Nocturnal enuresis decreased very clearly with increasing age from 36.9% (n=68) in children aged 5-6 years old to 3.8% (n=7) in children ≥ 13 years old, with significant association between the age and the sex of enuretic children (Table 1).

The results revealed that having extended families with more children and positive family history of nocturnal enuresis (including both parents side and siblings), were significantly higher among enuretic children compared to non-enuretic children. Also children with positive history of urinary tract infection and bad school performance (based on the final school report for the previous year) were significantly more among enuretic children (Table 2).

More than one third of the participated children 221 (35.5%) scored 13 or more by Rutter behavioral questionnaire indicating emotional and behavioral disturbances. A significant differences (P = 0.000) between the mean score for enuretic (13.712 ± 5.113 degrees) and non-enuretic children (9.751 ± 5.197 degrees) was found (Table 3).

Moreover, logistic regression design for nocturnal enuresis for almost all the studied contributors showed disturbed emotion and behavior among the studied children was the only responsible factor for having enuresis in this sample (Table 4).

Discussion

Enuresis is an important health problem both from medical and social perspective. It can be troublesome to normal family life and can generate stress between parents and children. Previous studies have shown varying prevalence rates of nocturnal enuresis in children, these variations may be attributed to differences in the objectives and definitions adopted by researchers. Worldwide, the prevalence of nocturnal enuresis among 5-15 year old children was reported as 4.6%–28.6% [8-15]. The prevalence found in this study was higher than that reported by most studies including other Iraqi studies that reported different prevalence of nocturnal enuresis ranged 6-24.7% [16-21], the rate reported by a study in Niger was 23.2% of (6-12 years old children) [13] and in Turkey 23.5% of (6-14 years old children) [14], while comparable to a study in Yemen, Mukala, a prevalence of 28.5% for 6-15 years old children was reported [15].

Possible reasons for such wide variation among countries and even those within Iraq might be due to socio-cultural variations between the countries and regions and the difference in study design starting from the definition of the study population, population based or health facility-based, and may be due to differences in selection criteria, including age ranges, definitions of enuresis, genetic predisposition, traditional and cultural background.

Table 1. Relation between the age and sex distribution of nocturnal enuretic children

Age (years)	Males		Females		Total		Significance
	No.	%	No.	%	No.	%	
5-6	33	29.7	35	47.9	68	36.9	
7-8	22	19.8	18	24.7	40	21.7	
9-10	24	21.6	16	21.9	40	21.7	X^2 = 17.149
11-12	25	22.5	4	5.5	29	15.7	P = 0.002
≥ 13	7	6.3	0	0	7	3.8	
Total	111	100	73	100	184	100	

Table 2. The relation of nocturnal enuresis and some selected factors

Factors	Categories	Nocturnal enuresis				*P* Value
		No		Yes		
		No.	%	No.	%	
Mother education Level (n=617)	Primary school or less	255	58.8	104	56.8	0.658
	High school or more	179	41.2	79	43.2	
Father education level (n=595)	Primary school or less	179	42.6	77	44	0.757
	High school or more	241	57.4	98	56	
Mother occupation (n=617)	Housewife mother	415	95.6	177	96.7	0.527
	Working	19	4.4	6	3.3	
Father occupation (n=595)	Unemployed	12	2.9	7	4	0.705
	Employed	277	66	111	63.4	
	Self-employed	131	31.2	57	32.6	
Dead parent (n=623)	No	415	94.5	174	94.6	0.987
	Yes	24	5.5	10	5.4	
Family composition (n=623)	Nuclear	254	57.9	85	46.2	0.008
	Extended	185	42.1	99	53.8	
Number of children (n=623)	≤ 4	205	46.7	57	31	0.001
	5-8	175	39.9	97	52.7	
	≥ 9	59	13.4	30	16.3	
Family history of nocturnal enuresis (n=623)	No	372	84.7	52	28.3	0.000
	Yes	67	15.3	132	71.7	
Polygamy (n=623)	One wife	414	94.3	168	91.3	0.168
	More	25	5.7	16	8.7	
Consanguinity (n=623)	Yes	177	40.3	85	46.2	0.175
	No	262	59.7	99	53.8	
History of Recurrent UTI (n=623)	Yes	119	27.1	93	50.5	0.000
	No	320	72.9	91	49.5	
School Performance (n=440)	Good	163	53.1	55	41.4	0.002
	Moderate	129	42	60	45.1	
	Fail	15	4.9	18	13.5	
Emotional and behavioral disturbance (n=623)	Yes (score ≥ 13)	105	23.9	116	63	0.000
	No (score < 13)	334	76.1	68	37	

Table 3. Rutter score distribution according to nocturnal enursis

Nocturnal enuresis	Rutter score Mean ± SD	Total	t-test	*P* Value
Yes	13.712 ± 5.113	184	8.719	0.000
No	9.751 ± 5.197	439		
Total	10.92±5.47	623		

Table 4. Multivariate analysis of the risk factors for nocturnal enuresis

Factors		Coefficient	Hazard ratio	95% CI	*P* value
Age (years)		0.006	1.006	0.930-1.088	0.885
Sex	Female Male	0.160	1.173	0.789-1.745	0.429
Mother education Level	Primary school or less High school or more	0.030	0.970	0.646-1.458	0.884
Father education level	Primary school or less High school or more	0.036	0.965	0.646-1.440	0.860
Mother occupation	Housewife Working	0.135	1.144	0.408-3.207	0.798
Father occupation	Unemployed Employed	0.074	1.077	0.374-3.099	0.891
Dead parent	No Yes	20.942	1.245E9	0.000	1.000
Family Composition	Nuclear Extended	0.232	1.261	0.812-1.960	0.302
Number of children		0.022	1.023	0.960-1.090	0.487
Polygamy	One wife More than one	0.268	1.307	0.608-2.808	0.492
Consanguinity	No Yes	0.280	1.323	0.886-1.974	0.171
Behavioral disturbance	No Yes	1.664	5.279	3.562-7.822	0.000

Most of enuretic children in the present study were of primary type 84.8% and 15.2% had secondary enuresis, this was comparable with most of previous studies [9,11,13,18,20]. While the severities of enuresis happened every night in more than half of the children, this was comparable to studies in Basra, Iraq [20], Nassiryiah city, Iraq [18], and in Yemen, Aden [11]. While a study in Jordan [8] reported only 27.5% had every night bed wetting.

Many studies demonstrated that the prevalence of nocturnal enuresis tended to decrease with increasing age [10,13,17] as nocturnal enuresis is mostly expected to improve spontaneously [22], and this was comparable with a study in Nassiryiah city, Iraq which reported the prevalence of nocturnal enuresis decline from 40.6% at 5-6 years old to 5.4% at 13-15 years old [18], and with a study in Baghdad [21] that reported most of enuretic children (64.5%) were observed at 8-9 years of age and 23.5% at age 10-12years while only 12% occurred at age 6-7 years.

The results of this study showed the prevalence of nocturnal enuresis was significantly more among males than females but females were younger in age group while more than quarter of the enuretic males was older than 10 years old. This was comparable to findings of a study from Sudan, Niger, and Iraq [10,13,19,20], while significant predominance among females was reported by a study done in Yemen, Mukala [15] and a study in Baghdad [21].

In this study, the results revealed that the education level, unemployed and death of parents played no role in the prevalence of nocturnal enuresis and this was in agreement with studies from Turkey [14] Sudan [10] and a

study in Baghdad [21], but in contrast with results of studies done in Jordan [8], Yemen, Aden [11], Niger [13], and other Iraqi studies [17,19] which stated that low education level of parents was significantly associated with the prevalence of nocturnal enuresis. And a study in Egypt, Menofia [9] reported that working mothers were found to have less enuretic children than housewives and this was thought to be due to the higher educational level of working mothers.

This study showed the prevalence of nocturnal enuresis was significantly associated with positive family history of nocturnal enuresis, large family size and increased number of household children, and this agreed with several studies [15,17,19,20], probably because nocturnal enuresis is commonly a familial disorder, which often has strong genetic roots with higher frequency in parents and sibling of bed wetter than in general population. On the other side, a study in Yemen, Aden [11] did not agree with this result and reported there was no significant association between the prevalence of nocturnal enuresis and the number of household children or family composition.

In this study, the personal factors which included the school performance and history of urinary tract infection had a significant association with the prevalence of nocturnal enuresis, and this agreed with other studies in Iraq [17,21].

The results of this study found three times higher rate of the behavioral disturbance among enuretic children (63%, n=116) than among non-enuretic children (23.9%, n=105) with a significant correlation between the presence of behavioral disturbance and nocturnal enuresis (P= 0.000), with four times increase risk of nocturnal enuresis among disturbed children.

Chang et al found that enuresis was associated with childhood behavioral problems, in particular attention problems, aggressive behavior, lower social competence and low school performance [23].

Comparable to our results a study in UAE [5] found a significant association between the nocturnal enuresis and behavioral disturbance by using Rutter behavioral questionnaire, and a study in Egypt, Menofia [9] reported four times increase risk of nocturnal enuresis among children with emotional and behavioral disturbance. On the other hand, many studies reported that bedwetting had adverse impacts on children's mental health [15,19,24,25] as children with nocturnal enuresis may experience social isolation, fear of detection, sense of immaturity, and loss of self-esteem, all of which act as a psychological stressors that may increase the risk for behavioral and emotional problems. Furthermore, nocturnal enuresis and behavioral problems may share the same biological, social, and psychological causes [26].

In conclusion, enuresis is a pediatric public health problem that associated with male gender, low age, familial factors, personal factors and emotional and behavioral disturbance. Most of the families don't have adequate attention about enuresis and most of the enuretic children don't receive professional treatment.

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Author contributions

Conception and design, data collection, analysis, interpretation, writing and revision of the manuscript were performed by both authors.

Conflict of interest

None

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Immunopathological Study of *Fasciola Hepatica* and Hydatid Fluid Antigens on Hydatid Cysts development in Mice

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Abstract

Background Hydatidosis is a chronic cyst forming helminthic disease of human, domestic and wild animals. The immune responses play a pivotal role in limiting cystic larval development.

Objective To investigate the immunopathological effects of *Fasciola hepatica* antigens (FHAgs) and hydatid cyst fluid antigens (HCFAgs) on the immune response against hydatidosis in mice.

Methods One hundred mice were divided into 5 groups; the 1st three were immunized subcutaneously with 0.2 ml of HCFAgs, FHAgs and mixed antigen (MAg = HCFAgs+FHAgs), respectively. The last two regarded as control groups. On day 27 post immunization, the skin test was done for the 1st four groups. On day 30, half the animals of each group were scarified to perform E-rosette test, agar gel diffusion (AGD), and ELISA test. The rest of the mice with the 5th group were challenged with 2000 viable protoscolex / mouse. After 3 months, they were scarified for gross and histopathological examination.

Results The immunized group with MAg was hypersensitive prior to challenge the skin test, while E-rosette test indicated the highest ratio of active lymphocytes before and after challenge in comparison with other groups. The AGD test showed severe reaction between the sera of mice immunized with MAg against the FHAgs against HCFAg and between sera of immunized group with FHAgs against HCFAg and vice versa. The highest level of antibodies was recorded in the immunized group with MAg. Histopathological examination for the internal organs of immunized groups revealed granulomatous lesions, reduction in the number of cysts with lymphocytic hyperplasia and presence of degenerative protoscolices. Control infected group revealed growth of cysts in the internal organs, with degenerative and necrotic lesions.

Conclusion The 3 types of Ags stimulate humoral and cellular immunity was proved by immune-histopathological investigations. The MAg was highly immunogenic in comparison with each Ag alone. This antigenic activity may be due to the presence of synergistic interactions and cross-reactivity between the 2 parasites and this can be used as protective value against hydatidosis in the intermediate hosts.

Keywords Parasitic infections, immunological cross reaction, isolation parasitic antigens, *Fasciola hepatica* antigens, Hydatid fluid antigens.

List of abbreviation: CE = cystic echinococcosis, AE = alveolar echinococcosis, Ags = antigens, Ab = antibodies, HC = hydatid cyst, PSC = protoscolices, HCFAg = HC fluid antigen, FHAg = *Fasciola hepatica* antigen MAg = mixed antigen, PBS = phosphate buffered saline, SFHFAg = soluble filtrated hydatid fluid antigens, SFHAgs = sonicated *Fasciola hepatica* antigens, SSFHAgs = soluble sonicated *Fasciola hepatica* antigens, NO = nitric oxide.

Introduction

Echinococcosis is a zoonotic parasitic disease caused by the adult or larval stages of the cestodes belong to the genus *Echinococcus* (family Taeniidae). Larval infection (hydatid disease, hydatidosis) is a chronic cyst forming helminthic disease of human beings as well as domestic and wild animals [(1)]. The important medical and public species are *Echinococcus granulosus,* and *Echinococcus multilocularis,* which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), both are serious diseases [(2)]. The AE

especially with poor prognosis if careful clinical management is not conducted [2,3].

The immune responses play a pivotal role in limiting cystic larval development. Lightowlers *et al* [4] reported that the vaccine conferred a high degree of protection against challenge with different geographical isolates of *Echinococcus granulosus (E. granulosus)*. Then results indicate that vaccine could have wide applicability as a new tool for use in hydatid disease control campaigns [5].

In another experiment, Hashemi and Razmi [6] reported that the antigens (Ags) of whole body of *E. granulosus*, might be a good candidate for immunization and diagnosis of hydatid cyst (HC) in an intermediated host. *Fasciola hepatica* (*F. hepatica*) has a wide distribution especially in Iraq, and sometimes there is mixed infection of hydatidosis and fascioliasis; and in an attempt to suggest a defined vaccine against HCs and *F. hepatica* infection, this study were designed [7].

The aims of current study are to evaluate the role of *F. hepatica* Ags and hydatid fluid Ags against HC infection in mice by performing immuno-pathological studies plus the correlation and assessment of the immunological and the pathological changes with the 3 types of Ags that used for immunization to get a good candidate for immunization and diagnosis of HC in the intermediate hosts.

Methods

Laboratory animals

Total number of (100) mice (BALB/C) of both genders ranged 4-6 weeks old, and weighted between 19-24 g. They were divided into five groups; each group had 20 mice, 1st, 2nd and 3rd groups were immunized with HC fluid Ag (HCFAg), *Fasciola hepatica* Ag (FHAg) and mixed Ag (MAg) respectively, 4th and 5th groups were considered as control groups, both treated with phosphate buffered saline (PBS), then the fifth infected with HC and was left for 3 months.

Preparation of Ags

Hydatid cyst fluid antigens

According to Moosa and Abdel-Hafez [8], four hundred ml of hydatid fluid was aseptically aspirated from 35 fertile HCs lodged in sheep livers. The fluid containing protoscolices (PSC) and membrane fragments was pooled and centrifuged at 5000 g for 30 minutes at 4 °C to precipitate PSCs. The supernatant was dialyzed and processed according to McVie et al [9] to get HCFAgs. The protein concentration was measured using UV-visible spectrophotometer at wave lengths 280 and 260 nm, respectively, it was 3.3 mg/ml. HCFAg antigens was stored at -20 °C till use (immunization), and 20 ml of antigens was centrifuged with ultra cold centrifuge (13000 rpm for 30 minute), supernatant was called soluble filtrated HFAg (SFHFAg) was stored at -20°C till use for skin test.

Fasciola hepatica Antigens

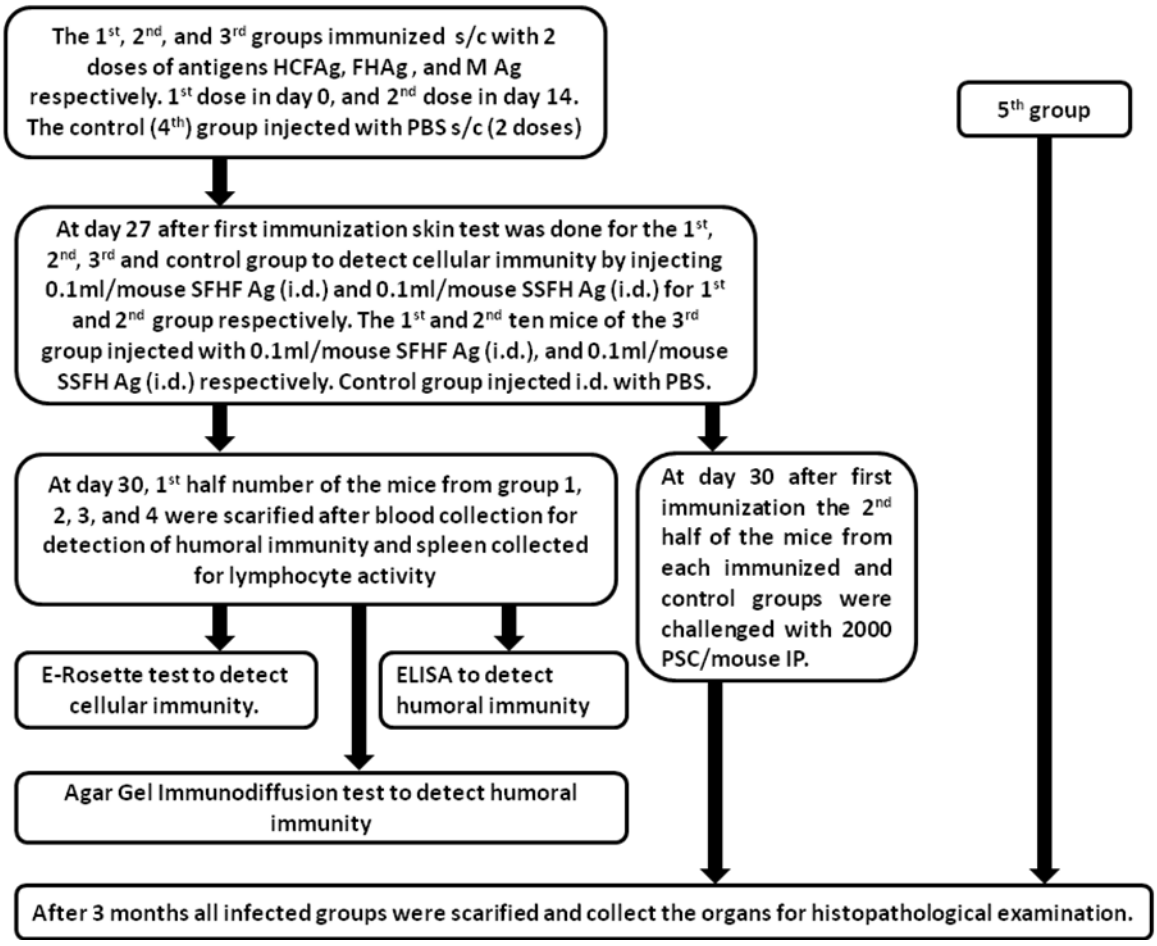
Thirty-five adult flukes were collected from livers samples of sheep infected with fascioliasis. The worms were identified morphologically according to Threadgold [10]. They were washed and homogenized in PBS pH 7.4 using an electrical homogenizor (ultra-turrax) and processed according to Abdel-Rahman et al [11] to get sonicated *Fasciola hepatica* antigens (SFHAgs). Protein concentration was 4.1mg/ml using UV-visible spectrophotometer at wave length 280 nm, 260 nm respectively, then it was diluted with distal water in order to make its concentration 3.3 mg/ml. SFH Ags was stored in a deep freezer until use. Fifteen ml of the supernatant was centrifuged with ultra cold centrifuge at 13000rpm for 30 min, to get soluble sonicated FHAg (SSFH Ags) then was stored at -20 °C till use.

Preparation of protoscolices and estimating the challenge dose

PSCs were collected aseptically from 10 fertile *E. granulosus* cysts, size (4-6 cm) from the liver of infected sheep. The collected PSCs were washed in PBS (PH7.4) containing 100 µg/ml of gentamycin, then the viability and the quantity of PSCs was determined according to Morel [12].

Experimental design

One hundred mice divided into five groups as shown in the box below:



Delayed type hypersensitivity test

Delayed type hypersensitivity test was done according to Ohta et al [13] for the all animals at 27th day post immunization as shown in fig.1.

Erythrocytes -Rosette test

This test was performed according to Braganza *et al* [14] to evaluate the viability and the activity of isolated T-lymphocytes from blood of immunized and control groups.

Agar gel diffusion test

This test was done according to Bombardier and Ggiordano [15]. 50 µl of pooled sera from each group of mice were isolated from their blood samples, they were diluted serially (1:50, 1:100, 1:200) in carbonate-bicarbonate buffer. Also 10 µl from each antigen was diluted serially (1:2, 1:4, 1:8). Best dilutions for the sera and antigens that gave visible precipitate line in the agar gel diffusion test were used in enzyme linked immunosorbent assay (ELISA) [16].

Enzyme Linked Immunosorbent Assay

Determination of specific antibodies was carried out as described by Ferragut and Nieto [17], with simple modifications briefly, 96-well microtitration plate was coated with 200 µl/well of antigens (HCF, FH or Mix) diluted 1:8 in carbonate-bicarbonate buffer pH 9.6 and incubated over night at 4 ^{o}C in a humid chamber. Excess antigen was removed and washing the wells four times with PBS – Tween 20 pH 7.4, and blotting by using a filter paper, then blocking was done with 200 µl of 1% Bovine serum albumin in PBS at 37 ^{o}C for 1hr. Wells were dried, and added 200 µl/well diluted sera at a 1:200 dilution in PBS –T20 and incubated 2 hr at room temperature, the plate was washed with PBS – T20 as described above, 200 µl of freshly diluted conjugate (HRP-Goat anti mouse IgG) 1:10000 in diluent reagent 1% BSA-PBS was added in each well and incubated for 3hr at 37^{o}C in humid chamber. The plate was washed as

described above to remove excess conjugate; 200µl of substrate solution (OPD and H_2O_2) was added to all well and left for 30 min in a dark place. The reaction was terminated by adding 50µl H_2SO_4 2.5 M, the optical density was read on (630) nm with a by bio-tek micro plate reader (model 450).

Preparations of tissue sections

For histopathological study tissue samples were taken from internal organs of immunized and control groups. They were fixed in 10% formalin and processed routinely according to Banccroft and Steven [18].

Statistical analysis

Data were analyzed statistically using the Microsoft Program (SPSS). Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of (P<0.05). Specific group differences were determined using least significant differences (LSD) as described by Snedecor and Cochran [19].

Results

The results of delayed type hypersensitivity showed a higher significant (P < 0.05) increase in the means of foot pad thickness of immunized groups as compared with the control. A higher increase of thickness detected in the mice treated with mixed antigen (HCFAg + FHAg) in comparison with immunized and control group as shown in table 1.

Table 1. Foot pad thickness of immunized and control groups at 27th day post immunization.

Group	Skin test before challenge with PSCs		
	24hr	48hr	72hr
Control	1.8±0.1 A c	1.8±0.1 A c	1.8±0.1 A c
HCFAgs	2.4±0.2 B a	3.7±0.2 A b	3.4±0.2 A b
FHAgs	2.06±0.06 C b	2.6±0.1 A b	2.4±0.2 B b
MAgs	2.7±0.1 B a	3.12±0.1 AB a	3.4±0.2 A a

HCFAg = Hydatid cyst fluid antigen, FHAg = *Fasciola hepatica* antigen, Mag = mixed antigen

The results of erythrocytes-Rosette test showed significant differences (P < 0.05) within the immunized groups, also between the immunized and the control groups, a higher stimulation (0.85±0.05) observed in immunized mice with M Ag (Table 2).

Table 2. E-rosette formation after 18hr incubation in RPMI-1640 (before challenge)

List	Group	E rosettes (% ± s.d.)
1	FHAgs	0.37±0.05
2	HCFAgs	0.59±0.06
3	HCFAgs+FHAgs	0.85±0.05
4	Control	0.25±0.06

HCFAg = Hydatid cyst fluid antigen, FHAg = *Fasciola hepatica* antigen

In the technique of agar gel diffusion test, diffusion of the antigen and anti-sera took place through the agar. Visible precipitin lines are formed in the gel at the point of equivalence. The results showed precipitin bands between sera of immunized mice with M Ag and the HCF Ags and FH Ags. Also precipitin bands observed between sera of infected mice with PSCs and HCF Ags and FH Ags (Fig. 1).

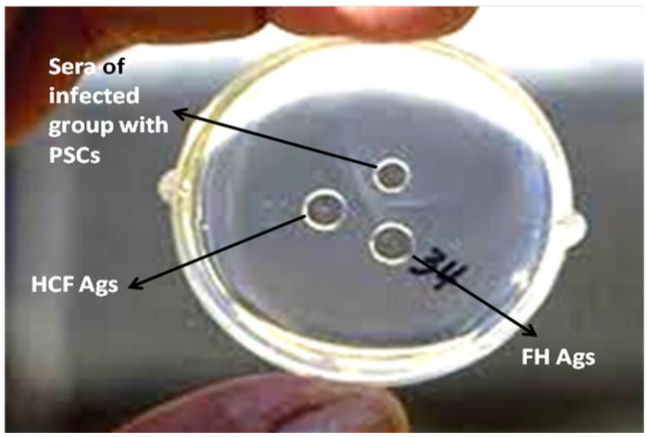


Fig. 1. Agar gel diffusion

ELISA of all immunized mice showed a strong response to the three antigens. The results reveal a significant difference (P < 0.05) between immunized and control groups. A higher level of

Abs (0.462±0.009) is found in sera of mice immunized with M Ag (Table 3).

Table 3. Antibody titer (OD) in sera of immunized and control groups by ELISA

List	Group	OD (mean±SE)nm/ml
1	FHAgs	0.293±0.007
2	HCFAgs	0.272±0.004
3	MAgs	0.462±0.009
4	Control	0.06±0.004

HCFAg = Hydatid cyst fluid antigen, FHAg = *Fasciola hepatica* antigen, MAg = mixed antigen

Gross pathological changes

A number of secondary HCs were observed in mice 3 months post infection, unilocular white fluid filled cysts, measuring from 1-2mm mainly in liver and abdominal cavity (Fig. 2). No gross lesions were seen in the examined organs of immunized animals that were scarified after challenge except the presence of variable degrees of splenomegally and hepatomegally with congestion of some organs mainly in the immunized mice with MAg.

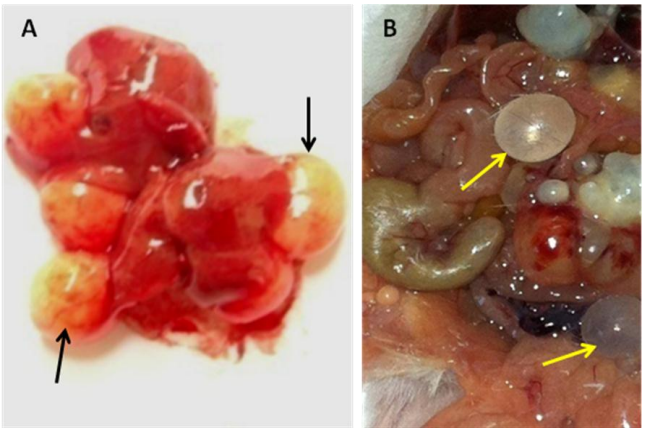


Fig. 2. Macroscopical examination of control group showed multiple unilocular, small secondary hydatid cysts on liver surface (A) and other internal organ (B) three months post challenge

Histopathological changes

In non-immunized infected animals (positive-control group) (Fig. 3) and immunized infected animals were found in different internal organs as shown in fig. 2 through 10 which revealed appearance of HCs in the liver and other internal organs of control infected group, with focal and diffuse amyloid deposition in the spleen. The immunized groups revealed absence of cysts in the liver, presence of granulomatous lesions, appearance of degenerated PSCs, appearance of lymphoid proliferation in spleen and lung (BALT), suppurative pneumonia, and presence of extensive fibrous capsule in the spleen and kidney of mice immunized with M Ag.

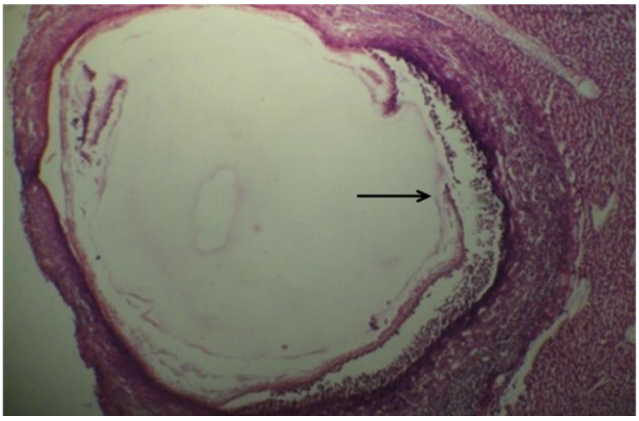


Fig. 3. Microscopical section in the hydatid cyst of control infected animal three months post challenge showed hydatid cyst with all three layers (germinative, laminated and adventitia (H&E X10)

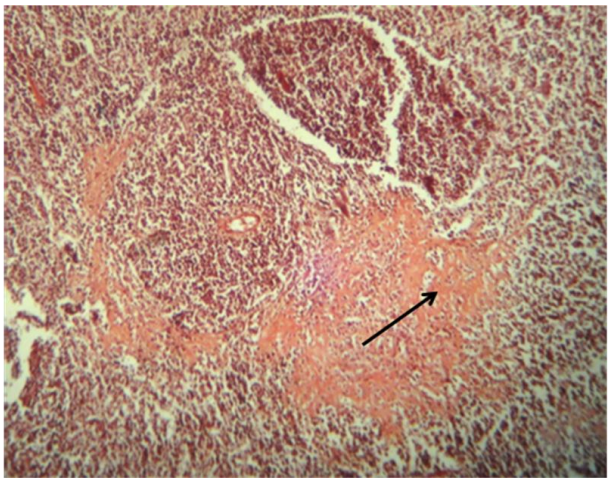


Fig. 4. Microscopical section in the spleen of control infected animals three months post challenge showed positive Congo red result (pale pink to red color) with focal and diffuse amyloid deposition (Congored X40).

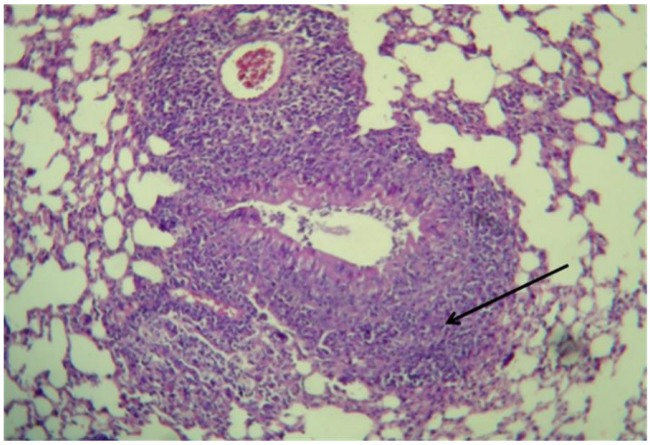


Fig. 5. Microscopical section in the lung of immunized mice with HCFAgs showed Hyperplasia peribronchiolar associated lymphoid tissue three months post-challenge (H&E X20).

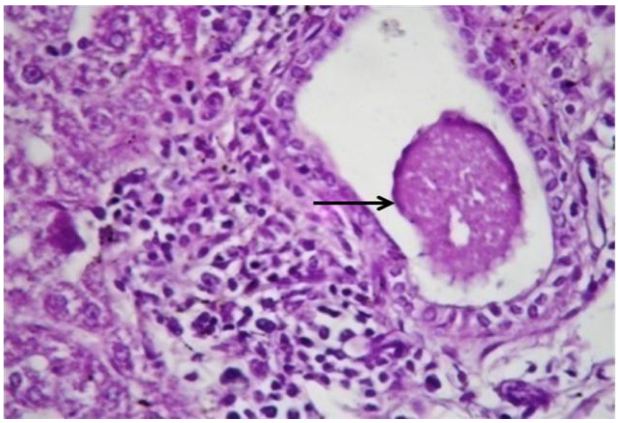


Fig. 6. Microscopical section in the liver of immunized animal with HCF Ags three months post challenge showed moderate MNCs aggregation in liver parenchyma mainly around dilated bile duct with appearance of degenerated protoscolex (H&E X40)

Discussion

Skin test (delayed type hypersensitivity-DTH)

We suggest that the parasitic Ags (immunization or mixed infection) synergistically interact with each other and probably an important influence on the immune system [20,21]. Also this could be due to presence of similar epitops in both parasitic Ags that is responsible for the cross-reactive effect against hydatidosis in sheep [11,22]. The formation of rosettes is a specific test for cells of T lineage. The results can be due to that these antigens stimulate cellular immune response through activation of T-lymphocytes [8,23].

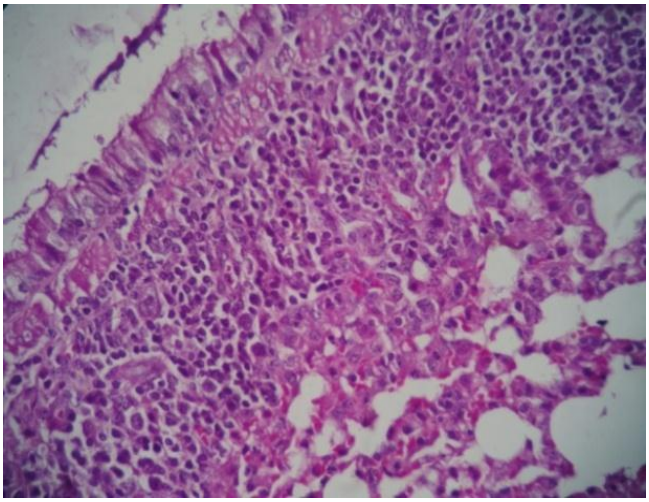


Fig. 7. Microscopic section in the lung of immunized animal with FH Ags showed suppurative pneumonia three months post challenge (H&E X40).

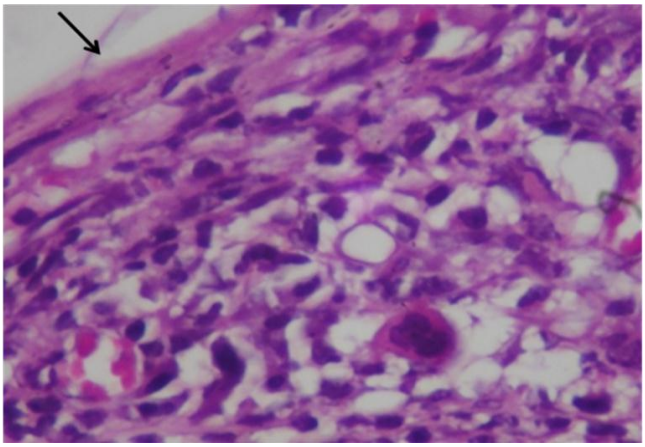


Fig. 8. Microscopical section in the spleen of immunized animal with M Ag, three months post challenge showed increase thickening of capsule with fibrosis together with congestion of red pulp (H&E X40)

The results of agar gel diffusion test may be due to the presence of common Ags in different parasites react against same antisera [24].

The results of ELISA were in agreement with Babba *et al* [25], Poretti *et al* [26], Lin *et al* [27], and Gaudier *et al* [28].

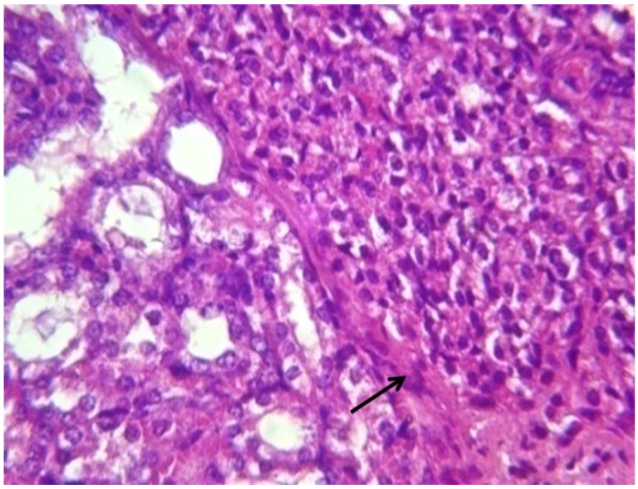


Fig. 9. Microscopical section in the kidney of immunized animal with M Ag three months post challenge showed increase Thickening of renal capsule with fibrosis (H&E X20)

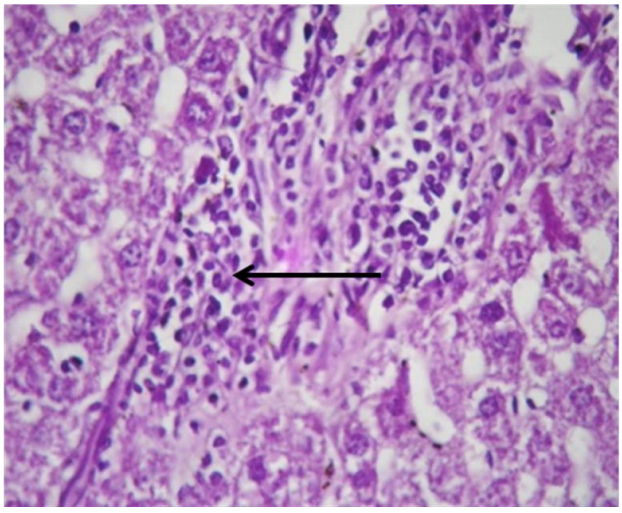


Fig. 10. Microscopical section in the liver of immunized animal with HCF Ag three months post challenge showed multifocal graulomatus lesion in liver parenchyma associated with vacuolar degeneration (H&E X40)

The results of this study proved a synergism and across reaction between two economically important zoonotic helminthes belong to different classes. This cross reaction was observed by ELISA in which Abs raised against one Ag cross reacted with the other antigens [11,20,21,22].

The appearance of HCs in the liver of control infected group associated with vacuolar and necrotic changes as well as atrophy of splenic white pulp indicate that PSC reached the liver through the peritoneum where the parasite developed and overcome the host defense with its secretion of immune complexes that inhibit immune mechanism of infected host [29]. The absence of cystsin the liver of immunized groups with HCF Ags and M Ag indicate that the immune response prevent the PSC to develop in the host [7]. Vuitton (30) suggested that parasite may avoid the host immune system because of low immunogenicity by masking surface Ags as well as interfering with Ag-presenting mechanism.

As well as granulomatous lesion (Fig. 10) may also be seen in the examined organs at three months post challenge in the immunized groups due to the host defense mechanism which attempts to localize and destroy the PSC through inducing proliferation and aggregation of phagocytic cells mainly alternative activated macrophage which produce cytokines IL- 8 and IL-12. Also the present results reveal the appearance of degenerated PSCs in the liver of immunized group with HCF Ags (Fig. 6) this may be due to the crucial and important role of IL-12 in the inhibition of larval growth, and was originally termed natural killer cell [31]. The appearance of lymphoid proliferation in spleen and lung (BALT) (Fig. 5) showed hyperplastic changes as a result to its persistent stimulation by HCF Ags especially 3 months P.I that revealed a good immune response following PSC infection which act as a mitogen stimulate lymphoid cells. Suppurative Pneumonia which was noticed in the present study (Fig. 7) may be due to the deposition of immune complexes as a result of pulmonary tissue injury [7,32].

The spleen revealed lymphoid depletion in the immunized group with *Fasciola hepatica* Ags, this may be due to the role of nitric oxide (NO), the FH excretory/secretory antigens decrease nitrate production by host peritoneal cells, a mechanism to avoid an immune response during the first stage of liver penetration. This could explain the transient suppression observed in spleen mononuclear cell proliferation response; on the other hand NO production could also be

one of the strategies of parasite to avoid potential killing effect of NO during peritoneal migration [33].

The presence of extensive fibrous capsule in the spleen (Fig. 8) and kidney of mice immunized with M antigen (Fig. 9) refers to the potential role of M antigen in activating and stimulating the host immune response and this capsule protects the host against PSCs effect that killed them before development so the results of kidney and spleen fibrosis would tend to cast doubt on the idea that the resistance developed against a challenge of PSC was due to physical barrier presented by extensive capsular fibrosis in kidney and spleen. In conclusion, this study showed that the three antigens (FHAgs, HCFAgs, and M Ag) stimulate both cellular and humoral immune response, and this is clear in the histopathological observations by preventing the development of HCs in the immunized group with 3 types of Ags. The mixed Ag gave high response for both humoral and cellular immunity. Using mixed Ag gave higher response or reactivity in skin test, E-rosette, ELISA and in agar gel diffusion, while other groups showed less response than the M Ag in comparison to control group due to the synergism and cross-reactivity between the two parasites.

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Author contribution

Dr. Faleh suggests the study and reading the histopathological findings; Dr. Khalil performs the immunological part and Dr. Al-Maliki collects and identifies the parasites and preparing the antigens.

Conflict of Interest

No author declares any interest in publishing this article or competitive intentions.

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Immunophenotypic Comparison between Reactive Bone Marrow B-Lymphocyte Precursor (Hematogones) and B-Neoplastic Lymphoblast Leukaemia Using Cd 34, Cd 123 by Flowcytometry

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Abstract

Background Flow cytometric study found that lymphoblasts of B acute lymphoblastic leukemia exhibited multiple aberrant antigens by which they can be distinguished from hematogones. These antigens are CD34 and CD123.

Objective To determine the immunophenotypic pattern of CD34 and CD123 expression in hematogone of reactive bone marrow and in neoplastic lymphoblast in B-acute lymphoblastic leukemia (ALL) patients and to evaluate the impact of that pattern in the residual disease detection after chemotherapy.

Methods This is a case control study to determine the expression of CD34 and CD123 in 30 patients newly diagnosed with B-ALL. Re-assessment was done for 20 patients of them after 4-6 weeks of chemotherapy; in addition to 10 patients with reactive bone marrow to assess hematogones.

Results In (93.4%) of the newly diagnosed B-ALL cases, leukemic blasts expressed both CD34 and CD123, Conversely, in (6.6%) cases, neither antigen was expressed. In hematogones; the immature hematogones (dim CD45, CD34 +) did not express CD123 while the mature hematogones (moderate CD45+, CD34-) expressed CD123. The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and hematogones respectively in post chemotherapy patients remain stable.

Conclusion The distinct pattern of CD34 and CD123 expression on hematogones (discordant) and B-ALL blasts (concordant) is useful in correctly classifying immature B cells as residual leukemic blasts or hematogones in the bone marrow of patients treated for B-ALL.

Keywords B-Acute Lymphoblastic leukemia, Flow cytometry, Immunophenotypic aberrancy, Hematogones, CD34 and CD123.

List of Abbreviations: ALL = Acute lymphoblastic leukemia, BM = Bone marrow, FAB = French-British-American, HGs = hematogones, RD = residual diseases, WBC = white blood cell count, PAS = Periodic Acid Schiff, FC = Flow cytometry, SBB = Sudan Black B.

Introduction

Acute lymphoblastic leukemia (ALL) is a clonal hematologic disorder. It involves excessive proliferation and impaired differentiation of leukemic blasts that lead to inadequate normal hematopoiesis. Thus, patients usually present with symptoms resulting from bone marrow failure [(1)]. It comprises approximately 80% of pediatric acute leukemias and 20% of adult cases [(2)].

The non-neoplastic counterparts of leukemic B lymphoblasts, normal bone marrow B-cell precursors, are commonly referred to as hematogones [(3)]. Hematogones may be abundant in healthy infants and children and there was a significant decline in hematogones

with increasing age, but a broad range was found at all ages [3,4].

Hematogones, especially if present in large numbers, may confound the diagnosis of B-ALL in 1 of 2 ways: (1) Hematogone hyperplasia in a background of cytopenias may be mistaken for B-ALL at initial diagnosis. (2) Increased hematogones in a patient treated for B-ALL may be mistaken for residual or recurrent leukemia. By flow cytometry all cases of precursor B-lymphoblastic leukemia/lymphoma (B-ALL) demonstrate multiple immunophenotypic aberrancies relative to normal maturing B-cell precursors (hematogones) [3-5].

CD123 is the α-chain of IL-3 receptor (IL-3R), a member of the cytokine receptor super family [6]. Earlier experiments have shown that IL-3 plays an important role in the leukemogenesis of lymphoid and myeloid cells, inducing these cells to grow autonomously [7]. CD34 is a human stage-specific hematopoietic differentiation antigen, in leukemia cells; it remains expressed over several stages of lymphoid and myeloid maturation [8]. Various authors have shown that hematogones display surface staining for CD123 only in the more mature fraction that lacks CD34 expression. This “discordant” pattern is in contrast with the almost invariable “concordant” expression of these 2 antigens in B-ALL blasts [6,7,9].

While a morphologic and immunophenotypic overlap exists between hematogones and leukemic lymphoblasts, we demonstrate that morphologic review combined with evaluation of immunophenotype using (CD34, CD123) can help distinguish hematogone from leukemic blasts of B-ALL.

Methods

This case control study was conducted from November 2013 to June 2014 and included thirty patients newly diagnosed with B-acute lymphoblastic leukemia collected randomly in relation to age and gender. T- ALL and L3 were excluded from the study; Re-assessment was done for 20 patients of them after 4-6 weeks of chemotherapy (they were included according to the availability of their bone marrow aspirate BMA sample after chemotherapy). In addition, 10 patients with reactive BM were enrolled to assess hematogones. The patients were selected from 4 different hospitals in Baghdad. For each patient, peripheral blood and BMA from left over samples were taken to perform full blood count by automated device, peripheral blood film, BMA morphology, cytochemical stain using Periodic acid-Schiff (PAS) and Sudan black B (SBB) stains, the results of PAS stain were recorded as a percentage of such cells showing any PAS-positivity then they were classified into 3 groups, corresponding to less than 1% PAS positive cells, 1-10%, and over 10 %, respectively for correlation purposes [10].

For every patient at least 0.3 ml of K2 EDTA anti-coagulated bone marrow sample was collected for flow cytometry and tested within 48 hours. All flow cytometric analysis in this study was done by Cyflow® cube 6 flow cytometer from Partec Company in a private laboratory. Flow cytometry analysis was performed as in the fallowing: in cases of B-ALL, leukemic blasts were gated in the CD45 versus side scatter (CD45/SSC) histogram, and the expression of CD34, CD123 on this population was then assessed. For reactive BM (hematogones):the population was identified as hematogones by gating on CD45/very low SSC events [11-14] and then hematogones were subdivided into 2 groups. The first group comprised less mature hematogones that expressed CD34 and had dim CD45. The second group was composed of more mature hematogones lacking CD34 but with moderate CD45 expression, then the expression of CD123on these group were assessed. For post chemotherapy patients, two gates on blast region of CD45/ SSC plot were used and the expression of CD34, CD123 was then assessed. Clusters of at least 10-20 events must be captured and interpreted [15].

Statistical analysis

All statistical operations were done by SPSS version 18 programs. The measurement and tests were: mean and slandered deviation; chi

square (X^2) test for qualitative data, student t test for independent data. An association or difference was considered significant if the probability value (*P* value) was ≤ 0.05.

Result

Patients were divided into 2 groups: Adults (≥ 15 years old) comprised 10 patients and children (< 15 years) comprised 20 patients. The mean age of pediatrics B-ALL group was 4.75±3 years (mean ± SD); ranging (2 months – 12 years) at diagnosis, with male: female ratio of 1.5:1 and the peak incidence was found in the age group ≥ 5 years. The mean age of B-ALL adults' group was 35.9±11.76 years, ranging (18-50 years) with male: female ratio of 1.5:1.

According to FAB classification; L2 was the most common subtype (80% and 60%) for adult and children respectively followed by L1. Regarding PAS studies on diagnostic blast cells, 9 (30%) patients had no PAS-positive material, while 21 patients (70%) had more than 1% PAS positivity, with 16 cases (53.33%) showing a strong reaction in over 10% of cells. All B-ALL cases included in this study were SBB negative (Table 1).

Table 1. Distribution of B-ALL patients according to WBC count, Hemoglobin concentration, platelet count, blast % in BM, FAB subtype and PAS %

Feature		Children (N = 20) N (%)	Adults (N = 10) N (%)	P value
WBC count x10^9/L	< 50 ≥ 50	15 (75) 5 (25)	7 (70) 3 (30)	0.548
Hemoglobin (g/l)	< 80 80-100 > 100	12 (60) 1 (5) 7 (35)	4 (40) 2 (20) 4 (40)	0.360
Platelet x10^9/L	< 50 50-100 > 100	9 (45) 10 (50) 1 (5)	6 (60) 3 (30) 1 (10)	0.47
Blasts % in BM	< 90 ≥90	4 (20) 16 (80)	3 (30) 7 (70)	0.542
Blasts % in blood	Present absent	18 (90) 2 (10)	10 (100) 0 (0)	0.301
FAB	L1 L2	8 (40) 12 (60)	2 (20) 8 (80)	0.419
PAS %	< 1 1-10 > 10	2 (10) 3 (15) 15 (75)	7 (70) 2 (20) 1 (10)	0.001

PAS = Periodic acid-Schiff, < 1% PAS positive cells (negative PAS), 1-10% (positive PAS), and over 10% (strongly positive PAS), FAB = French–American–British classification, WBC = white blood cell count.

Immunphenotyping of newly diagnosed B-ALL: in 28 (93.4%) cases, leukemic blasts expressed both CD34 and CD123, Conversely, in 2 (6.6%) cases, neither antigen was expressed. Thus, the expression of CD34 and CD123 was found to be concordant (either either positive or both negative) in all B-ALL cases (Fig. 1).

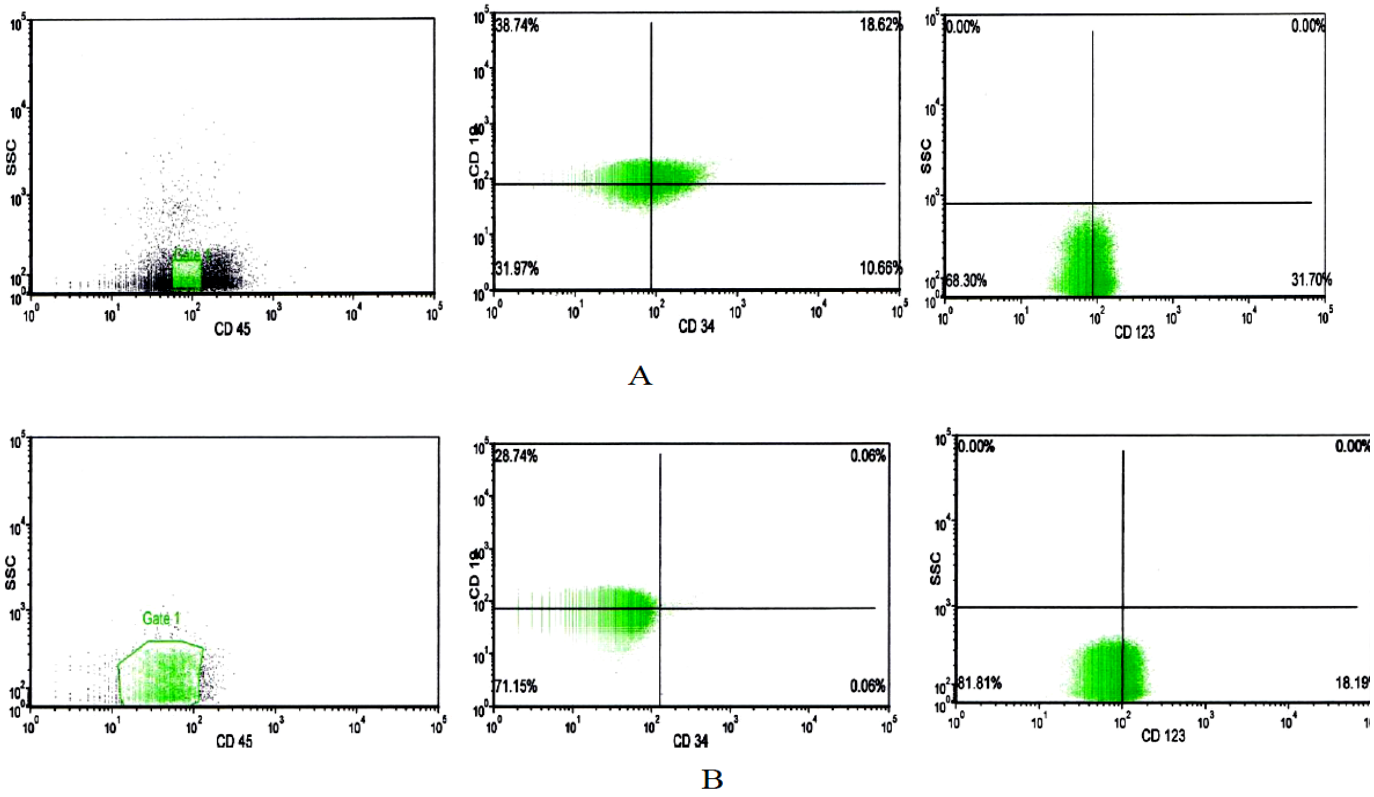


Fig. 1. Expression patterns of CD34 and CD123 on (B-ALL) blasts cases of B-ALL with pattern in which blasts express CD34 and CD123 antigens (A) blasts lack both antigens (B).

Regarding patients with hematogones (HGs), Age of patients ranged between 1.5 to 30 years, the median age was 2.75 year (hematogones occur in larger numbers in most normal marrow specimens of infants and young children but they are found in low numbers in most normal adult marrow specimens analyzed by flow cytometry (FC)) [(9)] with males: females ratio was 1:2.3. Hematogones cells by morphology show a spectrum of size and the exhibited features varied from mature lymphocytes to lymphoblast; HGs did not exhibit the PAS block- positive characteristic pattern of B- ALL blast and they were negative for Sudan black B (SBB). All cases of reactive BM specimens studied, hematogones were identified by flow cytometry, the less mature hematogones that had dim CD45, CD34+, not expressed CD123, whereas the more mature hematogones (moderate CD45+) lack CD34 but expressed CD123.Thus the expression of CD123 was found to be discordant in relation to CD34 in both groups of hematogones (Fig. 2).

The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and hematogones respectively had been studied in 20 B-ALL patients (4-6) weeks post chemotherapy (7 females and 13 males). In three (15%) of twenty cases, residual leukemic blasts were detected by FC. Five (25%) cases had residual leukemic blasts and late hematogones detected by FC (Fig. 3). In all of the above cases, the expression pattern of these two antigens (in blasts) remained constant after chemotherapy. For the remaining 12 cases (60%) HGs were detected only by FC.

In order to study relationship between expressions of both CD123, CD34 after induction therapy and the gender, age, (PAS% and WBC at initial diagnosis) and BM morphology after chemotherapy; the patients were divided after induction course of chemotherapy into two groups depending on response to induction therapy assessed by FC: First group included patients not achieved remission who had residual blast (concordant expression of CD34

and CD123), they were eight (40%). Second group included patients in remission that had only hematogones (discordant expression of CD34 and CD123) and they were twelve (60%) cases. A statistically significant difference in response to induction therapy ($P < 0.05$) was recorded with age of patients, initial PAS% and BM morphology after chemotherapy while the gender of patients or initial white blood cell count had no significant influence on response to chemotherapy (Table 2).

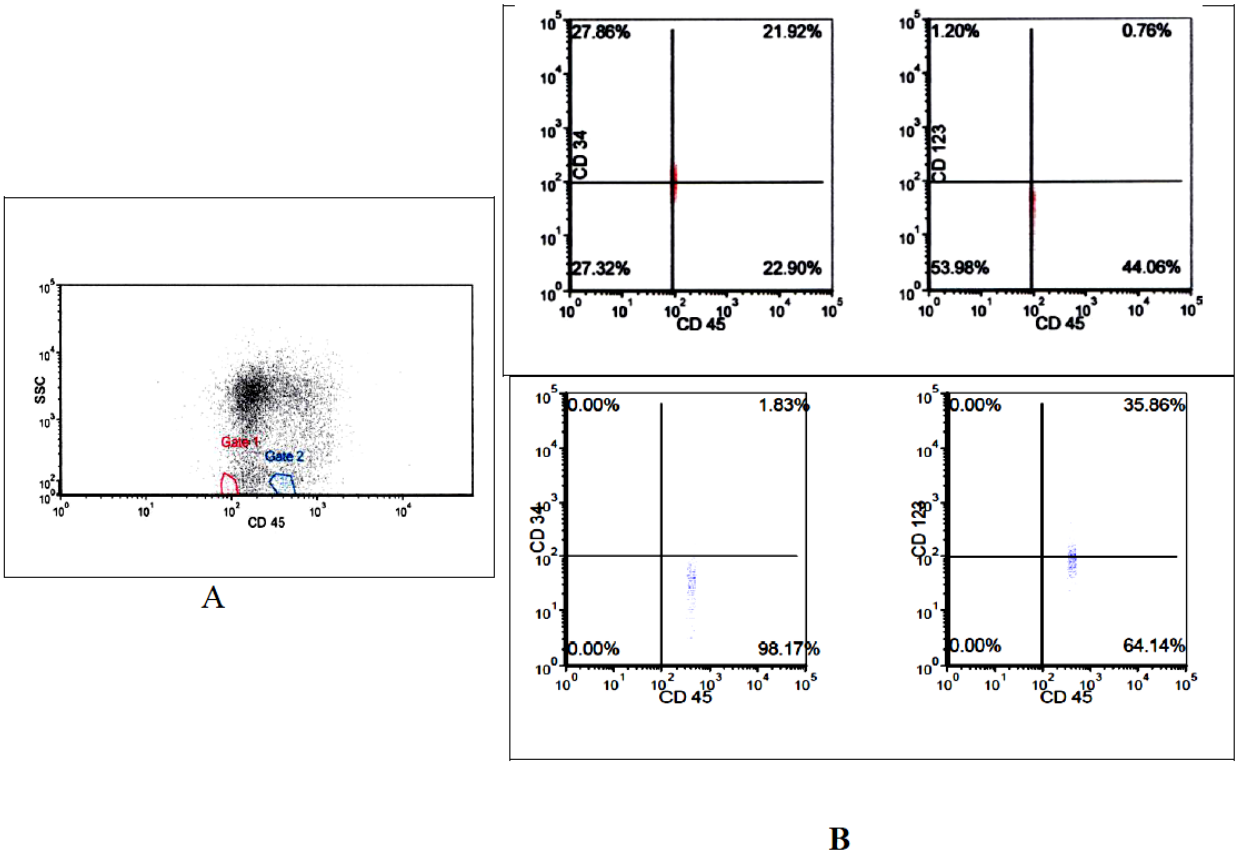


Fig. 2. Expression patterns of CD34 and CD123 on immature and mature hematogones. *A*, hematogones;the less mature hematogones with dim CD45 expression are gated in (gate 1 or red dots). The more mature hematogones with higher CD45 expression are gated in (gate 2 or blue dots). *B*, The upper left histogram shows the immature hematogones express CD34. The upper right plot shows that the same population is negative for CD123 expression and the lower left histogram show that the mature hematogones is negative for CD34 expression but express CD123 (lower right).

Discussion

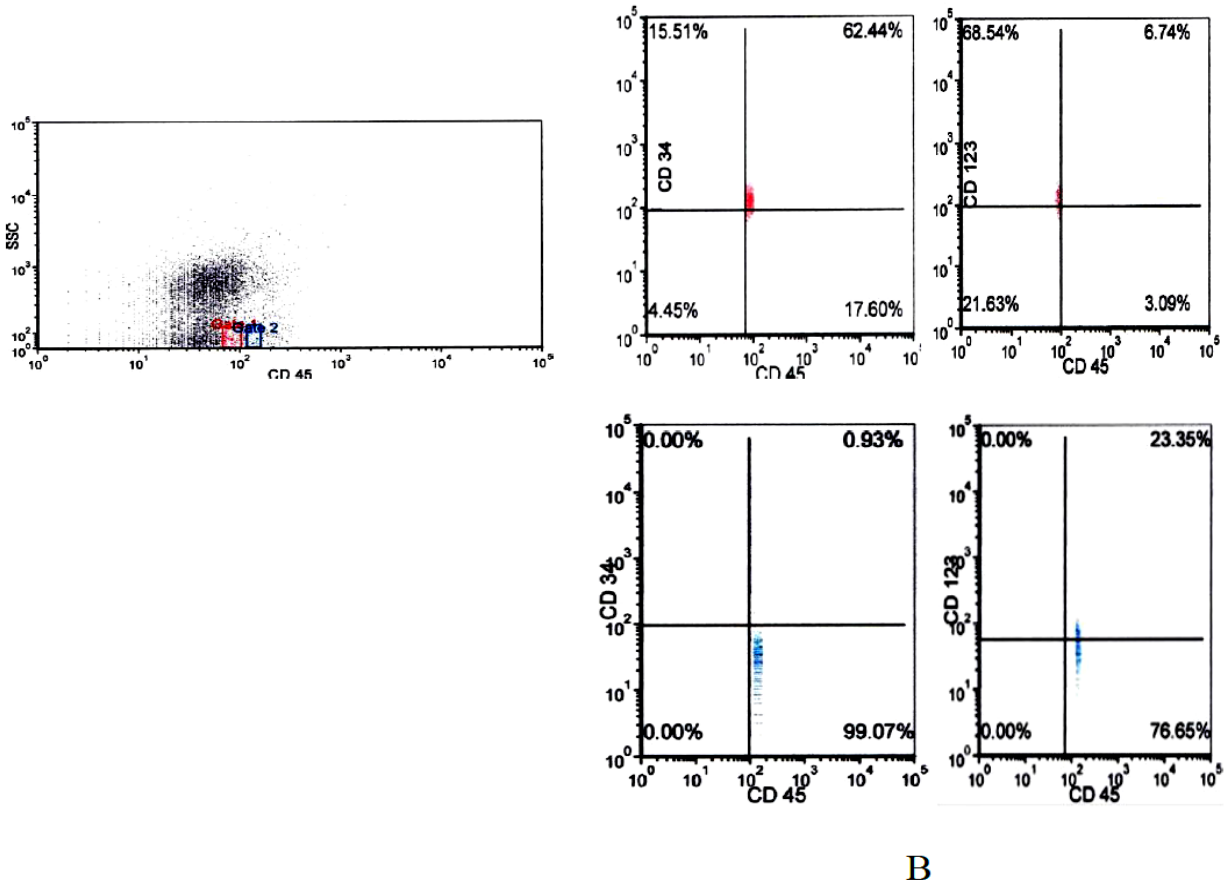
In this study, the mean age of pediatric B-ALL cases was 4.75 year with peak age of incidence was less than five years, these finding were comparable with other national study [16] and other literature in international populations [17]. The mean age of adult B-ALL patients was (35.9 year) lower than other Iraqi study [18] which was 46 years. This difference may be due to small sample size in current study.

In the present study, 25 % of pediatrics patients had WBC counts at diagnosis higher than $50x10^9$/L which confer poor prognosis; these findings go in line with many other studies [8,19] which showed that the WBC count was higher than 50 $x10^9$/L in 26%, 29% of pediatric ALL patients, respectively.

In current study, the WBC count among adult patients in 70 % of them was less than 50 $x10^9$/L while in 30 % of cases were over 50 $x10^9$/L These

figures were near the result recorded by Mancini *et al* [20].

Periodic Acid Schiff (PAS) studies on diagnostic blast cells showed that (30%) patients had no PAS-positive material, whereas 70% of patients had positive PAS material, these finding go in line with other observation [21] which showed that the PAS positive was in 66.66% of B-ALL cases.



B

Fig. 3. CD34 and CD123 expression patterns in treated B-acute lymphoblastic leukemia (B-ALL). A, Representative histograms shows residual blasts in (gate 1or red dots) and hematogones in (gate 2 or blue dots). B, residual blasts (red dots) that express CD34 and CD123 in the upper parts. The hematogones (blue dots) in the lower parts show discordant patterns of CD34 and CD123 expression.

Immunphenotyping of newly diagnosed B-ALL

It had been found that in 93.4% of cases leukemic blasts expressed both CD34 and CD123. Conversely, in 6.6% cases, neither antigen was expressed. These results confirmed the results obtained by Hassaneien and coworkers [9] who found that in 80% of B-ALL cases CD123 expression was associated with CD34 expression; whereas 11% expressed neither. Hematogones (HGs) in current study showed that small percentage of cells was indistinguishable morphologically from the lymphoblasts of B-ALL, this morphologic feature go in line with other investigator [4,7].

In some patients, the increase in HGs can be pronounced, resulting in confusion with ALL lymphoblasts. This is particularly true following treatment of ALL because hematogones are often expanded in regenerating marrow and can potentially be mistaken for residual disease [22,23].

Table 2. Responses to induction therapy and association with age, gender of patients, (WBC and PAS % at presentation) and BM morphology after chemotherapy

Feature		Patients in remissions (N = 8) N (%)	Adults (N = 12) N (%)	P value
WBC count x10^9/L	< 50 ≥ 50	15 (75) 5 (25)	7 (70) 3 (30)	0.548
Hemoglobin (g/l)	< 80 80-100 > 100	12 (60) 1 (5) 7 (35)	4 (40) 2 (20) 4 (40)	0.360
Platelet x10^9/L	< 50 50-100 > 100	9 (45) 10 (50) 1 (5)	6 (60) 3 (30) 1 (10)	0.47
Blasts % in BM	< 90 ≥ 90	4 (20) 16 (80)	3 (30) 7 (70)	0.542
Blasts % in blood	Present absent	18 (90) 2 (10)	10 (100) 0 (0)	0.301
FAB	L1 L2	8 (40) 12 (60)	2 (20) 8 (80)	0.419
PAS %	< 1 1-10 > 10	2 (10) 3 (15) 15 (75)	7 (70) 2 (20) 1 (10)	0.001
Age	≤ 15 > 15	11 1	4 4	0.035
Gender	Male Female	5 3	8 4	0.199
WBC count at initial presentation	< 50×10^9/L ≥ 50×10^9/L	4 4	10 2	0.06
BM morphology after chemotherapy	Blast < 5% blast ≥ 5%	5 3	12 0	0.021
PAS % at presentation	< 1% 1-10%	5 2	2 1	0.023

PAS = Periodic acid-Schiff, <1% PAS positive cells (negative PAS), 1-10% (positive PAS), and over 10% (strongly positive PAS), WBC = white blood cell count, Blast < 5% = complete morphological remission, blast ≥ 5% = incomplete morphological remission.

Because of these potential diagnostic difficulties, awareness should be taken for distinction of leukemic lymphoblasts in B acute lymphoblastic leukemia (B-ALL) from their non-neoplastic counterparts in bone marrow (hematogones).

One important distinguishing characteristic of HGs in our patients was In bone marrow smears, HGs did not exhibit the block-positive of PAS pattern characteristic of ALL, these finding accepted by other investigator [14,24] and negative for SBB, Other investigators have shown that HGs are nonreactive with SBB [23].

This study had used four-color flow cytometry to define precisely the patterns of normal antigen expression on a series of normal bone marrows using CD34, CD123 and it has been found that the less mature hematogones (dimCD45+) that expressed CD34 lack CD123 expression, whereas the more mature hematogones (moderate CD45+) lacked CD34 but always express CD123.

These findings were in agreement with other studies [7] who found that CD123 was negative in normal lymphoid progenitors (CD34+CD33-CD19 + CD10+) and with Djokic *et al* [6] study who found that the early B-cell precursors were CD123 negative while intermediate precursors and mature B cells showed weak CD123 expression.

Other investigators have also reported methods for discriminating between normal B-cell precursors and neoplastic lymphoblasts. Farahat and associates [25], using quantitative double-labeling flowcytometry, found B-lineage ALL lymphoblasts to express fewer TdT and CD19 and more CD10 molecules than did hematogones. While McKenna *et al* [22] found that the concurrent expression of earliest and last antigen e.g. CD34 and CD20 in B-ALL but discordant expression in HGs.

The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and HGs respectively had been studied in twenty of B-ALL patients 4-6 weeks post chemotherapy. In 8/20 (40%) cases in whom remission was not achieved, residual blasts were presented by FC while 12/20 (60%) of remitted cases, had only hematogones assayed by FC. This finding is comparable to that recorded by Delbuono *et al* [26]; who found that about half of the patients had detectable residual daises (RD) in the BM (44% on day 14 and 39% on day 28) and was lower than that recorded by Hassaneien *et al* [9], who reported that 62% of cases had residual blast.

The current study tested the relationship of response to induction therapy with respect to other prognostic factors and found that there was significant correlation between response to induction therapy and BM morphology after chemotherapy, PAS % at initial diagnosis and the age of patients but no significant correlation presented with gender of patient and initial WBC. Delbuono *et al* [26]; agreed in his study with fact that there was correlation between RD and BM morphology but he found that RD was not significantly associated to gender, age and white blood cell count .

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Author Contribution

Research proposal was done by Musa, collection of samples, patients' interview, sample analysis, and patients follow up were done by Shallan and the final printout of article was done by both authors.

Conflict of Interest

The authors declare no conflict of interest.

Finding

Self funding.

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Evaluation of Plasmid-Mediated Quinolone Resistance associated with the *Qnr* Genes in Clinical Isolates of *Shigella* Spp. in Baghdad

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Abstract

Background Although quinolone resistance results mostly from chromosomal mutations in *Enterobacteriaceae*, it may also be mediated by plasmid-encoded *qnr* determinants. *Shigella* harboring the novel *qnr* plasmid-mediated mechanism of quinolone resistance has been described worldwide.

Objective To understand the distribution of serogroup of *Shigella* spp, as well as antimicrobial susceptibility and to investigate the plasmid mediated quinolone-resistant *qnr* genes in clinical isolates of *Shigella* spp. resistant to quinolone.

Methods Fifty nine clinical isolates of *Shigella* spp. were collected from two hospitals in Baghdad. Antimicrobial susceptibility tests were performed using disk diffusion test and minimum inhibitory concentration. The isolates were screened for the plasmid-mediated *qnr* genes of *qnrA*, *qnrB*, and *qnrS* by Multiplex polymerase chain reaction.

Results The isolation rate of *Shigella* spp. was 14% and observed to be high among children < 10 years and low in teenagers and adults. The highest percentage was *Sh. flexneri* (54.2%) followed by *Sh. sonnei* (37.3%) then *Sh. dysenteriae* (8.5%), while no *Sh. boydii* was found in this study. Antimicrobial susceptibility tests revealed that 54.23% and 49.2% of both *Sh. flexneri* and *Sh. sonnei* were resistant to nalidixic acid and ciprofloxacin, respectively, while *Sh. dysenteriae* isolates were fully susceptible to these antibiotics. The minimum inhibitory concentration value of resistant isolates of *Sh. flexneri* and *Sh. sonnei* ranged between 2-64 µg/ml and 32-512 µg/ml for ciprofloxacin and nalidixic acid, respectively. Multiplex polymerase chain reaction amplification of plasmid-borne *qnrA, qnrB, qnrS* genes revealed that the overall percentage of *qnr*-genes were (52.9%) distributed as (29.4%) *qnrA*, (20.6%) *qnrS* and (2.94%) *qnrB* detected alone or in combination. The genes were identified in (44.1%, 15/34) of quinolone resistance *Shigella* isolates.

Conclusion To our knowledge, this is the first report detected fluoroquinolone resistance due to the *qnr* gene among *Shigella* isolates in Iraq which is indicated that plasmid-mediated quinolone resistance has emerged in Iraqi pediatric patients.

Keywords Drug resistance, *Shigella* spp., Plasmid; Quinolone, *qnr*

List of abbreviation: FQ = Fluoroquinolone, QR = quinolone resistance, Cip = ciprofloxacin, DNA = Deoxyribonucleic acid, PMR = plasmid-mediated resistance, PMQD = plasmid-mediated quinolone determinants, PCR = polymerase chain reaction, NA = nalidixic acid, MIC = minimum inhibitory concentration,

Introduction

Shigellosis, an acute diarrhoeal disease, remains a major public-health problem especially in children in developing countries [(1)]. Worldwide, an estimated 165 million cases of Shigellosis, of which 163 million are in developing countries [(2)]. Two-thirds of them concern less than 10 years-old children [(3)]. There are four species of *Shigella*: *Sh. dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei* [(4)]. Appropriate antimicrobial therapy shortens the duration of symptoms and can prevent life-threatening complications [(5)]. Fluoroquinolones (FQ) are broad-spectrum

agents that have excellent activity against most enteric pathogens particularly against gram-negative bacteria. Ciprofloxacin (Cip) perhaps the most important as well as the most used FQ [6]. According to the World Health Organization (WHO) revised guidelines for the control of Shigellosis, Cip is now the drug of choice for all patients with bloody diarrhoea [7]. However, the increased use of FQ has led to increasing resistance to these antimicrobials [8].

Quinolone resistance (QR) in *Enterobacteriaceae* results mainly from mutations in type II Deoxyribonucleic acid (DNA) topoisomerase genes [9] or changes in the expression of outer membrane and efflux pumps [10]. Studies have shown that plasmid-mediated resistance mechanisms also play a significant role in QR, and its prevalence is increasing worldwide [11-13].

The plasmid-mediated resistance (PMR) gene *qnr* is a member of the pentapeptide repeat family of proteins and has been shown to block the action of Cip on purified DNA gyrase and topoisomerase IV [14]. Several members of *qnr* determinants were identified and labeled as *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD*, while amino acid variations are indicated in numbering [15].

These genes have a wide geographic distribution mainly in *Enterobacteriaceae* [16]. *qnrA* is encoding a 218 amino acid protein of the pentapeptide family. The first *qnrS* gene was detected in 2003, in single clone of *Shigella flexneri* 2b was resistant to FQ caused an outbreak of enterocolitis in Japan [17]. The most heterogenous cluster of the *qnr* gene family is *qnrB*, having 47 different alleles [18]. The mechanism of the *qnr* protective effect is not completely understood. It has been shown that *qnrA* can bind to the DNA gyrase holoenzyme as well as to its respective subunits, *gyrA* and *gyrB*. This binding occurred in the absence of relaxed DNA, Cip, or ATP, indicating that the binding of *qnrA* to gyrase did not require the presence of the ternary complex of enzyme, DNA, and quinolone [14]. Similar findings were also reported for *qnrA* and topoisomerase IV [19]. The direct effect of *qnrA* is the reverse of the inhibition of gyrase-mediated DNA supercoiling caused by Cip minimizing opportunities for these agents to stabilize the lethal gyrase-DNA-quinolone complex [11]. This study is designed to determine the susceptibility of *Shigella* species isolated from two hospitals in Baghdad against quinolone group and to detect the prevalence of plasmid-mediated quinolone determinants (PMQD) like *qnrA*, *qnrB*, *qnrS* by polymerase chain reaction (PCR) in *Shigella* spp.

Methods

Patients and microbial identification

A total of 59 *Shigella* spp. were isolated from 420 fresh stool specimens were collected from patients presenting with acute diarrhea from two hospitals in Baghdad; Children Welfare Hospital (Al-Mansour) and Al-Imamain Al-Kadhimain Medical City hospital during a period between 1st Jun. 2010 and 31st May 2011.

The patient's age were ranging from 5 months to 62 years. All specimens transferred to the laboratories of Al-Nahrain Medical College and incubated overnight in Selenite F broth then plated onto MacConkey, XLD and *Salmonella-Shigella* agar and incubated at 37 ^{o}C for 24 hr in aerobic environment. The colorless non-lactose-fermenting colonies suggestive of *Shigella* were sub-cultured on nutrient agar and broth and were biochemically identified. Api20E was used to confirm the diagnosis and further identification at a group level by slide agglutination test with specific antisera was done.

Antimicrobial susceptibility tests

A total of 59 *Shigella* isolates were tested for susceptibility to quinolone group [Nalidixic acid (NA) and Cip)] by Disk diffusion method in accordance to Clinical and Laboratory Standard Institute [20] using *E. coli ATCC25922* as a standard strain and decided as susceptible (S) and resistant (R). The minimum inhibitory concentrations (MICs) of NA and Cip for the resistant *Shigella* isolates were performed using agar dilution method according to Clinical and Laboratory Standards Institute [20] and Wiegand *et al* [20] recommendations.

Plasmid DNA extraction

Plasmid DNA was isolated from *Shigella* spp. according to Heringa *et al* [22] using modified alkaline lysis method. The supernatant containing plasmid DNA subjected to electrophoresis and used as a template for PCR experiments to detect the presence of *qnr* genes. To estimate the size of plasmid DNA and PCR products, 1kb and 100bp DNA Marker (Lambda DNA cut with Hind-III) were used respectively.

Multiplex PCR-based screening for *qnr* genes

Multiplex PCR was done by modification of previously described PCR protocol [23] for PCR amplification of PMQR *qnrA*, *qnrB* and *qnrS* genes. The amplification was performed using GoTaq Green Master Mix, specific primers sequences for *qnr*A, *qnrB* and for *qnrS* (1.5 forward and 1.5 reverse for each primer) and plasmid DNA of *Shigella* isolates as a template for PCR experiments (Table 1 and table 2).

Table 1. Sequences and products of PMQR determinants (*qnrA*, *qnrB*, and *qnrS*)

qnr genes		Nucleotide Sequences (5' → 3')	Products bp	References
qnrA	**F**	GATAAAGTTTTTCAGCAAGAGG	593	Jacoby *et al* 2003 [24]
	R	ATCCAGATCGGCAAAGGTTA		
qnrB	**F**	GATCGTGAAAGCCAGAAAGG	469	Robicsek *et al* 2006 [11]
	R	ACGATGCCTGGTAGTTGTCC		
qnrS	**F**	TGGAAACCTACAATCATACATATCG	656	Pu *et al*., 2009 [25]
	R	TTAGTCAGGATAAACAACAATACCC		

F = forward, R = reverse.

Table 2. Concentrations of the components of PCR master mixture of different *qnr* genes used for multiplex PCR

Components		Volume/ µl	Final concentration
Green Master Mix, 2x		12.5 µl	1x
qnrA *qnrB* *qnrS*	Forward Reverse	1.5 µl 1.5 µl	30pmol 30pmol
DNA template ddH2O Total		2 µl 1.5 µl 25 µl	

The cycling was performed using protocol showed in table 3. Multiplex PCR products were resolved by horizontal agarose gel electrophoresis and visualized under UV trans-illuminator using digital camera (Sony-Japan).

Table 3. The conditions of PCR amplification steps for *qnr* genes

Steps	Temperature	Time	Cycles
Initial denaturation	95 °C	10 min	
Denaturation Annealing Elongation	95 °C 60 °C 72 °C	45 sec 45 sec 1 min	35
Final extension Hold	72 °C 4 °C	10 min	

Statistical analysis

The significance of differences in proportions was analyzed by the Chi-square test using statistical package for social sciences (SPSS) version 15 and *P* values equal or less than 0.05 were considered statistically significant.

Results

Shigella spp. was isolated from 59 (14%) of 420 stool samples. Isolation rate of *shigella spp.* was observed to be high among children 5 m - 10 yr (93.2%, 55/59) and low in teenagers and adults (6.8%, 4/59). Statistically, the highest proportion of stool specimens infected with *Shigella* spp. was in the age group (5 month – 10 years) and there is significant association between this age group and *Shigella* infection. The highest percentage of *Shigella* isolates were *Sh. flexneri* (54.2%, 32/59) followed by *Sh. sonnei* (37.3%, 22/59) then *Sh. dysenteriae* (8.5%, 5/59), while *no Sh. boydii* was found in this study.

Antibiotic resistance of *Shigella* isolates

By the disc-diffusion method, 16 and 18 isolates of *Sh. flexneri* and13 and 14 isolates of *Sh. sonnei* were resistant to Cip and NA in percentage reached to 49.2% and 54.23% (29 and 32 out of 59), respectively (Table 4).

Table 4. Number and percentage of resistant *Shigella* isolates to quinolone group

AB	*Sh. flexneri* (32)		*Sh. sonnei* (22)		*Sh.dysenteriae* (5)		Total (59)	%	
	No.	%	No.	%	No.	%		R	S
CIP	16	50.0	13	59.1	0	0	29	49.2	50.8
NA	18	56.3	14	63.6	0	0	32	54.23	45.8

Of these, 15 isolates of *Sh. flexneri* and 12 isolates of *Sh. sonnei* were resistant to both antibiotics. The MIC values of Cip and NA ranged from 2 to 64 µg/ml and from 32 to 512 µg/ml, respectively in all spp. except for *Sh. dysenteriae* which was susceptible to these antibiotics (Fig. 1 and Fig. 2).

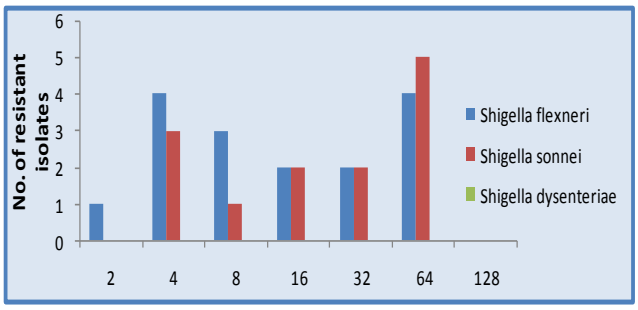

Fig. 1. MIC results of *Shigella spp.* resistant to Ciprofloxacin

Plasmid profile

In this study, analysis of plasmid DNA revealed that, all of the isolates contained multiple plasmids (2-8 plasmid bands), their molecular size ranged from (0.5 kb to more than 10 kb) forming a number of unique banding patterns. The total number of plasmid profiles was 14 of *Sh. flexner* and 11 of *Sh. sonnei.* Mega Plasmids of the same size >10 kb which appeared before the chromosomal DNA were present in multiple strains (Fig. 3 & 4).

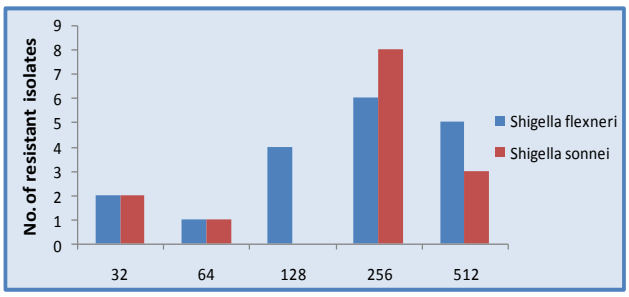

Fig. 2. MIC results of *Shigella spp.* resistant to Nalidixic acid

Multiplex PCR screening for *qnr* A, B, S genes

Nineteen isolates of *Shigella flexneri* and 15 of *Shigella sonnei* resistant to quinolone group and contain multiple plasmids were screened for the presence of the PMQR genes *qnrA*, *qnrB*, and *qnrS* by multiplex PCR. In this study, the

prevalence of overall *qnr*-genes were 18/34 (52.9%,) (Table 4).

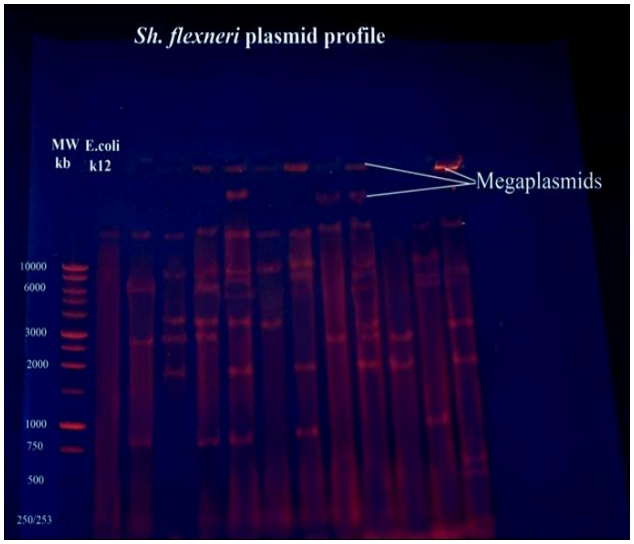

Fig. 3. Gel electrophoresis of *Shigella flexneri* isolates show plasmid profile (0.7% agarose, 7 v/cm, 2 hrs)

The genes were identified in 15/34 (44.1%,) of *Shigella* isolates.

As it is shown in table 5, the *qnrA* gene was the most common (29.4%) in *Shigella* spp. resistant to quinolone followed by *qnrS* (20.6%), whereas only one isolate of *Sh. sonnei* was positive for *qnrB* (2.94%) as shown on fig. 5 and fig. 6.

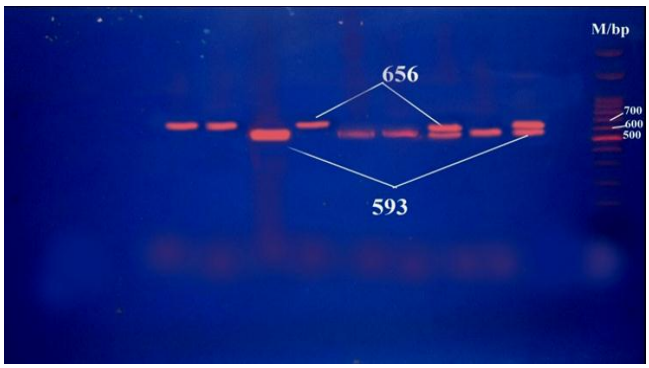

Fig. 4. Gel electrophoresis of *Shigella sonnei* show plasmid profile (0.7% agarose, 7 v/cm, 2 hrs)

Table 5. The prevalence of *qnr* genes in *Shigella* spp. resistant to quinolone

Genes	*Sh.flexneri* N= 19 N (%)	*Sh.sonnei* N = 15 N (%)	Total N = 34 N (%)
qnrA	6 (31.58)	4 (26.7)	10 (29.4)
qnrB	0 (0.0)	1 (6.7)	1 (2.94)
qnrS	5 (26.32)	2 (13.3)	7 (20.6)
Total	11 (57.9)	7 (46.7)	18 (52.94)

Discussion

Diarrheal disease is major cause of morbidity and mortality in the developing world [26]. Like many other developing countries, diarrheal diseases are among the main health problems in Iraq. This is attributable to personal hygiene and sanitary conditions which promote spread of organisms like *Shigella* and other enteric pathogens [27].

In the current study, the prevalence of Shigellosis among diarrheal patients was 14%, which is less than that of previous report from Iraq (26.1 %) [28]. The predominant species of *Shigella* isolated in the present study was *Sh. Flexeneri* (54.2%), followed by *Sh. Sonnei* (37.3%) then *Sh. dysenteriae* 5(8.5%), while no *Sh. boydii* was found. This distribution is very close to that seen in India by Bhattacharya *et al* [29] when they found that *Sh. flexneri* was the dominant strain isolated followed by *Sh. sonnei* and *Sh. dysenteriae* but not *Sh. boydii*.

The variation according to the geographical area suggested that the factors involved in natural selection may have been the main reason for these discrepancies. The average age of patients with *Shigella* infection in our study was similar to Ranjbar *et al* [30] who found that the highest frequency of isolation of *Shigella spp*. was seen among the patients with 1 to 5 years old and our results of other age groups are similar to other studies [31]. Children within this age-group are most susceptible because of poor resistance,

lack of previous exposure, poor personal hygiene [32]. The antibiotic resistance of *Shigella* spp. has been hindering the treatment of Shigellosis, particularly in children [1].

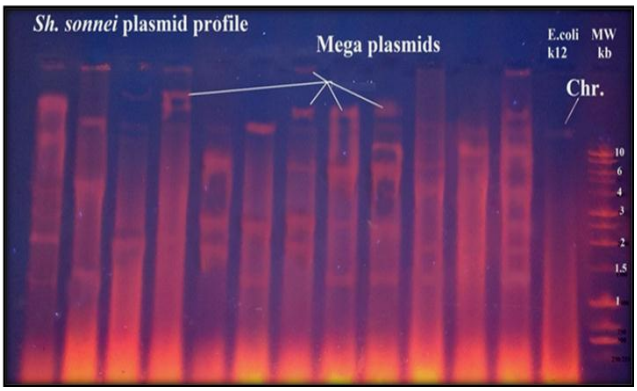

Fig. 5. Gel electrophoresis of Multiplex PCR positive products for *qnrA* (593 bp) and *qnrS* (656 bp) of *Sh. flexneri* isolates. Lane 1 (M/bp): 100bp DNA ladder; lane 2: Negative control (DW); lane (3,4,5,6,7,9): *Sh. flexneri* positive isolates for *qnrA* gene; lane (3,5,8,10,11): *Sh. flexneri* positive isolates for *qnrS* gene; (1% agarose, 7 v/cm^2, 1.5hrs)

The current study showed that all *Shigella spp*. presented a similar resistance profile for quinolone except for *Sh. dysenteriae, which* was fully susceptible to Cip and NA. This result was far from the findings of Talukder *et al* [33] when they previously found that nine strains *of Sh. dysenteriae* between 2002 and 2003 from South Asia were resistant to NA and Cip, with high MIC values. On the other hand, in Bangladesh [34] reported results nearly similar to ours when they found that, about 51% of *Shigella* isolates were resistance to NA, and Srinivasa *et al* [35] reported that, the rate of FQR including Cip in 2004 was 5.9% and gradually increased to 48.5% in 2007.

In Iraq, Munim *et al* [28] found that 18.18% of *Shigella* isolates are resistant to Cip in 2008. Furthermore, Bhattacharya *et al* [29] found that 96% of *Sh. spp*. was resistant to NA and 82% of them to Cip.

The MIC values of NA and Cip, in the current study were (2-64 µg/ml) and (32- 512 µg/ml), respectively in all spp. These results was included in the result of Bhattacharya *et al* [29] who found that MIC values were 0.5 to >256 µg/ml for NA and 1 to >256 µg /ml for Cip in different *Sh.* spp. These results may reflect the broad MIC values of quinolone group in *Sh.* spp. and these ranges of resistance are alarming. If *Sh.* spp. become resistant to such high levels of antibiotics, the treatment of disease with antibiotics would become quite difficult.

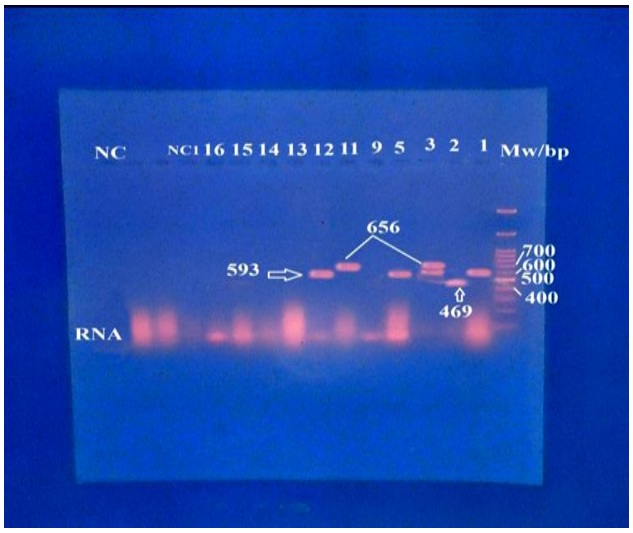

Fig. 6. Gel electrophoresis of Multiplex PCR products for *qnrA*, *qnrB*, *qnrS* of *Sh. Sonnei* positive *isolates*. Lane M/bp: 100bp DNA ladder; lane (1,3,5,12): *qnrA* gene (593 bp) positive isolates; lane (2): *qnrB* (469bp) positive isolate; lane (3,11) *qnrS* (656 bp) positive isolates; NC1: Negative control (DW); NC: quinolone negative isolate; (1% agarose, 7 v/cm^2,1.5hrs).

The results showed that all *Shigella* isolates contained multiple plasmids (2-8 plasmid bands), their molecular size ranged from 0.5 kb to more than 10 kb forming a number of unique banding patterns (14 of *Sh. flexneri*, 11 of *Sh. Sonnei*). This result was nearly similar to that of Ahmed *et al* [32] who found that (96.7%) of *Sh.* isolates harbored (1 to 9) plasmids and the number of plasmids were highly variable for all spp. This finding was also reported by Munim *et al* [28] who they identified numerous plasmid patterns in the 11 *Sh.* isolates in Iraq. Plasmids are

present at one or more copies per cell. This provides an additional survival mechanism for the bacteria [36]. PMQR determinants (*qnrA*, *qnrB* and *qnrS*) were recently reported worldwide in many strains of *Enterobacteriaceae*, including *Shigella* [37,38].

In this study, multiplex PCR technique showed that, PMQR determinants were present in (44.1% 15 of 34) of the isolates with *qnr* gene detected alone or in combination (3 isolates carried two types of *qnr*), and in a significant percentage (*P* = 0.01) reached to (52.9%, 18 gene) distributed as (29.4%, 10 *qnrA*), (20.6%, 7 qnr*S*) and (2.94 %, 1 *qnrB*). Similar findings were reported by Xiong *et al* [39] who found that PMQR determinants were present in (53.8%, 14 of 26) of *Sh. flexneri* isolates and *qnrA*1, *qnrS*1, *qnrS*2, were present in (30.8%, 11.5% and 3.8%), respectively.

Hata *et al* in 2005 [17] was the first to describe *qnrS* in *Sh. flexneri* isolates resistant to NA and Cip in Japan. The *qnrS* gene has ~60 and ~50% homology to the gene of *qnrA* and *qnrB*, respectively. The progenitor of *qnr* gene is bacteria widely distributed in aquatic environments and rarely involved in human infections.

The genes of these determinants are plasmid localized and can be horizontally transferred. Although these PMQR determinants confer low-level resistance to quinolones and / or FQs, they are a favorable background for selection of additional chromosome - encoded QR mechanisms [40].

In conclusion: *Sh. flexneri* was the predominant spp. in Iraq. All spp. contains multiple plasmids and the prevalence of overall PMQR *qnr*-genes were (52.9%,18/ 34), these genes were identified in (44.1%, 15/34) of *Shigella* isolates.

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Author contribution

Dr. Abdulrahman prepare, perform and did the tests and interpret the results of the research; Jamal and Kadhim help in sampling and Dr. Belal supervise this paper as part from a thesis.

Conflict of Interest

No conflict of interest

Funding

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Evaluation of Interleukins 12 and 13 Levels in Beta Thalassemia Major Patients and their Relations to Viral Hepatitis C

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Abstract

Background Several immunological defects can be found in patients with β-thalassaemia, among them is disturbance in the production of some cytokines.

Objectives To evaluate the levels of interleukin-12 and 13 and its relation with Hb, packed cell volume, serum ferritin and between them in β-thalassemic major patients with or without viral hepatitis.

Methods A case control study was conducted on 48 patients with β-thalassemia major divided into two groups; Group I comprised 24 infected with viral hepatitis C and group II comprised 24 patients with no infection; in addition twenty healthy age- and sex-matched subjects were studied as control group. Five ml of venous blood sample were collected; two ml put in EDTA tube for complete blood count and 3 ml in plain tube for biochemical lab investigations; 300 µm from the left-over serum was taken and divided into two tubes; one for estimation of interleukin-12 level and the other for estimation of interleukin-13 using ELISA Reader device.

Results All thalassemic patients with or without viral hepatitis had low level of interleukin-12 and had high level of interleukin-13. Interleukin-12 was much lower in those infected with hepatitis C virus than those with no infection and the reverse with interleukin-13.

Conclusion β-thalassemia major patients had decreased level of interleukin-12 and increased level of interleukin-13 which was more prominent in hepatitis positive thalassemic patients. Inverse correlation was noticed between interleukin-12 and 13 levels in thalassemic patients.

Key words IL-12, IL-13, β-thalassemia major, viral hepatitis.

List of abbreviation: IL-12 = Interleukin 12, IL-13 = Interleukin 13, Hb = Hemoglobin, PCV = Packed cell volume, EDTA = Ethylene diamine tetra acetic acid, CBP = Complete blood picture, HIV = Human immunodeficiency virus, HBV = Hepatitis B virus, HCV = Hepatitis C virus, ELISA = Enzyme-linked immunosorbent assay.

Introduction

β-Thalassemia is a heterogeneous hemoglobin (Hb) disorder characterized by the absence or reduced synthesis of the β-globin chain that causes globin chain imbalance [(1)]. Several immunological defects can be found in patients with β-thalassaemia, among them is disturbance in the production of some cytokines. Decreased interleukin (IL)-12 level and increased IL-13 level was documented in patients with β-thalassaemia major [(2)].

The mechanism of these abnormalities is not clarified, also the role of immunologic alternations on the clinical course of β-thalassemia is not established, although they have been considered relevant to infectious episodes that these patients suffer [(3)].

A major cause of morbidity and mortality in β-thalassemic patients is infections; these could be the results of functional alteration in the immune system due to multiple blood transfusions [(4)]. Repeated blood transfusions will

expose them to dangerous infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) [5]. HCV is responsible for 80-90% of post transfusion hepatitis in β-Thalassemic patients [6].

The objectives of this study was to evaluate the levels of interleukins-12 and 13 in β-thalassemic major patients with or without viral hepatitis

Methods

A case control study was conducted on 48 patients with homozygous β-thalassemia major who were attending Al-Karama Teaching Hospital for receiving blood transfusion and treatment for the period from December 2013 to April 2014.

β-thalassemia patients were divided into two groups; group I comprised 24 infected with HCV and group II comprised 24 with no infection. In addition, twenty healthy age- and sex-matched subjects were included in the study as control group. Peripheral venous blood sample was collected from anticubital fossa from each patient and control subjects. Patients sample were collected just before transfusion. Two ml put in EDTA tube for complete blood count and three ml was collected in the plain tube, which then immediately centrifuged for 10 minutes at 3000 rpm to separate the serum. From the leftover sample, 1 ml of serum was taken and divided into two tubes; one for estimation of IL-12 level and the other for estimation of IL-13, and they were stored at -20^{o}C in central lab. of Al-Nahrain Medical College until time of assay of IL-12 and IL-13. One hundred µl of serum was taken for IL-12 and other 100 µl for IL-13 assay by using ELISA kit applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-12 and the other specific for IL-13; and polyclonal antibody preparation were used manufactured by CUSABIO BIOTECH co.

The procedure was carried out in accordance with the manufacturer's instructions. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-12/P40 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3, 3', 5, 5'tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain IL-12/P40, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color.

The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured at a wavelength of 450 nm±2nm spectrophotometrically.

The concentration of IL-12/P40 in the samples is then determined by comparing the O.D. of the samples to the standard curve. The same procedure was done for IL-13 except that the microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-13 and then the same steps will be done.

Statistical analysis of data was performed with SPSS version 21 (2013) and Excel professional edition 2010 programs.

Results

The mean value of IL-12 in thalassaemic patients was lower in group I and group II than controls and it was much lower in patients belongs to group I than in those in group II ($P < 0.05$); accordingly 15 out of 24 patients (62 %) of group I versus 2 out of 24 patients (8%) of group II had markedly reduced IL-12 level (Table 1 and fig. 1). While 7 out of 24 patients (29%) of group I versus 10 out of 24 patients (41%) of group II had moderately reduced IL-12 level and only 2 out of 24 patients (8%) of group I versus 12 out of 24 patients (50%) of group II had minimal reduction of IL-12 level (Table 2 and fig. 3).

Table 1. Interleukins 12 and 13 in β-thalassaemia patients and control groups

Interleukin (pg/ml)	Group I mean±Sd N=24	Group II mean±Sd N=24	Controls mean±Sd N=20	P1	P2	P3
12	3.52 ± 2.2	6.32 ± 1.3	18.45 ± 6.41	0.033	<0.001	<0.001
13	1089.1 ± 618.4	864.5 ± 493.1	14.08 ± 66.3	0.11	<0.001	<0.001

P1: Group I vs. Group II, P2: Group I vs. controls, P3: Group II vs. controls

Table 2. Distribution of reduction in IL-12 categories according to the IL-12 quartiles

Reduction in IL-12	Group I		Group II		P Value
	No.	%	No.	%	
Markedly reduced	15	62.5	2	8.3	< 0.001
Moderately reduced	7	29.3	10	41.7	
Minimally reduced	2	8.3	12	50.0	
Total	24	100	24	100	

The mean level of IL-13 was significantly lower in controls than in group I and in group II ($P < 0.05$) and it was much higher in group I than in group II (Table 1 and fig. 2); accordingly 11 out of 24 patients (45 %) of group I versus 6 out of 24 patients (25%) of group II had markedly high IL-13 level.

Eight out 24 patients (33%) of group I versus 7 out of 24 patients (29%) of group II had moderately high IL-13 level and 5 out of 24 patients (20%) of group I versus 11 out of 24 patients (45%) of group II had minimal increase in IL-13 level (Table 3 and fig. 4).

Table 3. Distribution of increment in IL-13 categories according to the IL-13 quartiles

Increment in IL-13	Group I		Group II		P Value
	No.	%	No.	%	
Markedly High	11	45.8	6	25.0	0.15
Fairly high	8	33.3	7	29.2	
Minimally high	5	20.8	11	45.8	
Total	24	100	24	100	

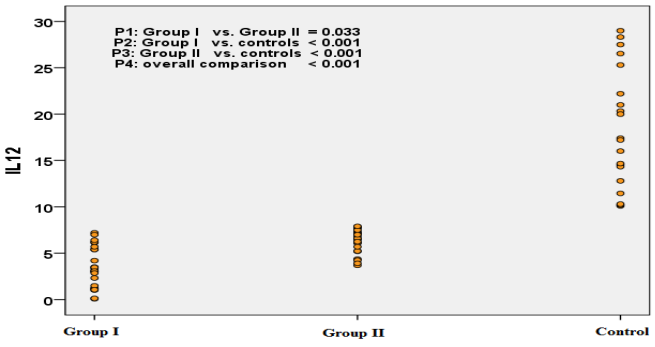

Fig. 1. Distribution of IL12 among the studied groups

There was an inverse insignificant correlation between serum ferritin and IL-12 in both studied thalassemic groups, in hepatitis C positive and in hepatitis C negative and positive insignificant correlation between serum ferritin and IL13 in both studied thalassemic groups, in hepatitis C positive and in hepatitis C.

Discussion

The current study showed that mean serum level of IL-12 in groups I and II β-thalassemic patients.

This may be attributed to many factors such as splenectomy, iron overload, repeated exposure to foreign antigens at the time of blood transfusion and the use of chelating agents may induce profound deleterious effects on the immune cells that secret this cytokines in β-thalassemia patients [2,3,7].

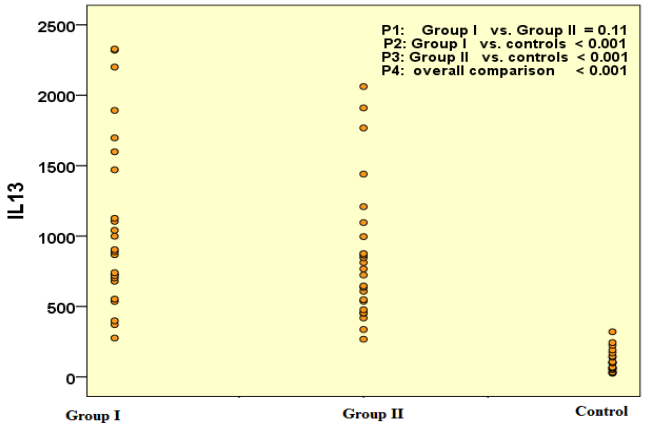

Fig. 2. Distribution of IL-13 among the studied groups

The decrement in the level of IL-12 in β-thalassemic patients with HCV infection as compared to those who were free from infection may be attributed to that HCV infection may suppress IL-12 production [2,8-10]. The inverse, yet, insignificant correlation between serum ferritin and IL-12 in both β-thalassemic groups may be ascribed to that iron overload per se had a negative effect on IL-12 p40 gene expression in neutrophils [11].

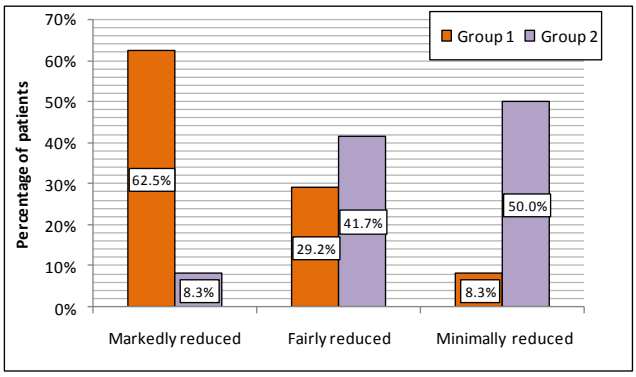

Fig. 3. Distribution of patients according to the reduction in IL-12 level

The current study showed that mean serum level of IL-13 was significantly higher in both groups of β-thalassaemia than that in controls, this could be ascribed to that in thalassemia the chronic transfusion program will result in continuous antigenic stimulation and iron overload with consequent abnormality in cell mediated immunity such as reduce CD4/CD8 ratio, T-cell subset anomalies and alteration in T-cell number and function [2,3,7]. This iron overload will induce oxidant stress and inflammation and will lead to organs injury [12,13]. Since IL-13 is an anti-inflammatory cytokine so its secretion will increase as aconsequence to inflammatory processes that occurs in thalassemia. In the current study serum iron along with IL-13 were higher in group I compared to group II; this may be attributed to the oxidative effect of iron overload which increase the secretion of anti inflammatory cytokines (IL-13) [12,13].

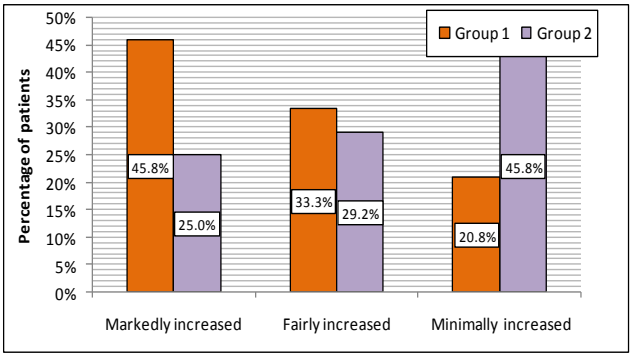

Fig. 4. Distribution of patients according to the increment in IL-13 level

There was positive insignificant correlation between serum ferritin and IL-13 in both studied thalassemic groups. The oxidative effect of iron overload in thalassemia can initiate tissue injury and/or inflammation, and this may result increase the secretion of anti inflammatory cytokines (IL-13) [12,13].

In conclusion, β-thalassemia major patients had decreased level of IL-12 and increased level of IL-13 which was more prominent in hepatitis positive thalassemic patients. There was a significant inverse correlation between IL-12 and IL-13 level in β-thalassemic patients.

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Author contributions

Conception and design, data collection, analysis and interpretation, writing and revision of the manuscript by Hashim; collection of cases and lab investigations, checking medical and surgical history by Dr. Alrawi and Hashim; and evaluating the levels of interleukins 12 and 13 by Jamal and Hashim.

Conflict of interest

None.

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Surgical Treatment of Parkinson's Disease: A Clinical Prospective Study with Six Years Follow up

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Abstract

Background Deep Brain stimulation is well accepted now as amethod for treating refractory parkinsons disease.

Objective To describe deep brain stimulation in Iraq, emphasize the technically demanding procedure and to discuss the results on the patients after six years of follow up.

Methods A clinical prospective study of 8 patients with Parkinson's disease underwent deep brain stimulation surgery in the Neurosciences Hospital. We performed 18 multiple stages operations from October 2007 to June 2008. The procedure begins with proper selection of patients, pre operative radiological studies, planning for targeting the subthalamic nucleus, the operation stage and the programming stage, which usually starts one-week post operatively.

Results One patient developed subarachnoid hemorrhage, another one have miss targeting and required retargeting surgery. A part from these complications all the patients had satisfactory outcome in controlling their symptoms during the six years follow up period.

Conclusion Deep brain stimulation is indicated for the treatment of refractory Parkinson disease. However it needs a well skilled personnel working as a team. The future of deep brain stimulation is remarkable as the list of indications is continually increasing to include other disease modalities.

Key words Parkinson's disease, deep brain stimulation, functional neurosurgery.

List of abbreviation: PD = Parkinson's disease, DBS = Deep Brain Stimulation, GPi = Globus Pallidus Internus, STN = Sub thalamic nucleus, MRI = magnetic resonance imaging, CT = computed tomography, IPG = Implantable Pulse Generator, AC= anterior commissure, PC = posterior commissure.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease. The main clinical features composed of resting tremor, bradykinesia and rigidity. These tend to occur early in the course of the illness; postural instability, loss of balance and freezing of gait are later features [1-4]. As PD progress, intractable disability is commonly caused by medically unresponsive axial symptoms particularly gait and postural impairment, dysphagia, dysphonia and cognitive decline [3-5].

Deep brain stimulation (DBS) has become an accepted treatment for medically refractory PD. Those includes all the patients whom not adequately controlled with medications, disability resulting from hypokinetic fluctuations, dyskinesia and/or tremor [6]. It improves PD motor symptoms proportionally to the pre-operative response to levodopa; the improvement of motor disability allows a significant reduction in dopaminergic therapy with consequent regression of drug-related dyskinesia [7,8].

Both Globus Pallidus Internus (GPi) and Sub thalamic nucleus (STN) stimulation have shown similar efficacy rated. However the literature demonstrates a trend that STN stimulation may be more effective in managing the symptoms of

PD. The Choice of the STN over the GPi is often based on institutional experiences, surgical and programming expertise and preferences [9-11].

Method

Patients Selection

In this clinical prospective study, eight patients were selected in the period from October 2007 to June 2008 to perform the surgery. They were evaluated with certain inclusion criteria; the patient must pass it before accepting him to this therapy (Table 1). We performed 21 operations for 8 patients with PD. Two patients completed the whole procedure in one session, two patients completed the surgery in two sessions and four patients had three sessions.

Table 1. Inclusion and exclusion criteria for deep brain stimulation

Inclusion Criteria for Surgery/DBS [13]	Exclusion Criteria for Surgery/DBS [14]
1. Good general health 2. "Normal" cognitive and affective functions 3. Motor disability 4. Failure of all drug strategies 5. Good response to L- Dopa drug 6. Good level of functioning when "on" Brain MRI in the normal range	1. Multiple medical complications 2. Dementia, severe frontal lobe dysfunction, severe depression 3. No adequate drug trials 4. Poor levodopa response 5. Disability arising from levodopa-unresponsive symptoms 6. Significant MRI abnormalities

Radiological Studies

These studies include pre-operative magnetic resonance imaging (MRI), head frame fixation with computed tomography (CT) or MRI studies and intraoperative C- arm studies. New set of MRI studies must be done. These ideally be done with 1.5 tesla closed MRI. They must have certain requirements to be accepted for the software of the planning workstation (e.g. frame link of Medtronic or surgiplan of Elekta) like the square image, no overlap, equidistance, no space, no compression, real image (no scout), no gantry tilt, no head tilt, and maximum slice thickness 2 mm

Head Frame Fixation

By using the Leksell Stereotactic Frame System of Elekta. The fixation is done routinely in the radiological department. Taking axial 2 mm thickness volumetric scans (from the hard palate to the vault). These sets of images will not be used for the detection of the target; it will be used by the software program to determine the values of X (medial-lateral), Y (anterior-posterior), Z (superior-inferior) of the STN. We also perform the MRI images with the frame and apply fusion with the frameless images, but the image distortion with the CT-images is much less than with the MRI. During taking these images the surgeon must be sure that all the nine fudicials will appear in the image sections. All these studies should be transferred to the planning workstation (we use Medtronic workstation with frame link software version 4).

Planning For Targeting the STN

After transferring the image data to the workstation, it will be recognized automatically by the software. The images with frame signals will be the registered images and the others will be recognized as the working images. Determining the anterior commissure (AC) and posterior commissure (PC) points is done in the axial T1 MRI images, with simultaneously observing the other sagittal and coronal reconstructed ones. Mark the center of the posterior border of the AC and the center of the anterior border of the PC. The AC-PC line length must be within the acceptable limits (22-26 mm).

The STN will be determined in the T1 images using the indirect method (the formula based equation) i.e., 12 mm lateral to the mid-commissural point, 4mm (2 sections) below and 4mm posteriorly. This will be automatically revealed with single click. We record the values of the x, y, and z of the frame.

In axial views we consider the section with largest diameter of red nucleus. The STN is determined by drawing two tangential lines from the lateral border of the red nucleus and the anterior border of it. The STN is located at a point 2.3 mm away from the lateral border and 2.1mm from the anterior border (Fig. 1).

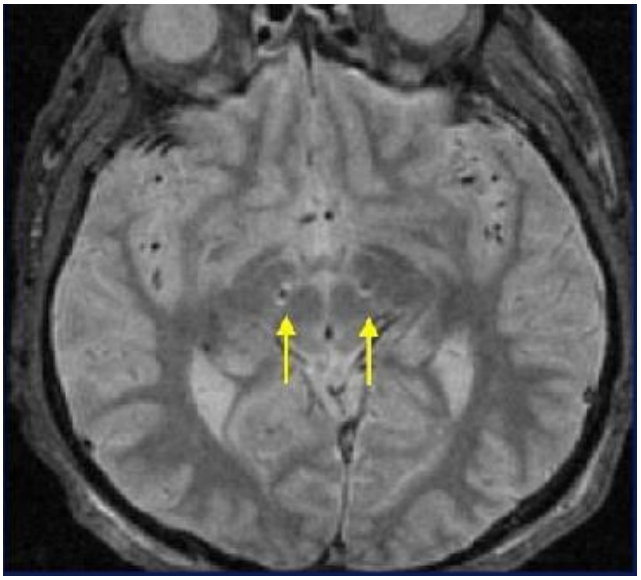

Fig. 1. Targeting the STN in the Axial views

The final agreed reading would be a combination of all the records by taking average reading aiming to include most of the recorded values within a circle of 4 mm diameter, which represents the circle of the 5 microelectrodes that will be used later for recording and stimulation.

We set the entry point just on or 2 cm anterior to the coronal suture (properly visualized through the CT images). It must be sufficiently laterally located to avoid the sinuses and entrance of the trajectory to the cerebral ventricles. In the probe's eye view we should go down to 5 mm below the target area because the recording and stimulation studied will involve these areas too.

Operating Theatre Stage

This stage is composed of application of the arc with the Micro targeting drive, burhole, insertion of the electrodes for recording and stimulation studies, implantation of permanent electrode and implantation of the Implantable Pulse Generator (IPG). The major steps of surgery are done under local anesthesia to check the response and side effects of the stimulation on the patient.

Intraoperative Neurophysiologic studies

Microelectrode recordings during stereotactic procedure for implantation of DBS electrodes in the STN have provided important data about the physiology and pathophysiology of this nucleus in the humans [16,17].

The criteria for identifying the neural activity of STN were the shape of the units, their firing frequency, the response to passive and active limb displacement and the tremor-locked activity [18].

Recording begins at various distances from STN target from several millimeters up to 40 mm.

The maximal length of STN recorded varies from patient to patient and depends also on the approach angles, but ranges from 4.2 to 5.4 mm.

We chose the most reliable electrode, which reveals the site of the STN to start its stimulation. Stimulation begins with examination for baseline resting tremor and rigidity. All the team most commonly performs testing the DBS electrode for efficacy and side effects.

The final position of the permanent electrode is a shared decision of the neurosurgeon and neurologist with the neurophysiologist. The macro electrodes are introduced into the deepest desired location. The cannula with the accompanying electrode of the desired DBS electrode placement is removed and its length is calibrated with the DBS electrode which will be introduced into the brain and remove it's stylet. Finally it is fixed to the burhole fitter slit and cover it with its plastic cover. C – Arm image is

taken to assure no kink occurred along the pathway of the implanted electrode.

The connection of the electrode with the extension wire and the IPG is implanted in the right subclavicular area. This part of the surgery is done under general anesthesia. The patient's preoperative PD regimen should be restarted immediately after surgery to avoid problems with dopaminergic withdrawal. Patients should undergo postoperative CT scans and/or MRI scans to assess the electrode location and intracranial status. Patients can be discharged as early as 24 hours after surgery, depending on their neurological and cognitive status [19].

Programming of the patient starts usually one-week post operatively in order to avoid the period of lesioning effect of the surgery.

Results

We performed 18 operations for 8 patients and the surgery for each patient was individualized, i.e., some patients complete the whole procedure in one session (2 patients) which includes the electrode implantation on both sides and IPG implantation. Others either in two (2 patients) or three (4 patients) sessions with one week interval for electrode implantation and 3 days later for the IPG implantation. This variation in the number of sessions is due to the long duration of the surgery. The average length of the whole sessions is eight hours; seven hours of them are under local anesthesia, so not every patient can withstand this long duration of awakened surgery. The sessions were delayed upon the request of each patient individually.

The final targeting of the STN was determined as in table 2. All the patients showed significant improvement during the intraoperative stimulation studies. The final location of the permanent electrode was determined as the site with maximum response with the least side effects.

All the patients started programming one week following the surgery to avoid the period of lesioning effect (this is a period in which the patient get relief of the symptoms just because of the lesion that was resulted by the electrophysiological studies). The patient then gets regular visits on monthly period to re evaluate for three months and later on every 6 months.We followed the patients for an average of six years. We start the stimulation with Monopolar program and when we need a higher voltage we shift to bipolar programming to try to length the life of the battery. The voltage for all the patients was in a range between 2.0 to 4.5 volts.

Table 2. details of the operative electrode replacement

Patient	Eelctrode		Final targetig	
	Left side	Right side	Left STN	Right STN
1	Anterior	Medial	-2+5	-3+4
2	Medial	Anterior	-3.5+3.5	-2+5
3	Anterior	Medial	-4+3	-2.5+4.5
4	Anterior	Medial	-1+6	-2+5
5	Medial	Anterior	-1+6	-3+4
6	Anterior	Medial	-3+4	-1+6
7	Anterior	Medial	-3+4	-2+5
8	Anterior	Medial	-4+3	-3+4

STN = subthalamic nucleus, - No. = millimeters proximal to the target point, + No. = millimeters beyond the target point. Each permanent electrode has four attachment point for stimulation making 7mm length of active area to stimulate the subthalamic nucleus.

In the follow up period, 4 patients we reduce their medication and the others with the same dose of l- Dopa but with better symptomatic relief (Table 3).

We had one patient developed intravenricular hemorrhage. This enforced us to delay one face of electrode implantation for one month. Another patient developed errors during programming which necessitate retargeting procedure. Six of our patients had to change their IPG because of the end of battery life

Table 3. The followup results over six years period

Patients	Duration of follow up (Months)	Starting mode	Recent mode	Post operative drug dosage (+)
1	75	Monopolar	Monopolar	Reduced 40%
2	72	Monopolar	Bipolar (6 months)	Reduced to 25%
3	70	Monopolar	Bipolar (8 months)	Not changed
4	78	Monopolar	Bipolar (3 months)	Not changed
5	68	Monopolar	Bipolar (8 months)	Not changed
6	62	Monopolar	Bipolar (8 months)	Not changed
7	62	Monopolar	Monopolar	Reduced 40%
8	60	Monopolar	Bipolar (3 months)	Reduced 30%

(+) = The drug reduction in the table is related to the L- Dopa. For all patients the dopamine agonist were reduced but the neuroprotective medications remained unchanged

Discussion

To establish the DBS services in Iraq, many challenges were faced. The time of surgery was challenge per se; our first surgery last for 13 hours but with splitting the operation into 2 to 3 stages and with increment in the learning curve the time reduced to 5-6 hours. Our average time was eight hours. This is close to the readily accepted average time of six hours [19].

As noticed in the results, 50% of our patients had significant reduction in the dose of their medication with significant relief of the tremor, because they were in continuous contact with us for follow up better than the others. Compared with other data, the reduction in the dose was ranging from 20, 35 to 60 % [9,10,20]. The reduction of the medication alone is not the only indicator of the success of surgery but the better control of the symptoms even with the same medication is the main gain to all our patients [20,21].

Those patients who have no change in their dose of medication, they have axial symptoms and postural instability, which is in many literatures, does not respond to STN stimulation as tremor and rigidity [20]. It was concluded that the postural instability usually not relived with the DBS [20-22]. The risk of intracerebral or intravenricular hemorrhage is well recognized in the literatures [20,21]. It is estimated to be 2.5-5% in most centers. It can be avoided by proper planning of the trajectory by using higher resolution MRI imaging like 3 tesla MRI.

In conclusion, DBS is a reliable control measure for PD. It does not affect the fate or progress of the illness. In contrast to the other ablative procedures; DBS is completely reversible that can remove the whole stuff with no permanent lesion to the patient's brain.

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Conflict of interest

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Electroencephalographic Assessment of Cerebral Activity in Patients with Spinal Muscular Atrophy

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Abstract

Background Spinal muscular atrophies are a group of degenerative diseases primarily affecting the anterior horn cells of the spinal cord and motor cells of cranial nerve nuclei. Even if the clinical picture is mainly dominated by the diffuse muscular atrophy, in some cases, patients may show associated, atypical clinical features. There have been a few reported cases of spinal muscular atrophy with central nervous system involvement; in particular, the association with progressive myoclonic epilepsy has been rarely described.

Objectives To clarify the impact of this degenerative disease on central nervous system and specifically on cerebral activity by using electroencephalographic exam.

Methods Thirty two patients with spinal muscular atrophy and 20 control subjects were included in this study. Their ages were between two months and one year. Brain CT and MRI, electroencephalography was done for all of them and cerebral activity was precisely assessed with emphasis on normal brain waves frequency and distribution.

Results The frequency of brain waves recorded from patients with spinal muscular atrophy who show abnormal electroencephalography were 1.49±0.4 and it is significantly lower than that of control subjects (4.11±1.1). The mean frequency of brain waves recorded from patients with spinal muscular atrophy with normal electroencephalography were 4.13±1.2 which is higher than the mean frequency of brain waves recorded form patients with abnormal electroencephalography (1.49± 0.4).

Conclusion Central nervous system could be affected in patients with spinal muscular atrophy specifically cerebral activity, which might show diffuse slowing in brain waves as revealed in this study.

Keywords Spinal muscular atrophy, cerebral activity, electroencephalography

List of Abbreviation: SMA = spinal muscular atrophy, EEG = electroencephalography, CT = computerized topography, MRI = magnetic resonance imaging, CNS = central nervous system, PML = progressive myoclonic epilepsy.

Introduction

Spinal muscular atrophies (SMAs) are a group of degenerative diseases primarily affecting the anterior horn cells of the spinal cord and motor cells of cranial nerve nuclei [(1,2)]. Clinically, it is classified into three sub-types based on the age of onset and severity. Type one is the severe form with onset before the age of six months, and the patient is unable to sit without support; type two is the intermediate form with onset before eighteen months, and the patient is unable to stand or walk without aid; and type three is the mildest form with age of onset after eighteen months, and the patient is able to stand and walk [(3)].

Genes for all the three subtypes of SMA have been mapped to chromosome 5q13 [(4,5)]. Even if the clinical picture is mainly dominated by the diffuse muscular atrophy, in some cases, patients may show associated, atypical clinical features ("SMA plus"). There have been a few reported cases of SMA with central nervous

system (CNS) involvement, in particular, the association of SMA and progressive myoclonic epilepsy (PME) has been rarely described [6,7].
The aim of this study is to clarify the impact of this degenerative disease on CNS and specifically on cerebral activity using electro-encephalography (EEG) exam to see if there is any possible influence of the disease process on cortical neurons which might contribute later on to affect cognitive function and cause behavioral changes for those individuals who complain subtle changes on cerebral functions.

Methods

This is a cross sectional study carried out in the period from February 2012 to October 2014 in Basra Children Specialty Hospital. It includes thirty two patients with proved SMA (clinically and electrophysiologically; their ages were between two month and one year. Brain computed tomography (CT) and Magnetic resonance imaging (MRI) had been done for all of them and were normal. They were stopped using any type of medication especially sedative drugs for at least two weeks before doing EEG.
Digital EEG had been done for them using digital EEG machine (Nihon Kohden EEG 1200 J/K) according to the 10-20 International system. The measured impedances were less than 5 Kohms at all electrodes. All studies utilized both bipolar and referential montages. Initial analog signal conditioning included a 0.3-1 Hz high pass filter, a 35-70 Hz low pass filter and a 50 Hz notch filter. The digital sampling rate was 200-500 per second. EEG recordings last for 30 minutes. Activating techniques including intermittent photic stimulation were used and the record obtained during awaking and during sleep. The EEG record were re-analyzed and reviewed and background cerebral activity were assessed by calculating the frequency of brain waves and their distribution manually and by specific analysis program software (EEG examination support software:QP-150AK). According to the results patients were divided in to two groups:
1. Group A: patients with normal EEG.
2. Group B: patients with abnormal EEG .

We include twenty infants as a control group aged between one month and one year whom referred to the neurological outpatient clinic in Basra General Hospital or the Neurophysiology Outpatient Clinic in Basra Children Specialty Hospital in the same period of our research for conditions other than flaccid paralysis or suspected CNS disorders. After taking the acceptance of their parents about the aim of our further investigations, a brain imaging studies was done for them and those with completely normal results and on no medical treatment of any kind for at least the last two weeks were selected. Then we did EEG study for them using the same protocol that had been used for the patients. This study was conducted in accordance with a protocol approved by the Committee on Clinical Investigations at Basra College of Medicine and Basra Health Directorate. All patients were informed about the aim of study and their acceptance obtained.
Data analysis was done using Statistical Package for Social Sciences (SPSS) version 20 computer software. Descriptive statistics for all data of each set was expressed as mean ± 2SD. The difference in mean frequency of brain waves between groups was assessed by independent sample t-test, $P < 0.05$ considered statistically significant.

Results

The total number of patients included in the study were thirty two patients with SMA, fifteen of them (46.9%) were males and seventeen (53.1%) were females. Twenty control subjects age and gender- matched were also included in the study (Table 1).

Table 1. Number of patients with spinal muscular dystrophy and control subjects

Gender	SMA patients		Control Group	
	No	%	No	%
Male	15	46.9	10	50
Female	17	53.1	10	50
Total	32		20	

SMA = spinal muscle atrophy

Table 2 show the number of patients with SMA and have abnormal EEG pattern (group B) they were thirteen patients (40.6%), six males and seven females, and patients belongs to SMA who have normal EEG (group A) were nineteen patients (59.4%), no significant difference between males and females patients were obtained regarding their EEG findings.

Table 2. Number and percentage of patients with normal and abnormal EEG findings

Gender	Group A		Group B		Total
	No	%	No	%	
Male	9	47.4	6	46.2	15
Female	10	52.6	7	53.8	17
Total	19	59.4%	13	40.6%	32

The comparison in mean frequency of brain waves recorded by EEG from patients with SMA in group (A) and control subjects show no significant difference obtained and the p. value was not significant. When we compare the frequency of brain waves recorded by EEG from patients in (group B) and control subjects, the results show that the mean frequency of brain waves for patients in (group B) were (1.49±0.4) which is significantly lower than that of control subjects (4.11±1.1) as observed in table 3.

Table 3. Frequency of brain waves in control subjects and patients with and without EEG abnormalities

Subject	Group A		Group B	
	No	mean±SD	No	mean±SD
Patients	19	4.13±1.2	13	1.49±0.4
Controls	20	4.11±1.1	20	4.11±1.1*

* *P* = 0.001

The results in table 4 showed that the mean frequency of brain waves recorded by EEG exam for patients in group (A) were (4.13±1.2) which is higher than the mean frequency of brain waves recorded form patients in group (B) (1.49± 0.4).

Table 4. Frequency of brain waves between spinal muscle atrophy patients with abnormal and patients with normal electro-encephalogram

Group	No.	Mean ±SD
A	13	1.49±0.4
B	19	4.13±1.2*

*P = 0.001

Discussion

We found that 46% of SMA patients have significant slowing in their brain activity as compared to control group and this high percentage cannot be explained by an accidental finding or an incidental condition.

When the pathology of this disease was revised; an obviously no evident pathology had been found in both corticospinal and corticobulbar tracts at autopsy [8,9], yet this is not necessarily means a normal function of these systems or the cerebrum during their lives, specially there are only several studies investigate this issue [5,21]. So we have to revise our knowledge about the believe of the only affection of motor neurons of brain stem and spinal cord by SMA and cortical and subcortical structure have to be further investigated and analyzed functionally and structurally (either by autopsy or histologically if possible at life) for possible effect of the degenerative disease process on these organs [10,5].

In one study by Striano *et al* there was an association between SMA and PME but it was unclear whether it was a separate conditions (genetically independent syndromes) or a variant from SMA [6,11].

In another study by Anderson *et al*, a clear association between Duchenne muscular dystrophy, which should be purely muscle disease and brain pathology was found. Despite of the fact that Duchenne muscular dystrophy has nothing to do with our study, nevertheless, it can give an exemplary of how we have to restudy these diseases using modern tools to see how these diseases actually affect different

systems of our body and not only the targeted system of the degenerative processes [12,13].

In conclusion, although SMA affects mainly motor neurons in bulbar and spinal cord region but CNS could be affected specifically cerebral activity, which might show diffuse slowing in brain waves as revealed in this study.

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Author contributions

Dr Mohammed did the study conception and design, in addition to the EEG recording; Dr Hammady collect the cases, made the diagnosis and neurological examination. The authors share the responsibility in preparing and completing this work.

Conflict of interest

There is no conflict of interest that could influence the objectivity of the research reported.

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Elliptical Rotation Flap for Complicated Pilonidal Sinus

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Abstract

Background Pilonidal sinus is one of the most common sinuses seen in general surgical practice and usually seen in the natal cleft. Postoperative wound complications have always been the main cause of concern followed by the risk of recurrence in the surgical treatment of the pilonidal sinus disease. Various techniques evolved so far mainly aimed at solving these problems. This clinical study conducted to discuss the results of elliptical rotational flap in chronic pilonidal sinus.

Objective To assess the outcome of patients operated by rotational flap procedure in chronic pilonidal disease in term of healing time, complications and recurrence rate.

Methods The study was conducted in two different places from December 2007 till April 2011. Eighty two patients with chronic pilonidal sinus were treated by rotation flap. The setting was in two different places, at Nizwa General Hospital, Sultanate of Oman and from Al-Imamain Al-Kadhemain Medical City, Baghdad. Evaluation of operative and post-operative results, complications and recurrence in addition to demographic data.

Results Out of 82 Patients included in study, 2 cases (2.4%) failed to heal due to partial necrosis of the flap and underwent redo surgery, while 80 patients (97.4%) were cured completely and no further surgery was needed. Eighteen patients (22%) went home in the end of same day and the rest (78%) discharged in second day. Forty six Patients (56%) had dry dressing till suture removed after 8 days, while 20 Patients (24.4%) continued to discharge serosangunious fluid and spent more than 10 days to heal. Eight Patients (9.8%) developed seroma needed further time to becomes dry and the last 6 Patients (7.3%) developed wound infection and treated by proper antibiotics and surgical drainage in some case.

conclusion Flap procedures may be promising especially in complex and recurrent pilonidal diseases as it is simple to be done and carry low recurrence rate and relatively shorter hospital stay in comparison to other surgical procedure

Keywords Chronic pilonidal sinus, elliptical rotation flap.

List of Abbreviation: PNS = pilonidal sinus, SSI = surgical site infection

Introduction

Pilonidal sinus (PNS) was first described by Hodges in 1880, who described the sinus as a characteristic epithelial tract in the skin of the natal cleft, generally contains hair [(1)], The Latin name pilonidal means literally a nest of hair. Chronic PNS is a common disabling disorder that affects mainly young adults and the men are affected twice as often as woman [(2)]. It has a high incidence in some countries, particularly in the Middle East and Gulf region owing to differing hair characteristics and growth patterns [(3,4)].

Today's most widely accepted explanation for the pathogenesis of PNS was suggested by Karydakis [(5)] who attributed the occurrence of PNS to three main factors: the invader (loose hair), the force (causing insertion) and the skin vulnerability (depth of the natal cleft).

Many surgical and non-surgical procedures have been suggested for treatment of PNS; however, in most of cases, an operation is advised. There are various operations adopted to cure this problem. Each one has its own pros and cons. The following is the main surgical procedures in clinical practice and there are many other procedures and modifications.

1. Wide excision and healing by secondary intention. The wound is left open to heal by second intention. This usually means that the wound can take several weeks to heal and requires regular dressing [6].
2. Excision and primary closure (to form an ellipse shape around the sinus), taking out the sinus, and stitching together the two sides of the ellipse. The advantage, if successful, that the wound heals quite quickly, however, the risk of a recurrence, or of developing an infection of the wound after the operation, is higher than the above procedure [7,8].
3. Wide excision of the area containing the sinus and all branches after discolored by blue dye, and to remove all sinus bearing areas, then hemostasis is achieved and the defect is covered by tension free local advancement flap from adjacent healthy region (rotation or limberg flap) which ensure excellent healing result and make an unfertile bed for the recurrence of new sinus in the future [9,10].

The management of chronic PNS, the best method is still controversial. Many surgical and nonsurgical treatment modalities have been suggested, but the ideal and widely accepted treatment has still not yet been established. In this regard, low recurrence rate, shorter hospital stay, less cost, minimal inconvenience and time off work are important considerations [11,12].

This study was carried out to evaluate the usefulness of elliptical rotation flap technique in treatment of chronic PNS in our setup.

Methods

This is prospective study of 82 patients with complicated PNS, we mean "complicated" by either recurrent or chronic PNS with midline and lateral pits, the setting was in two different places; one in Nizwa General Hospital Sultanate of Oman, the other in the surgical department of Al-Imamain Al- kadhemain Medical City, Baghdad. The study started in period between Dec 2007 till Nov 2011.

All patients were operated after preliminary investigations and anesthetic fitness. Patients received prophylactic antibiotic in form 500 mg metronidazole and 1 gm third generation cephalosporin, taking in consideration that a colonic microorganisms are the most probable microorganisms than that of skin flora.

The exclusion from this study was for all patients with severely infected sinus or have PNS abscess till the infection cured, in addition simple acute PNS and patients with under 16 year old were also excluded.

Under general or spinal anesthesia, the sinus discolored by methylene blue dye to color all side branches beside the main tract, an asymmetrical elliptical incision was made with wide margin to remove all diseased area deepen to presacral fascia, then we incise nearby skin (local flap reconstruction) in the same size measured by a ruler, including the subcutaneous fat and in the same thickness of the removed tissue, the flap is detached from one side of surrounding tissue deep to gluteal fascia and keep 2 finger breadth in the inferior aspect for nourishment of flap, the flap is transferred to cover the defect and sutured to the deep tissue by 2/0 vicryl suture and to the skin by non absorbable 3/0 nylon (figures 1-4). The flap should be wide enough to completely obliterate the midline natal cleft and to reduce potential risk of flap`s corner ischemia Suction drain was used for drainage and removed in the second or third post operative day.

The patients were followed up for an average of about 20 months, the follow up visits were scheduled at 3rd, 8th ,14th, 28th post operative day then 4-6 monthly or on need in case of wound complications. The Chi square test was used for statistical analysis, a *P* value < 0.05 was considered significant.

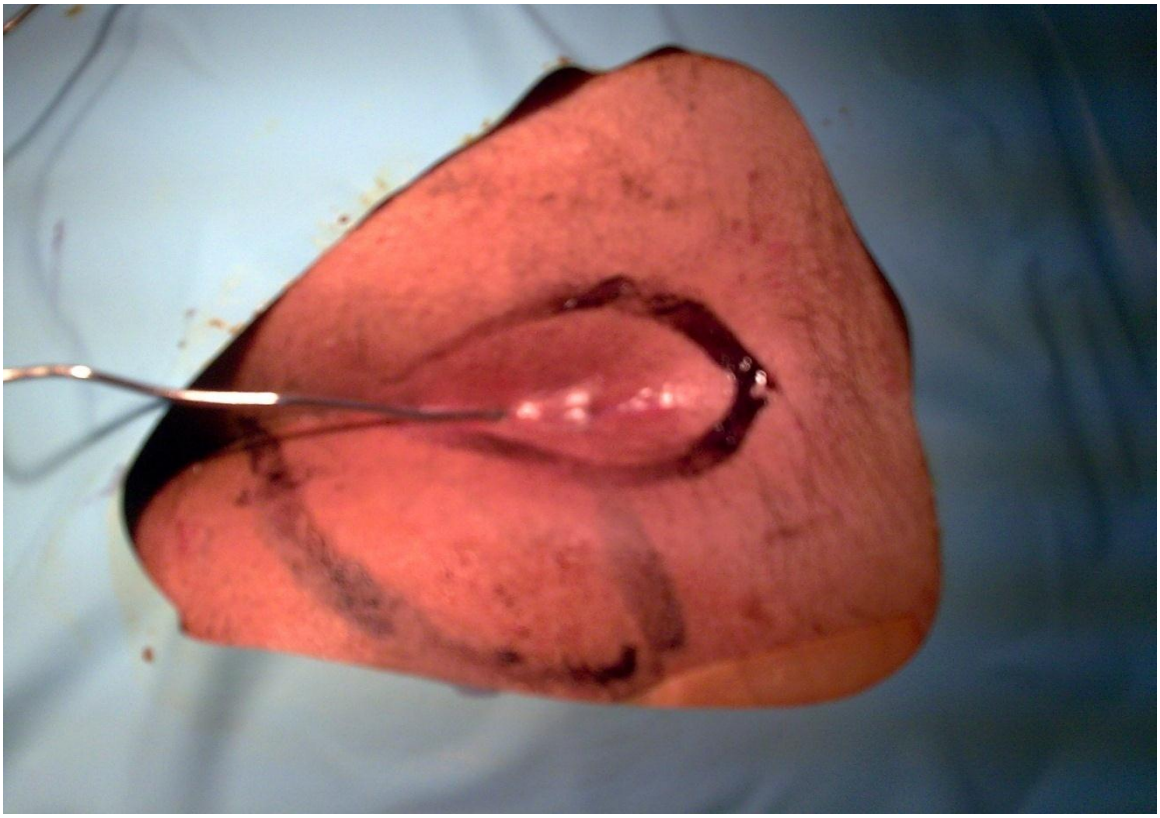

Fig. 1. The sinus was probed and the skin was marked by marker, the upper flap for removal and adjacent flap for reconstruction

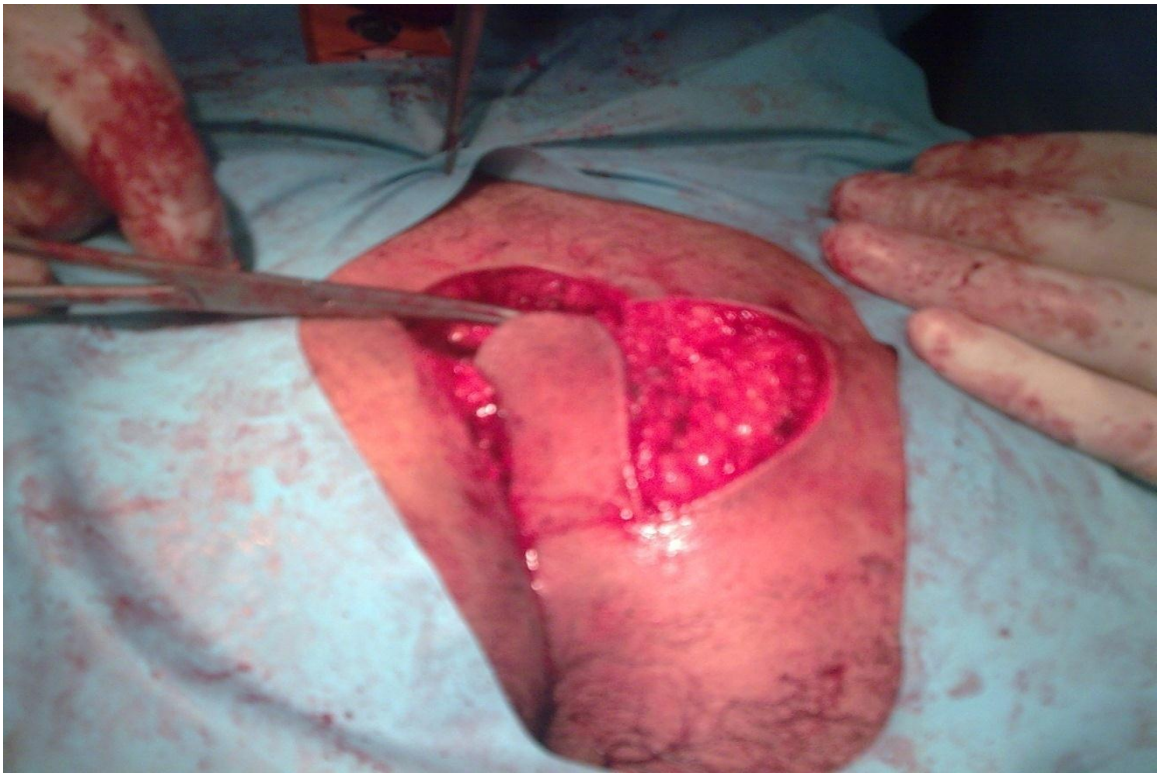

Fig. 2. The sinus bearing area excised and adjacent flap with its sufficient pedicle mobilized to cover the defect

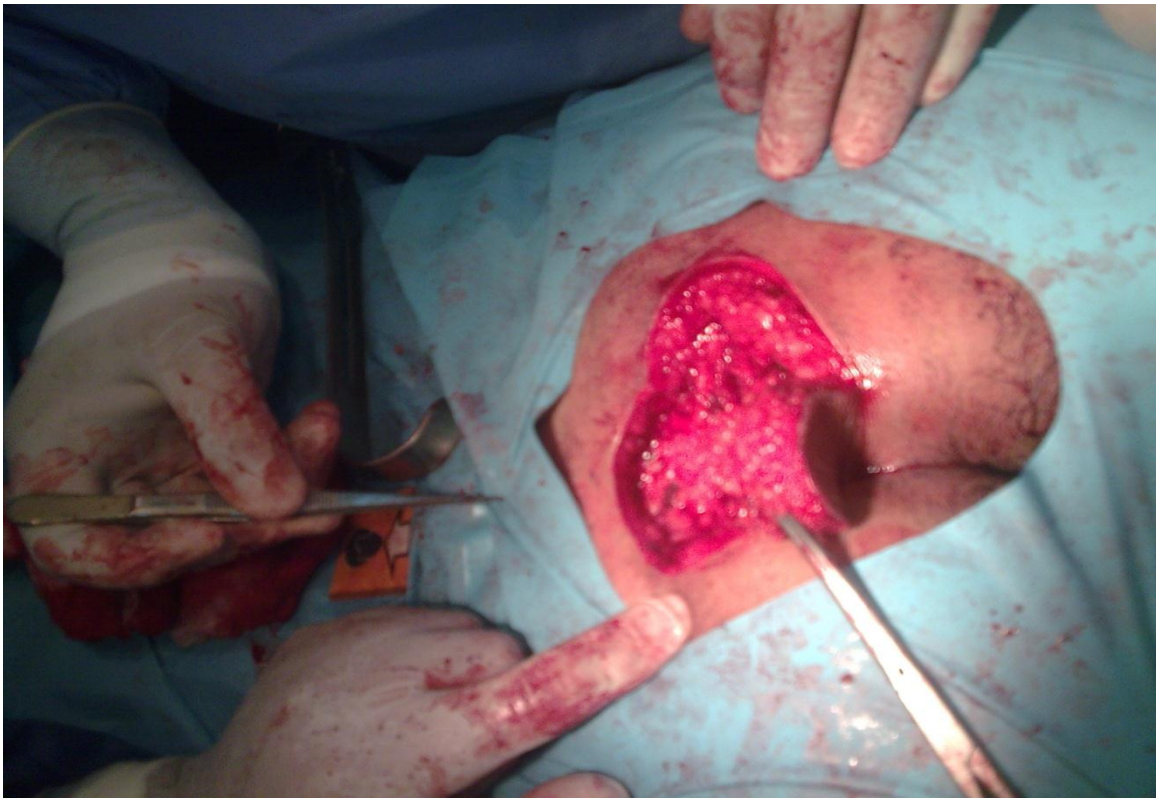

Fig. 3. After hemostasis the flap ready for rotation and defect closure

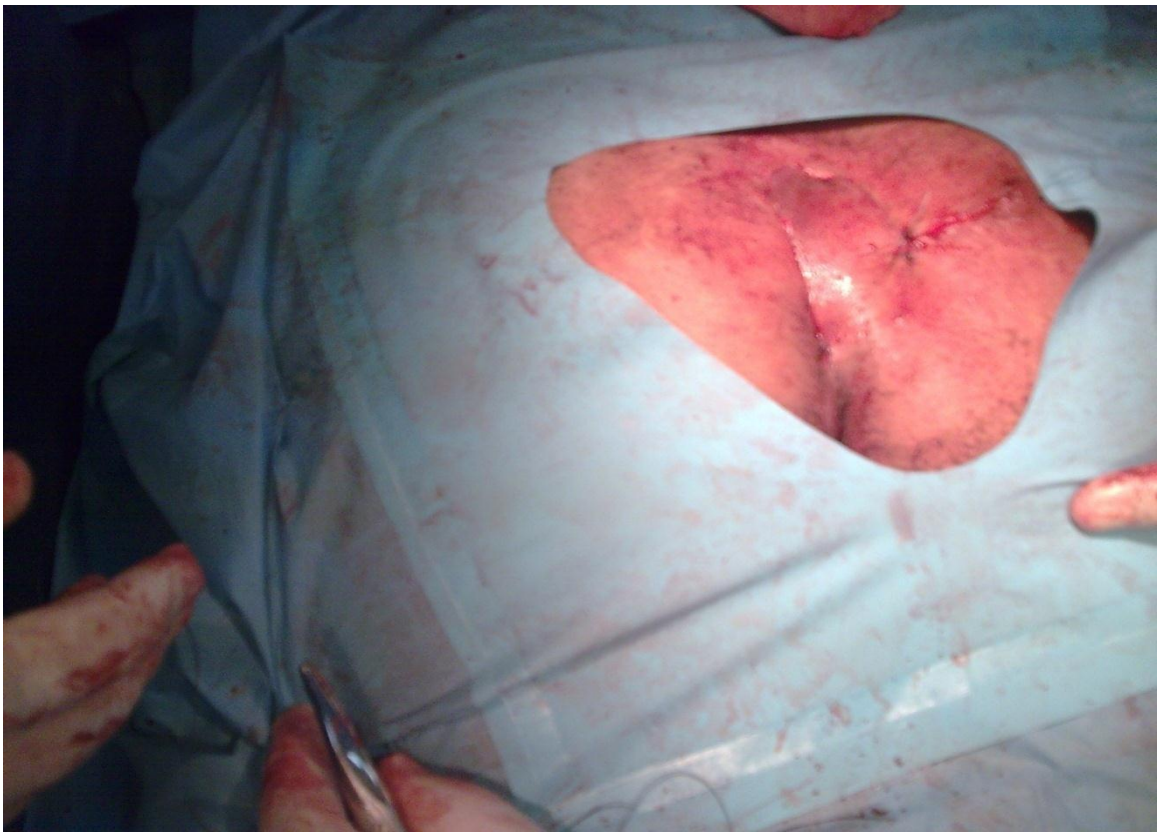

Fig. 4. The end of the end result of surgery the defect created closed by adjacent flap and the gluteal defect closed by direct suture the edge of the skin

Results

Out of 82 Patients included in this study, there were 56 males (68%) and 26 females (31%). The male: female ratio in Iraqi patients was 16.6:1, while among Omani patients was 1.4:1, the difference was statistically significant (P<0.0001) (Table1).

Table 1. Sinus distribution among Iraqi and Omani patients

Gender	Omani patients		Iraqi patients		Total	
	No	%	No	%	No	%
Male	17	21	59	61	67	82
Female	12	14.6	3	3.4	15	18
Total	29	35.6	53	64.4	82	100
♂:♀ ratio	1.4 : 1		16.6 : 1			

The mean age was 20 years ranging from (16-28 years). Chronic PNS represented in 59 patients (72%) and the other 23 Patients with recurrent PNS (Table 2). In 72 Patients (88%) closed vacuum drains were used and the rest 10 patients (12%) sustained closure without drains. 18 patients (22%) were sent home in the end of same day and the rest 64 Patients (78%) discharged in the second day (Table 2).

Table 2: miscellaneous characteristics

Type of sinus	Chronic pilonidal sinus 59 Patients (72%)	Recurrent pilonidal sinus 23 Patients (28%)
Flap viability	Healthy viable (80 Patients)	Partial necrosis (2 Patients)
Need second surgery	No further definite operation.	2 Patients need second surgery.
The Use of Drain	72 Patients closed vacuum drain	10 Patients (12%) no drain used
Hospital stay	18 Patients as day case surgery	64 Patients one night sleep.
Time of return to daily work	8-16 days	
Recurrence rate	3 Patients (3.6%)	
Major Complication	4 Patients (4.8%)	

Regarding healing, 46 patients (56%) had a dry wound and their sutures removed usually in 8-14 days, twenty patients (24.4%) had continuous serosangious fluid discharge, needed further few days for healing, 8 patients (9.8%) developed seroma solved by simple drainage procedure, other 6 patients (7.3%) get wound infection that needed culture based antibiotics to complete cure, 2 of them were deep surgical site infection (SSI) dictated readmission for 7 days with surgical drainage and debridement and later secondary wound closure, the last 2 patients failed to heal and needed revisional corrective surgery. Nearly all patients (97.6%) operated upon had viable flap. The time of recovery and return to daily activity was with a range of 8-16 days and with a mean of 9.4 day (figure 5).

The major complications that require admission and surgical procedure occurred only in 4 patients (4.8%), two with major or deep SSI and the other two patients with partial necrosis of flap's corners, while minor complications like superficial or minor SSI and seromas were treated simply and effectively at outpatient clinic in less than 2 weeks period. As seen in table 3, the recurrence rate in our series within the first 18 months of follow up was 3.6% (3 patients).

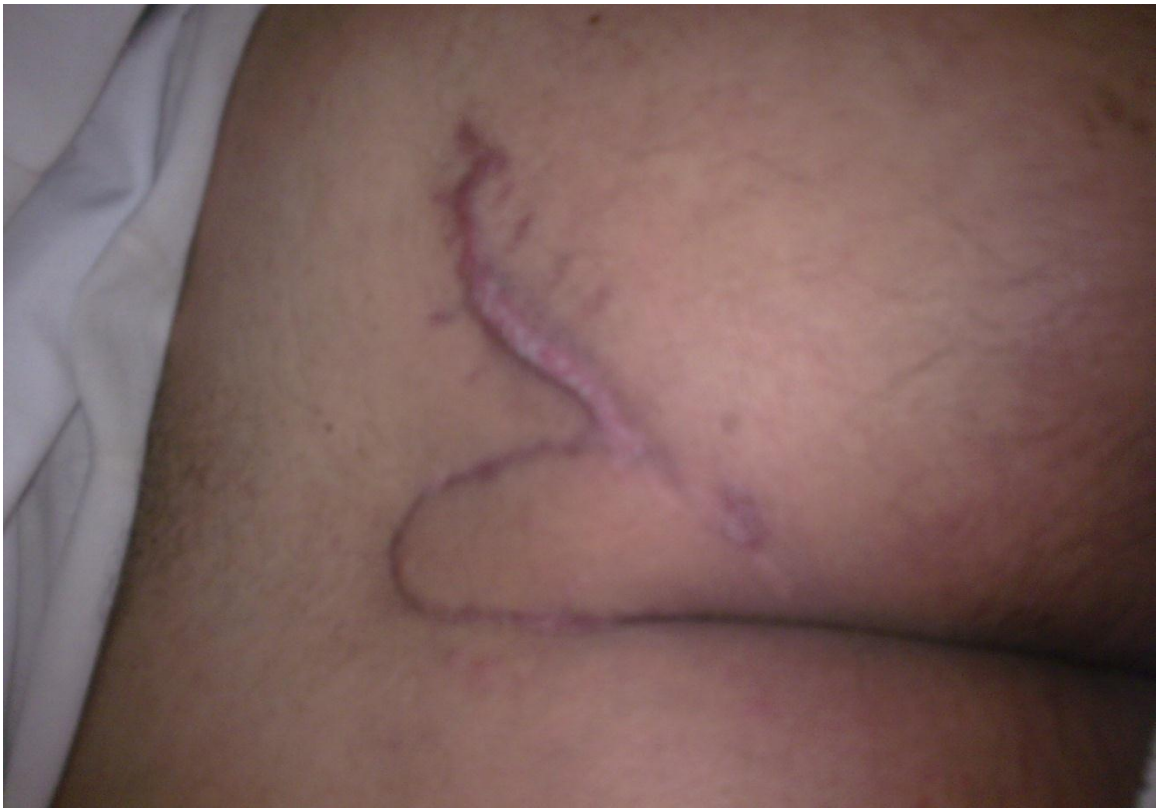

Fig. 5. The end result of healed flap one month post operatively

Table 3. The outcome and fate of our patients

Outcome of wound		No. (%) of Patients	The fate (end result)
Dry Wound		46 (56)	Healed in usual 10 days
Serosanguinous fluid		20 (24.4)	Become dry in 12-16 days
Seroma		8 (9.8)	Healed with repeated aspiration & dressing in 12-20 days
SSI	**Superficial**	4 (4.9)	Need just proper antibiotics
	Deep	2 (2.4)	Drainage and antibiotic, cured within14-21days
Fail to heal		2 (2.4)	Needed revision flap surgery after 12 weeks

Discussion

Pilonidal disease is a complex condition that causes both discomfort and embarrassment to sufferers. Direct costs to the healthcare system and indirect costs through absence from work are high. Regardless of the surgical technique concerned, standard principles of wound care are essential with repeated depilation of the natal cleft, removal of cut hair and any debris from the wound bed and keeping the wound edges dry and clean using an appropriate dressing [3,13].

In our study, nearly all patients included in this survey were completely cured apart from two of them (2.4%) who needed revision flap surgery to achieve good healing. This failure can be attributable to excessive scarring and ischemia of the vicinity area owing to repeated surgery and active recurrence of sinus (those 2 Patients had 3 times recurrence).

There is great discrepancy in gender distribution in between Iraqi vs. Omani patients" where male: female is 1.4:1 among Omani patients while 16.6:1 for Iraqi patients". This may be due to genetically determined factors related to type

of sacral hair among Omani women and/or may be due to ignorance and lack of medical advice among Iraqi woman.

Most of the patients treated by rotation flap have very little morbidity post surgery, as compared with other surgical methods, which incur prolonged healing time and a high recurrence rate, also it has many advantages. It is easy to perform, to design, and it flattens the natal cleft with large vascularized pedicle, sutured without tension. This in turn maintains good hygiene, reducing the friction, preventing maceration, and avoiding scar in the midline. It is a particularly useful technique for complex sinuses with multiple pits and extended tracts where radical excision leaves a large defect [14,15]. The healing with secondary intention would require prolonged supervised wound care. This operation is also suitable for cases where other simpler operations have failed. The use of local flap accelerates healing.

In the present study, the final healing was achieved in most cases (56%) within 10 days, the reminder get cured in a couple of weeks and only few of them 6 Patients (7%) needed 21 days hospital stay, the average mean time for recovery and return to daily activities and work was 9.4 days with a range of 8-16 days, these findings were more or less comparable with many other series [16-19] (Table 4), while flap's corner necrosis encountered in 2 patients (2.4%) as mentioned before recalled simple revision operation after 12 weeks to achieve full cure.

In our series, recurrence rate 3.6% and major complication rate 4.8% was comparable with other similar studies although hospital stay was short because most of our patients had tolerated the surgery well and they were able to be discharged in next day or at end of same day and the drain removed in next visit (Table 4).

Table 4. Comparison of current study with some similar studies

The study	No.	Hospital stay	Time of return to daily work	Complications %	Recurrence %
Jethwani et al [16]	67	2-3 days	10-16 days	11.94	1.49
Mentes et al [18]	238	2-3 days	4-17 days	2	1.3
Akin et al [17]	411	3 days	7-18 days	16	2.9
Mouhammed [19]	110	3 days	Within 21 days	5	1
The present study	82	12-36 hours	8-16 days	4.8	8

In comparison with other procedures, in case of excision and lay open method, one study included 150 patients showed an average healing time of 4 weeks and recurrence rate of 8% were recorded [20].

Another study used sinus excision and primary closure, 371 patients, recurrence rate was 12.1% (scar sited midline) [21] and 10% of recurrence rate (scar sited laterally) [22]. In Iesalnieks and his colleagues (German study) showed a high recurrence rate (42%) after excision of a pilonidal sinus and primary midline closure and (21%) after open procedure [23].

In conclusion, the results of our series support the PNS excision and rotation flap reconstruction as safe and effective definite treatment of the disease.

The technique can be mastered easily and provides an effective procedure for primary as well as recurrent disease. Few complications associated with it can further be reduced by meticulous skin closure and preventing skin edge inversion, especially at the lower midline.

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Author contributions

Dr. Al-Najjar did the data collection and analysis and Dr. Al-Helfy did the acquisition and interpretation of data, revising and supervision of manuscript.

Conflict of Interest

No potential conflict of interest

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Prospective Descriptive Study of Short-term Result of Ipsi-lateral Fracture Neck Shaft Femur Treated by Modified Traditional Ante-grade Interlocking Nailing and Lag Screw

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Abstract

Background Ipsilateral concomitant fractures of the femoral neck and femoral shaft are unusual (incidence 5-6%), and pose a difficult treatment problem. This injury usually resulting from high energy trauma, road traffic accident and fall from height. Many treatment methods have been invented and adopted, controversy exists about the optimal methods of treatment for such fracture.

Objectives To assess the short-term results of ipsilateral fracture neck and shaft femur treated by modified traditional ante-grade intra-medullary nailing.

Methods Fourteen patients with ipsilateral concomitant fracture neck and shaft femur treated by modified traditional ante-grade interlocking nailing. The surgery includes fixation of both neck and shaft fracture by interlocking nail (traditional type) and multiple long lag screws inserted via the nail itself toward the head and anterior or posterior to nail. Those patients followed for maximum 48 weeks to assess the risk of infection, knee stiffness, delayed union, mal-union, and non-union.

Results One patient had superficial infection treated by oral antibiotics and improved (7.1%). Two patients had knee stiffness of mild degree (the range is 0-120) improved by physiotherapy (14.3%). Two patients had Trendelenburg's gait (14.3%). One patient had non-union of femoral shaft (14.3%). One patient had mal-alignment of femoral neck (7.1%). One patient had mal-alignment of femoral shaft (7.1%). One patient had delayed union (failure to progress by 6 month as judged by serial radiographs) of femoral shaft (7.1%). Two patients need open reduction because of soft tissue interposed (14.3%). We lost follow up of 2 patients (14.3%).

Conclusion Modified method for fixation of ipsilateral neck and shaft fracture with traditional interlocking nails and multiple long lag screws still can be adopted especially in the absence of cephalo-medullary nails with good results regarding early results. Still the cephalo-medullary nails may be better and required other studies.

Key words Interlocking nailing, ipsilateral fracture neck and shaft femur.

List of Abbreviation: RTA = road traffic accident, FFH = fall from height, ATLS = advanced trauma life support, IMN = intramedullary nail, DHS = dynamic hip screw, PFNA = proximal femoral nail, Miss = minimal invasive surgery.

Introduction

Ipsilateral concomitant fractures of the femoral neck and femoral shaft are unusual and pose a difficult treatment problem [(1)]. Most of these are intra-capsular fractures, with a high shear angle similar to a Pauwels type III fracture of the femoral neck. Many of these are initially completely un-displaced and therefore are difficult to diagnose [(2,3).] Concomitant femoral neck fractures occur in 3% to 10% of patients with femoral shaft fractures, which are

usually non-displaced and missed injuries in 30% to 57% of cases [4-8].

The presumed mechanism of injury requires that the hip be flexed and abducted to a degree that the femoral head is well-seated within the acetabulum. The load is initiated at the knee and then applied longitudinally along the femoral shaft [9-13]. Most of the force is dissipated in the femoral shaft fracture, therefore often resulting in undisplaced or minimally displaced fractures of the femoral neck, which therefore have less soft-tissue damage and a much lower incidence of avascular necrosis of the femoral head than would be expected [2,14].

Most authors agree on the need for a high index of suspicion (especially for mid-shaft fractures), adequate antero-posterior views of the pelvis with femoral fractures, repeat films if suspicious and early accurate reductions of the femoral neck fracture [1]. Approximately 25% of femoral neck fractures are discovered during nailing of shaft fractures. Although most of these fractures have been discovered during preliminary positioning and preparation, nearly one third of femoral neck fractures initially missed are discovered at completion of operation [1].

Ipsilateral fracture neck shaft femur is often the result of high energy trauma and usually occurs in young patients [15,16].

Plates combined with femoral neck screws have been reported to be associated with a high incidence of infection and non-union [17,18].

The advantages of closed locked intramedullary nails are a low incidence of infection and non-union, minimal surgical trauma, and control of both length and rotation in comminuted unstable shaft fractures [19].

Technical complications have been encountered with reconstruction nailing, and a high incidence of non-union and complications related to the knee have been reported with retrograde nailing combined with lag screws or DHS [20,21]. The PFN-long has been shown to result in favorable outcomes, but the availability of only three nail lengths and one diameter presents certain drawbacks [22].

The PFNA is designed for peri-trochanteric femoral fractures. It provides reasonable biomechanical fixation and yields better purchase of the helical blade in the femoral head [23,24].

The objectives of this study was to To assess the short-term results of ipsilateral fracture neck and shaft femur treated by modified traditional ante-grade intra-medullary nailing.

Methods

Our prospective descriptive study involved 14 patients with ipsilateral basal fracture neck femur and middle 3/5 shaft fractures treated surgically between October 2010 until April 2012, in Al-Imamain Al-Kadhimain Medical City, by using traditional Targon interlocking nails with multiple screws (at least two) for neck fractures.

Those patients with skeletally mature bone (closed proximal and distal physis) and ipsilateral basal fracture neck femur and middle 3/5 of the shaft femur were included and treated surgically within 72 hours from the injury. On the other hand, those with the following criteria were excluded from the study:

1. Closed and open type I Gustillo fractures.
2. The exclusion criteria:
3. Multiple injured patients (i.e. other injuries like head, spine...etc.).
4. Previously traumatized limb, e.g. previous fracture or soft tissue injury in the same limb.
5. Peripheral vascular insufficiency.
6. Associated chronic medical illness, like Diabetes mellitus, hypertension, heart failure, and uremia.
7. Associated vascular injuries.

The surgery includes fixation of both neck and shaft fracture by interlocking nail (traditional type) and multiple long lag screws inserted via the nail toward the head and anterior or posterior to nail. All the operations performed under general anesthesia, with fluoroscopic control on traction table, static locking, closed and open reduction done for them. All the patients were followed up by regular intervals at five days, two weeks, and then monthly for a

maximum of forty eight weeks recording the following on a special formula for each patient:

a) Presence and type of infection (early and delayed maximum 12 weeks).
b) Mal-alignment (maximum acceptable limits were 1cm shortening and 5 degrees angulation for shaft fracture and 10 degrees for neck fractures, compared with the other normal hip joint).
c) Union rate, confirmed by clinical and radiological findings, delayed union if no signs yielded for maximum 32 weeks, non-union if passed the maximum follow-up period (48 weeks) with radiological findings of non-union.
d) Trendelenburg gait.
e) Knee joint stiffness, mild if 5-15 degrees, moderate 16-25 degrees, severe more than 25 degrees.

The patients managed initially in the emergency room according to ATLS protocol. The patients were prepared for elective surgical management within seventy two hours. Femoral shaft fixed by traditional method of locked IMN (Targon) and the neck is fixed by insertion of two cannulated long screws guided by k-wire, one inserted via the nail and the other screw inserted anterior or posterior to the nails (Fig. 1 and 2).

Fig. 1. Interlocking nail with proximal targeting device (conventional method)

Protected weight bearing was permitted as soon as possible postoperatively. Quadriceps-setting and straight leg raising exercises are begun before hospital discharge. During the postoperative interviews; the patient examined clinically and radio logically. Clinical examination includes general examination, local examination of the wounds and fracture site with neurovascular evaluation of the lower limbs, also the range of motion of hip, knee was measured, compared with the contralateral side and assessment of the hip musculoskeletal integrity through Trendelenburg's testing.

Fig. 2. Inter-locking nail (the modified method, the screw via the neck)

Radiological examination includes anterior-posterior view of pelvis and both hips and anterior-posterior and lateral view of the affected femur.

Results

Out of the 14 patients of the study sample, there were 9 males (64%) and 5 females (36%), (Table 1), with an average age of (36.79±8.84 years); range (25-50).

Table 1. Frequency distribution of age group

Age group (years)	Frequency	%
20-29	3	21.4
30-39	4	28.6
40-49	4	28.6
50-60	3	21.4
Total	14	100.0

Nine patients were injured by RTA and 5 patients had been injured by FFH (Fig. 3).

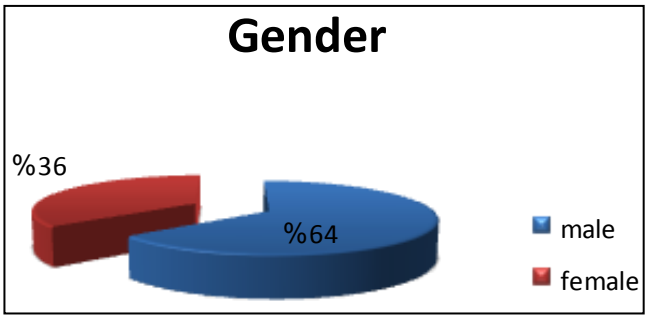

Fig. 3. Pie chart showing the percentage of the patients according to the gender

Table 2 showed that one patient had superficial infection treated by oral antibiotics and improved (7.1%). Two patients had knee stiffness of mild degree improved on physiotherapy (14.3%). Two patients had trendelenburg`s gait (14.3%). One patient had non-union of femoral shaft (7.1%). One patient had mal-alignment of femoral neck (7.1%). One patient had mal-alignment of femoral shaft (7.1%). One patient had delayed union of femoral shaft (7.1%). Two patients need open reduction because of soft tissue interposed (14.3%). We lost follow up of 2 patients (14.3%).

Discussion

Different modalities for treatment are invented and adopted, but in the majority of cases the full range of implants are not available (or the experience is not ideal) namely the cephalo-medullary long nails, dynamic hip screws, retrograde and ante-grade traditional intramedullary nails.

At the time of our study, the cephalo-medullary nail was not available; we used traditional interlocking nails with multiple lag screws to fix these fractures and we estimated the early results regarding union rate, early infection, knee stiffness, Trendelenburg's gait and the need for open reduction of femoral shaft fracture.

Table 2. Patients' number, age, mechanism of injury and side of injury

Case No.	Age (years)	gender	Mechanism of injury RTA/FFH	side
1	25	M	RTA	L
2	47	M	RTA	L
3	40	M	FFH	R
4	30	F	RTA	L
5	50	F	RTA	R
6	31	M	FFH	R
7	35	F	RTA	L
8	43	F	RTA	L
9	36	M	FFH	L
10	30	M	FFH	R
11	50	M	RTA	R
12	28	M	RTA	L
13	44	F	RTA	L
14	26	M	FFH	R

Shantharam et al (2007) stated that of the 14 cases of neck of femur fractures were treated with AO ASIF Group miss a nail technique, one patient had delayed union (7.1%) and one non-union (7.1%), two patients had knee stiffness (14.3%) and one patient had superficial wound infection (7.1%) [25].

Chang-Wug et al (2006), use retrograde nailing with subsequent screw fixation for ipsilateral femoral shaft and neck fractures, there were 16 patients, in femoral neck fractures one (6.25%) nonunion with avascular necrosis occurred and of femoral shaft fractures nonunion occurred in five (31.25%) patients [27].

Pavel et al (2011), in his study stated that out of five patients, three patients stabilizing with reconstruction nail, and two stabilized with 95 blade plate or with lag screws, there was one case of mal-union of diaphyseal fracture (20%) and one case (20%) in which the distal locking screws of the reconstruction nail broke which resulted in a fracture non-union [28].

Hossam et al., Koldenhoven et al., and Randelli et al. reported excellent results in patients with ipsilateral fracture of the femoral neck and shaft

treated with the Russell-Taylor reconstruction nail [29].

Wang et al (2010) stated that out of 21 patient 11 were treated with cancellous lag screws or dynamic hip screws (DHSs) for the fractured femoral neck and compression plate fixation for the fractured femoral shaft (group 1). 10 patients underwent surgery with PFNA-long (group 2).

In group 1, one patient developed a deep infection (9.09%) another patient experienced implant failure of the femoral shaft fracture (9.09%). In group 2, one patient developed a superficial wound infection (10%) and one patient (10%) had delayed union of the femoral shaft fracture [26].

In our study, we have 14 cases treated by modified traditional ante-grade interlocking nails; two cases have been missed on follow up. Two patients had knee stiffness of mild degree improved on physiotherapy (14.3%). One patient had superficial infection treated by oral antibiotics (7.1%). Two patients had Trendelenburg's gait (14.3%). One patient had non-union of femoral shaft fractures (7.1%).

One patient had delayed union of femoral shaft fractures (7.1%). One patient had mal-alignment of femoral shaft fractures (7.1%). One patient had mal-alignment of femoral neck fractures (7.1%). Two patients need open reduction because of soft tissue interposed (14.3%).

In conclusion, modified method for fixation of ipsilateral neck and shaft fracture with traditional interlocking nails and multiple lag screws still can be adopted especially in the absence of cephalo-medullary nails with good results regarding the rate of infection, knee stiffness, union progression, mal-alignment, and Trendelenburg's gait; still our method is better than fixing the two fractures by two implants like DHS and plate and screws or retrograde nailing as compared to international studies since there is no many studies using our procedure (miss anail technique); no conclusive treatment methods have been found; much better preoperative assessment to minimize the per-operative difficulties may necessitate preoperative CT-scan. We encourage further researches in Iraqi hospitals to compare the results of such methods of fixation and more meticulous management of the shaft fracture, neck reduction, postoperative weight bearing may improve the outcome and reduce complications.

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Author contribution

Both authors contribute equally in the practical surgical operations and literature review and preparing this paper.

Conflict of Interest

No conflict of interest of any type in the work.

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BK Polyomavirus-infected Decoy Cells in Urine Cytology Specimens of Renal Transplant Recipients

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Abstract

Background BK polyomavirus is one of the common post-transplant viral infections, affecting ~15% of renal transplantation recipients (RTR), leading to graft loss in more than half of cases.

Objectives Study the rate of detection of BK virus (BKV) in RTRs in Pap-stained urine cytology specimens.

Methods A single center study, urine samples were collected from 99 RTR patients, with 15 Living Donors (LD) and 15 patients with chronic kidney disease (CKD) were taken as controls. And urine cytology smears were Pap stained for detection of decoy cells (DCs).

Results Out of the 99 RTRs, 27 (27.3%) patients were decoy positive, 8 out of these 27 patients had uncommon DCs, and 5 out of these 27 cytology positive patients (18.5%) had biopsy proven BKV nephropathy (BKVN).

Conclusion This study suggests that the finding of BKVN in 18.5% of the DC positive patients stresses the importance of screening for BK polyomavirus with Pap-stained urinary cytology in RTR.

Key wards BK polyomavirus, renal transplantation, decoy cells

List of abbreviations: BKV = BK virus, BKVN = BK virus nephropathy, RTR = renal transplant recipient, LD = living donor, CKD = chronic kidney disease, DC = decoy cell.

Introduction

Opportunistic polyomaviruses infections mainly BK virus (BKV) and JC virus have become increasingly common problem among renal transplant recipients (RTR). Polyomaviruses are circular, double-stranded DNA viruses. The most important and commonest among these viruses is BKV infection, which was reported in ~15% of RTRs in the first post-transplant year in the absence of an effective prophylaxis strategy [1-3].

BKV presents with an asymptomatic gradual rise in serum creatinine with a tubulo-interstitial nephritis mimicking rejection, making a treatment dilemma. The decrease in immune-suppression that is needed to treat BKV infection is opposite to the increases in immune-suppressive drugs that are needed to treat rejection [4].

Once the virus has reactivated, there will be an ascending infection via cell-to-cell spread [5]. In the absence of an appropriate immunologic control, a progressive lytic infection could take place [6]. This results in large nuclear virus-containing inclusions in the tubular cells. Lysis of these urothelial infected cells leads to spread of the virus into the tubule lumen and then urine, as well as to the tubular interstitium and then spread to the surrounding cells. Subsequently, there will be tubular cell necrosis and cast formation [4,7].

Urine cytology screening for viral inclusion-bearing, so called decoy cells (DCs) allows for the early identification of BKV infection, and it has a relatively high sensitivity and a negative predictive value above 95%, besides being a cost-effective non-invasive assay [8-10]. Detection of DCs in the urine is one of the earliest assays, in this assay urine is Papanicolaou-stained and examined under light microscope to look for virus infected cells "decoy cells", which are epithelial cells with enlarged nuclei, and large basophilic ground-glass intranuclear viral inclusions [8-12].

Thus, the objective of the present study was to evaluate the prevalence of BKV infection in RTRs based on the detection of urinary DCs in Pap-stained urine cytology specimens.

Methods

A total of 99 RTR patients who attended the (Center of Kidney Diseases and Transplantation) in the Medical City of Baghdad, were enrolled in the study. A consent letter was signed by each patient, and the study was approved by the Ethical Committee of Al-Nahrain University.

Urine samples were collected from the patients, 33 of them had normal renal function, and the remaining 66 had impaired renal function. Two control groups were included in the study, 15 living donors (LD), and 15 non-transplanted patients with chronic kidney disease (CKD). Living Donors (who are apparently healthy individuals, not diabetic, not hypertensive, not receiving any medications, and their serum creatinine and creatinine clearance tests are normal).

Urine (10-ml aliquots) was centrifuged in Falcon tubes at 1500 rpm for 5 min for DCs screening. The supernatant was discarded and the sediment was re-suspended in the remaining urine. For each patient; two slides were prepared; one was immediately stained with the Papanicolaou method and examined under light microscope at 40 and 100X; the other was stored unstained at -20 ^{o}C for confirmation of diagnosis if required (slides preparation and staining were conducted in the Teaching Laboratories in the Medical City of Baghdad).

Identification and Quantification of Decoy Cells

Activation and replication of polyomaviruses was detected by identification of DCs, which are viral inclusion-bearing epithelial cells characterized by a ground-glass appearance with an enlarged nucleus, occupied by a basophilic inclusion surrounded by chromatin [10,11]. Some of the DCs appear resembling the tail of a comet [13]. For DCs quantification; a cut-off level of ≥ 10 DCs / (removed) slide, is defined as decoy positive [14]. In addition to the quantification of common ground-glass DCs, the uncommon (clumped) variants were also looked for; as their presence reflects the pathological stages of BKVN, if the uncommon (clumped) variants are more than 25% of the total decoy cell count; then BKVN can be predicted with more than 75% probability [15].

Statistical analysis

Statistical analysis was performed with the software SPSS version 21.0, and Microsoft Excel 2013. Categorical data formulated as count and percentage. Fisher exact test was used to describe the association of these data. Numerical data were described as mean, standard deviation of mean. ANOVA was used for comparison among more than two groups. $P \leq 0.05$ was considered statistically significant.

Results

This prospective study involved 99 RTR, 33 of them had normal renal function, and the remaining 66 had impaired renal function, 78/99 (78.79%) were males. Their mean age was 37±13years ranging between 18 and 67 years.

The mean serum creatinine value in the RTRs was 2.33±1.7 mg/dl, and their mean post-transplantation period was 17.5±9.7 months ranging from 2-30 months

Among these 99 RTRs, 19.2% had renal allograft rejection (biopsy-proven), five of them (5.1%) were receiving antithymocyte globulin (ATG) as anti-rejection therapy.

In addition, 5.1% had biopsy proven BK virus nephropathy (BKVN) (biopsy was studied in a separate laboratory), and 4.0% had ureteric stenosis (diagnosed by ultrasonography).

Papanicolaou-stained urine cytology smears revealed high rate of DCs shedding among RTR as compared with both control groups; LD and CKD that were all DCs negative, table 1.

On the other hand, uncommon DCs variants were present in 8 out of 99 RTR as shown in table 1 and fig. 1.

The most frequent variant of DCs was the amorphous, basophilic, ground-glass–like nuclear appearance. While in the other variants (uncommon type), the nucleus appeared eosinophilic and granular, and could be surrounded by a halo, or with a finely granular without a halo (Fig. 1).

Table 1. A; Decoy cells shedding, and B; Uncommon decoy cells shedding in renal transplant recipients

Feature		Study groups		
		LD	RTR	CKD
Decoy cells	Negative (%)	15 (100.0)	72 (72.73)	15 (100.0)
	Positive (%)	0 (0.00)	27 (27.27)	0 (0.00)
	Total	15	99	15
Uncommon Decoy cells	Negative (%)	15 (100.0)	91 (91.92)	15 (100.0)
	Positive (%)	0 (0.00)	8 (8.08)	0 (0.00)
	Total	15	99	15

LD = living donor, RTR = renal transplant recipient, CKD = chronic kidney disease

In addition, the results of this study revealed that 19 out of these 27 cases (70.4%) were males, their mean age was 34±7 years with no significant correlation with decoy cell positivity, and their mean post-transplant period was 18.2±8 months which also not significantly correlatedwith decoy cell positivity.

On the other hand, 21/27 (77.8%) of these DC positive patients had impaired renal function with a mean serum creatinine value 2.3±0.9 mg/dl, which is significantly correlated with DC positivity (p=0.01).

Table 2 demonstrates sensitivity and specificity of urine cytology in which all of the 5 patients who had biopsy-proven BKVN had positive urine cytology for DCs, i.e. 18.5% of them.

Two main standard immunosuppressive regimes are mainly followed in our transplantation center in Baghdad; the old regimen which includes cyclosporine A (CSA), mycophenolate (MMF), and prednisolone, the second regimen includes tacrolimus (TAC) instead of CSA, in addition to MMF and prednisolone.

Table 2. Sensitivity and specificity of Urine cytology as compared with renal biopsy in the diagnosis of BKVN

	cytology+	cytology-	Total
Biopsy +	5	0	5
Biopsy -	22	72	94
Total	27	72	
Sensitivity	100%		
Specificity	76.6%		

On comparing with the type of immunosuppression used, 55.6% of DC positive patients were on tacrolimus regimen, and 44.4% were on cyclosporine A regimen, which is not significantly correlated with DCs positivity and 4/5 (80%) of patients who were on ATG (anti-thymocyte globulin) were decoy positive, among the 26.3% (5/19) patients who had rejection. Finally, 3/4(75%) of ureteric stenosis patients (diagnosed by ultrasonography), were DC positive.

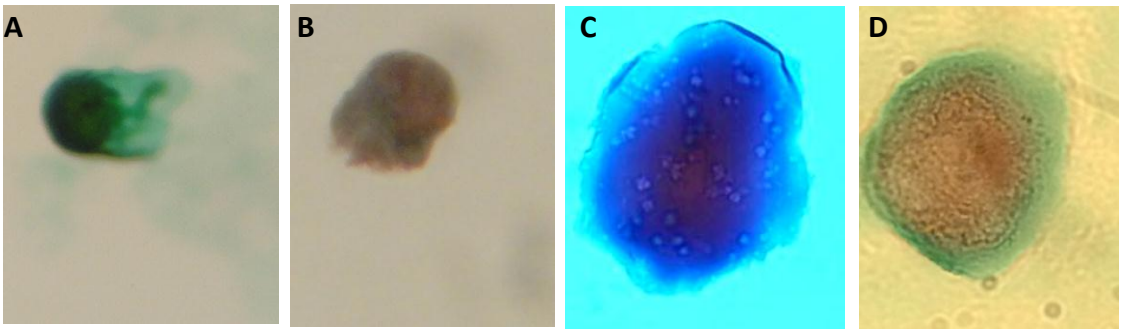

Fig. 1. Urine cytology: The activation and replication of polyomaviruses can be monitored by searching for viral inclusion-bearing epithelial cells, i.e., decoy cells (DC), in routine urine cytology specimens, (A,B) typical DC phenotype resembling the tail of a comet. And (C) uncommon (atypical) eosinophilic DC, (D) uncommon finely granular DC. Papanicolaou stain, (A&B) X400, (C&D) X1000.

Discussion

BKV shedding into the urine occurs in 10-30% of renal transplant recipients, and prospective monitoring of RTRs may identify patients with active infection before deterioration of the renal function. BKV cytopathic effect is a well-recognized entity in urine cytology specimens. Virus-infected cells termed (decoy cells) can be found in urine samples, and may mimic the nuclear changes that occur in urothelial cancer however, experienced cytopathologist could easily differentiate between them [8,9,16].

Decoy cells were found in the urine of 27.3% of the patients, mostly within 1-2 years following renal transplantation, and more than 50% were above 40 years age, matching findings from international studies reporting urinary decoy cells in 20-30% of patients from the 16th week of transplantation onwards [17-20].

Based on the morphologic features alone, one cannot always distinguish between BKV excretion and other viral infections. DCs might result from infection with BKV, JCV, and less commonly, adenoviruses [18,21]. However JCV and adenoviruses rarely cause nephropathy in RTRs [22,23]. The detection of uncommon DCs in about 30% of positive cases raises the possibility of BKV reactivation with more than 75% probabilityof BKVN [15]. This could support the specificity of this assay.

Detection of DCs in all of the five biopsy-proven BKVN cases indicates a high sensitivity of this screening method, a result that is in agreement with the majority of studies on DCs [8-10]. According to Drachenberg et al [24], the absence of DCs in urine rules out BK-associated nephropathy in up to 99.4% of instances. In addition, because urinary cytology is noninvasive, inexpensive, fast, and simple to perform, it remains a feasible alternative to immunohistochemistry and molecular biology for monitoring BKV infection in transplantation centers with limited resources [8].

Clinical manifestations associated with post-transplantation BKV infection include interstitial nephritis or BKV-associated nephropathy, ureteral stenosis, systemic infection, and bladder cancer [25,26]. In this study, 21/27 (77.8%) of positive cytology patients had impaired renal function with high serum creatinine, among which 5 patients had BKVN, and 3 patients had ureteric stenosis (diagnosed by ultrasonography).

Patients developing BK nephropathy oftenturn and remain 'DC positive' months before the initial diagnosis of viral nephropathy, repeating urine cytology is useful for proper risk assessment. Decoy cell positive renal allograft recipients fall into risk level 1; they have to be closely monitoredat 4-week intervals using repeat cytology examinations and additional quantitative (plasma) polymerase chain reaction tests [17]. DC can bedetected in urine when more

than 10^6 viral gene copies/ ml are excreted in urine [14].

Finally, 5 out of these 27 patients had rejection, and 4 of them were on ATG antirejection therapy, this could be explained either due to a concurrent BKV reactivation with rejection [14,27,28], though it is rare. Or more commonly, it is usually difficult to differentiate BKVN from the reaction of an interstitial cellular rejection (Banff 1 A/B) [29].

According to Pillai et al [30], in view of the increasing number of RTRs in South Indian states, Papanicolaou screening of urine cytology specimens for DCs is now a simple and efficient routine procedure for identifying patients at risk of developing BKVN and, of course, for ruling out disease. The test is now mandatory for all organ transplant recipients [31].

In conclusion, the finding of BKVN in 18.5% of patients with urinary decoy cells, stresses the importance of screening for BKV with urinary cytology. The test is sensitive, noninvasive, inexpensive, fast, and simple to perform, and is therefore highly indicated for transplantation services lacking immunohistochemistry and molecular biology testing facilities.

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Author contribution

Al-Obaidi and Kadhim collect the specimens, Abd refer the patients, Habib do the sample preparation and processing, Qasim primarily read the cytolopathology slides, and Hussain and Abdlqader revise the cytolopathology slides.

Conflict of Interest

Authors declare no conflict of interest.

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KTP (532 nm) Laser Enhances the Effect of ND:YAG (1064 nm) Laser in the Treatment of Nevus of Ota

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Abstract

Background Nevi of Ota is usually present at birth. It may be congenital but is not hereditary.

Objective To determine the efficacy and side effect profile of Q-switched Nd:YAG and KTP Laser on fourteen patients with nevus of Ota.

Methods Fourteen patients were treated with Q-switched ND: YAG (1064 nm) and KTP (532 nm) laser for a period of 21 months. Six-month follow-up was done after the last session. Response to the treatment was graded on quartile grading scale.

Results Near total improvement was observed (grade IV) in 36% patients, marked improvement (grade III) in 28.5%, moderate improvement (grade II) in 28.5%, and minimal improvement (grade I) in 7% of the patients. Regarding complication (not permanent), no textural change or scaring. When Nd:YAG laser was only used to treat nevi of Ota for three sessions the response was less than 10% improvement in the lesion. When KTP used in combination with Nd-YAG alternatively there was an increasing improvement in most cases up to more than (70%).

Conclusion Concurrent use of the Q-switched 532 in combination with the (1064 nm) Nd:YAG is more effective in pigment clearance than the Q-switch (1064 nm) alone for nevus of Ota.

Keywords Nevus of Ota, Q-switched Nd:YAG (neodymium-doped yttrium aluminum garnet).

List of Abbreviation:

QS = Q-switched, Nd:YAG laser = neodymium-doped yttrium aluminium garnet, KTP laser = Potassium titanyl phosphate, nm = nanometer, ns = nanosecond, mm = millimeter, Hz = Hertz, j/cm= joule/ centimeter, µm = Micrometer

Introduction

Nevus of Ota is also known as nevus fuscoceruleus ophthalmomaxillaris. The lesion may not appear until the ten years [(1)]. It persists throughout life. Eighty percent occur in females; 5% are bilateral [(1)]. This disorder is very common in Asian populations and it has been said to occur in 1% of dermatologic outpatients in Japan, it has been reported in east Indians, among black people and rarely in white population [(2)]. Nevus of Ota may be bilateral; it may be congenital but is not hereditary [(2)]. The entire patient in this study, have no family history.

The cause of Nevus of Ota is not fully known, it is generally believed that nevus of Ota, during early embryonic life, represents a failed migration of melanocytes from the neural crest to the dermo-epidermal junction and subsequent arrest within the dermis [(3)]. Some have hypothesized that sex hormones play a role in its pathogenesis, given the female predominance [(4-6)].

Nevus of Ota typically presents as unilateral blue-black or slate gray macules [7,8], that are located in the distribution of the first or second branches of the trigeminal nerve, tympanic membrane, oral, ear, scalp [9], cornea, iris and retina [9,10]. Nasal mucosal involvement is common [10].

Involvement of hard palate is rare [9,11]. As already stated, most patients tend to develop the nevus at birth or shortly thereafter, although some patients can develop it as late as 20 years of age [10]. Therefore, diagnosis is mainly clinical and a biopsy is rarely needed [10]. The condition is more common in female with male:female ratio 1:4.8 [10, 12]. Most patients have no family history [13].

In many cases the nevus consists of a melanotic nevoid cells [8] which only become pigmented after stimulating by triggering factors; sex hormone [14], infection, trauma, and ultraviolet light exposure. These factors have been reported to trigger the onset of nevus [8,15]. Histologically elongated dendritic melanocytes are scattered widely in the papillary to mid reticular dermis. Hirayama and Suzuki [16] examined the histological findings of 450 cases of nevus of Ota and classified the condition according to the distribution of the dermal melanocytes as follow:

- Superficial (S): dermal melanocytes are located in the superficial layer of the dermis
- Deep (De): dermal melanocytes are located in the deep layer of the dermis
- Diffuse (Di): dermal melanocytes are evenly spread throughout the dermis:

i. Superficial dominant (SD): diffuse distribution of dermal melanocytes, but with greater concentration in the superficial layer
ii. Deep dominant (DD): diffuse distribution of dermal melanocytes, but with greater concentration in the deep layer.

Complications in this nevus occur mainly in ocular involvement including glaucoma [8], uveitis, cataracts, and rarely orbital and cerebral melanomas [17-19]. Other complications may include psychological impact [10]. In this study 90% of the patients have psychological impact; they used camouflage to cover the nevus.

The objectives of this study was to determine the efficacy and side effect profile of Q-switched Nd:YAG and KTP Laser on fourteen patients with nevus of Ota. And to treat difficult cases by laser which cannot be treated with medical and surgical interruption.

Methods

Fourteen patients of nevus of Ota underwent multiple treatments sessions [14] carried at 2-3 week intervals over a period of 1 year and 9 months with a Q-switched Nd:YAG and KTP laser. These patients presented at the outpatient department of Al-Karkh General Hospital, other hospitals in Baghdad and physicians. Two patients had received surgical treatment for their lesions and failure occurred with scar. The study was done in Laser Medical Research Clinics at Laser Institute for Postgraduate Studies, University of Baghdad. The diagnoses were made on clinical appearance in the skin and the eye. Detailed history, clinical examination, and ophthalmoscopy had been performed in all cases. Most patients were in the age group (16-30) years, eleven females and three males. The nevus in nine patients started at (10-12) years old. In five patients was since birth, only three cases had no eye involvement (21%). The area within the orbital rim was not treated. Two patients had bilateral nevus of Ota (14%). Skin types included type III and IV according Fitzpatrick classification; Sun protection with a broad-spectrum sunscreen cream was advised at the start of therapy and continued throughout the duration of treatment. Topical anesthesia with Emla (eutectic mixture of lidocaine and prilocaine), 1 hr with occlusive dressing prior to laser irradiation was applied.

Patient and doctor in the laser room wore appropriate eye protection goggles specific for this type of laser during treatment sessions.

Therapy was initiated with (1064 nm) Q-Switched Nd:YAG Laser with (3 mm) spot size, at 3 Hz frequency and Pules duration (5-10 ns) and fluence (8.98-11.25 J/cm^2).

Regarding (532 nm) Q-Switched KTP Laser with (3 mm) spot size, (3 Hz) frequency pulse duration (5-10 ns) and fluence (3.98-5.31 J/cm^2). The fluence subsequently increased on subsequent sessions based on the therapeutic response and patient tolerability. The end point for treatment was taken as near total improvement or until a maximum of 14 sessions.

Evaluation of results had been made on visual inspection as well as by comparing serial photographs that had been taken before the treatment and after every laser irradiation session. Six-month follow-up was covered after the last session. Response to treatment in terms of clearance of the lesion was graded based on a quartile grading scale as minimal, moderate, marked, and near total as follow:

Grade I: < 25% improvement, minimal improvement

Grade II: 26%-50% improvement, moderate improvement.

Grade III: 51-75% improvement, marked improvement.

Grade IV: Greater than 75% improvement, near total improvement

No Patients had no improvement or worsening of symptoms. A complication such as hyperpigmentation, hypopigmentation, no scarring or textural change was recorded.

Results

Majority of the patients at the end of the treatment reported some improvement (Table 1).

Table 1. Results of treatment of (14 patient) by KTP and Nd:YAG laser

No. of patient	Improvement	Grade	%
5	Near total	IV	> 75
4	Marked	III	51-75
4	Moderate	II	26-50
1	Minimal improvement	I	< 25

Near total improvement (Grade IV) was seen in 36% as in (Fig. 1A-D).

Marked improvement (Grade III) was seen in 28.5% as in (Fig. 2A-C). Moderate improvement (Grade II) was seen in 28.5% as in (Fig. 3A and B) and 7% patients reported less than 25% clearing of the lesion, which was (grade I) minimal improvement as in (Fig. 4). We found a statistical correlation between the number of treatment sessions and the therapeutic outcome. The age and sex of patients did not in any way influence the treatment outcome. Two Patients who had bilateral involvement, one of them had marked response (Grade III) and the other had near total improvement (Grade IV).

The patient, who had recurrence of the pigmentation during the treatment session, gave history of an increase in pigmentation during menstrual cycle, which indicates the relation between nevi of Ota and hormonal cause.

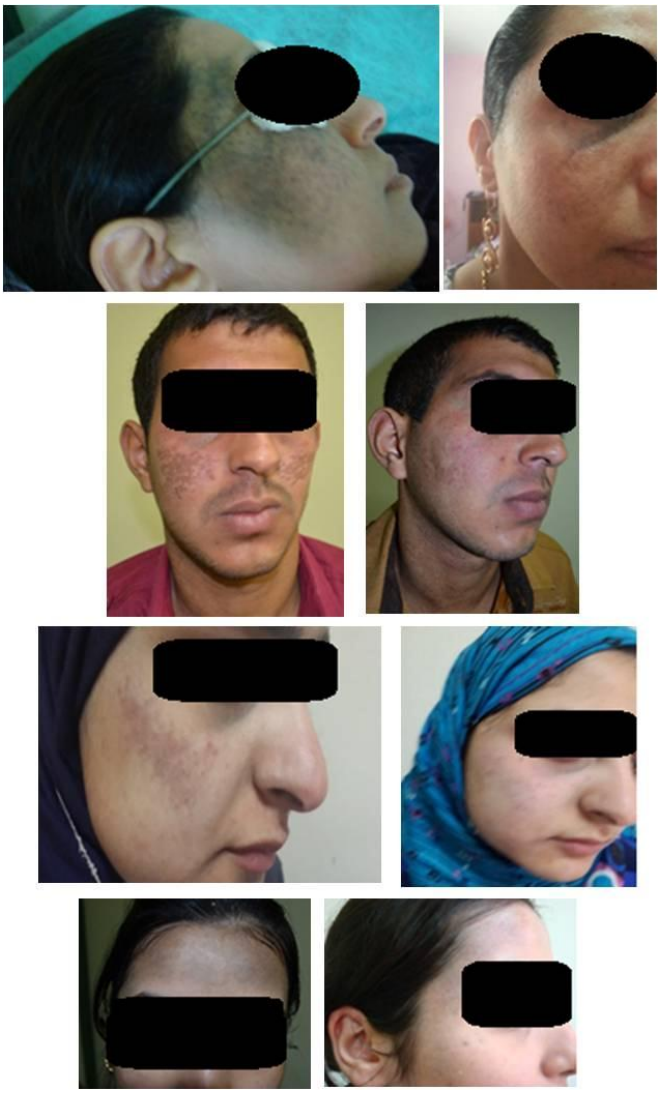

Fig. 1. Prelaser (Left) and postlaser (Right) fourteen sessions with grade IV (near total improvement)

Complications

We can state the following remarks that were observed during the course of treatment and follow up period (Table 2):

1. Pricking sensation during treatment and post-treatment were common, which was resolved within one day after using bepanthine ointment. This sensation always occurred when KTP laser was used.

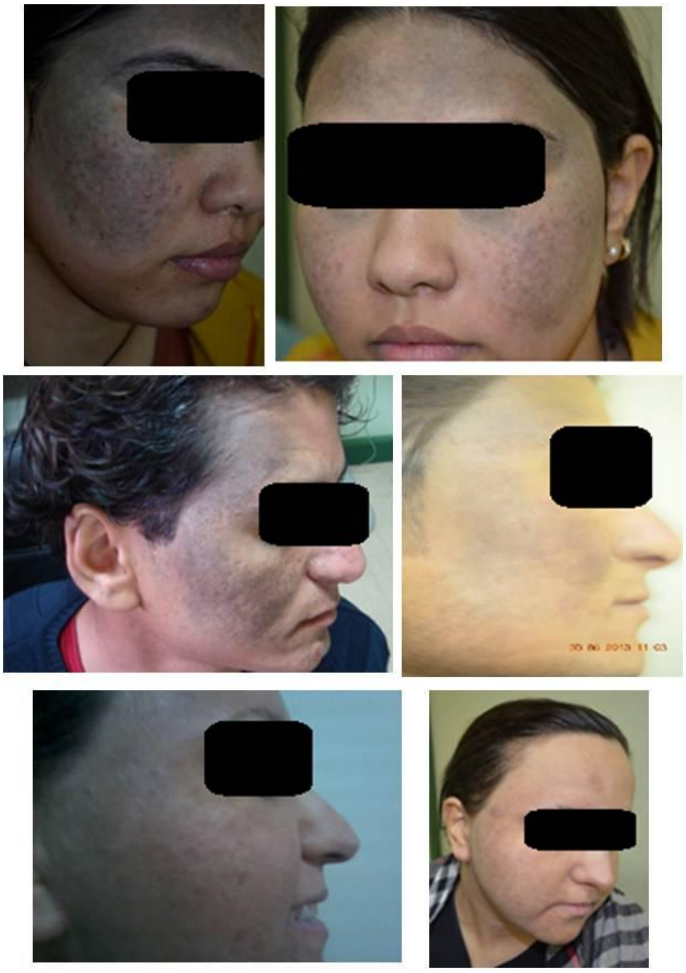

Fig. 2. Prelaser (Left) and postlaser (Right) fourteen sessions with grade III (marked) improvement.

2. Observed with either wavelength because of the short pulse duration of the Q-switched modes.
3. Transient post-inflammatory hyperpigment-ation was observed in one patient (7%), which was cleared with the use of sunscreens and bleaching agents after one month.
4. Hypopigmentation occurred in two patients (14%) after using KTP laser in high fluence, 5-25 j/cm^2 (Fig 5).

Fig. 3. Prelaser (Left) and postlaser (Right) fourteen sessions with grade II (moderate) improvement

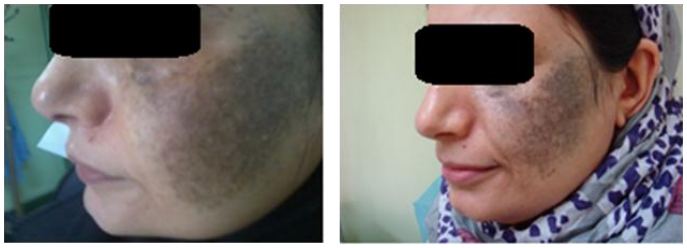

Fig. 4. Prelaser (Left) and Postlaser (Right) fourteen sessions with grade I (minimal) improvement.

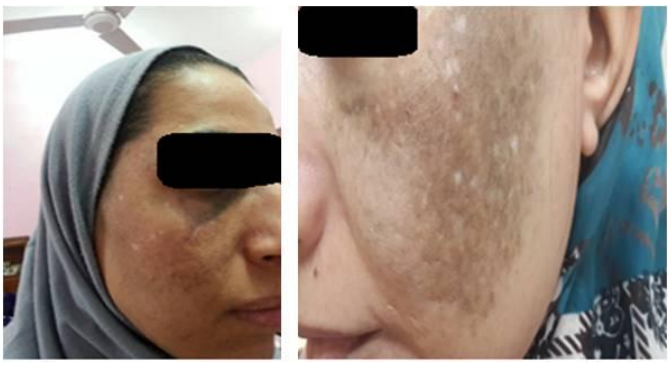

Fig. 5. Hypopigmentation after KTP laser in two patients.

5. Recurrence was seen in three patients (21 %).

Some of the complications were noticed and most of them were reversible, as listed in table 2.

Table 2. Some of the complications of laser treatment in patients with nevi of Ota

Complication	No. of patient/ Nd:YAG Laser	No. of patient/ KTP Laser
Pain sensation	-	14
Scarring or texture change	-	-
hyperpigmentation	-	1
Hypopigmentation	-	2
Recurrence	3	3

Discussion

The treatment options for nevus of Ota prior to the advent of laser were limited. These included cryotherapy [(20)], dermoabrasion [(21)], surgical excision [(22)], and cosmetic camouflage. The surgical treatment options were associated with significant scarring and permanent pigment alteration. Q-switched (QS) lasers have changed the management of pigmentary lesions to a great extent. In addition, it is ideal for treating pigmented skin lesions [(28,29,36,41)]. In Ota nevus Melanin absorbs and localizes the high-intensity irradiation from Q-switched lasers, thereby creating a sharp temperature gradient between the melanosome and other surrounding structures. This gradient leads to thermal expansion and the generation and propagation of acoustic waves, which can mechanically damage the melanosome-laden cells. Tissue repair following laser-induced melanosome disruption demonstrates a 2-staged initial transient cutaneous depigmentation followed by subsequent repigmentation weeks later [(23,24)].

According to Polla et al [(23)], and Dover et al [(24)], the destruction of melanosomes is pulse-width-dependent and the longer pulse durations (in microseconds) do not damage the melanosomes. This is consistent with the theory of selective photothermolysis, which states that the pulse duration of an emitted laser wavelength must be less than the thermal relaxation time of the targeted object. Melanosomes are (0.7 µm) in diameter in types I and II skin and (1 µm) or more in diameter in darker skin types. Melanosomes, due to their small size, have very short thermal relaxation times. Q-switched lasers, with pulses in the nanosecond range, provide the most destructive effects on melanosome with the least damage to surrounding cellular structures [(23,24)].

Because of the broad absorption spectrum of melanin (figure 6) [(25,27)], there are numerous lasers that can specifically target pigmented lesions; red-light lasers [e.g., (664 nm) ruby, (755 nm) alexandrite], green-light lasers [e.g., (510 nm) pulsed dye [(26)]. (532 nm) frequency-doubled Nd:YAG], and near-infrared lasers (e.g., (1064 nm) Nd:YAG) [(25)]. It had been found that the Q-switched laser is a treatment of choice for the nevus of Ota [(28)].

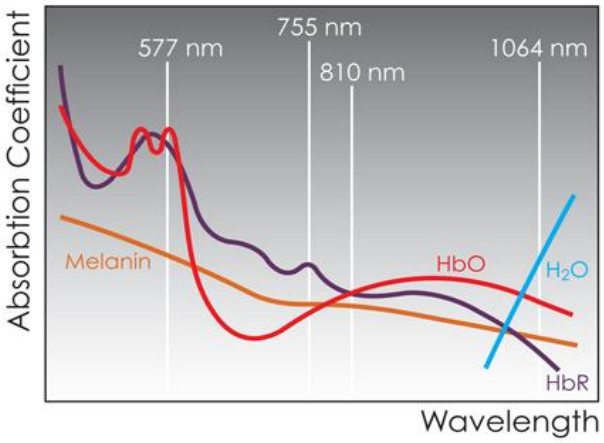

Fig. 6. Absorption characteristics of green and infrared beam Laser [(27)]

In this study, fourteen cases with nevus of Ota were treated, we started first by ND:YAG laser and theclinical improvement in pigment clearance were less than 10%, then we added KTP laser to the treatment alternatively with ND:YAG laser. There was increase in improvement to more than 70% in most cases. We selected both wavelengths (1064 nm, 532 nm) because they fall within the broad absorption spectrum of melanin [(29)].

The result in this study may be explained first, because there is weak absorption by the natural skin chromphores, including melanin at (1064 nm) [30], which may explain the low Incidence of pigmentary changes in nevus of Ota when used (1064 nm) in the treatment only [30]. Second, according to study done by Sherwood et al; the (504 nm) wavelength produced the most pigment-specific injury because the longer wavelengths caused disruption of the basement membrane with pigmentary incontinence [31]. Third, according to Anderson et al study that evaluated the effects of a Q-switched Nd:YAG laser. They show that longer wavelength (which are less well-absorbed by melanin) require a higher energy fluence to induce melanosome changes [32]. Due to these above three reasons, there were less pigmentary changes when we used Nd:YAG (1064 nm) alone at first 3 sessions; but, there was one casethat still had improvement less than 25% after using KTP laser with Nd:YAG. This result may occurr because the (532 nm) laser had epidermal and upper dermal affect (Table 3). Due to its short wavelength the KTP laser cannot reach deep dermal layers [33].

According to the Suzuki histopathology [16], the distribution of the dermal melanocytes in nevus of Ota occurs either in the superficial dermal layer or in the deep dermal layer. So that the lesion with superficial dermal melanocyte improved quickly after four sessions by KTP laser (532 nm), while the lesion with deep dermal layer melanocyte had little improvement after four sessions by KTP laser.

Lastly, according to different studies had done in the laser management of Ota, the use of two wavelengths gave good result in pigment clearance than the use of one wavelength laser radiation [34,36].

In this study; the complication were few and most of them were reversible:

- Transient post-inflammatory hyper-pigmentation, this may be due to possible neglecting the use of sunblock by the patientsafter the laser session.
- Hypopigmentation which may be due to melanocytic damage [33] after using KTP laser in high fluence, (5-25 j/cm^2); because dark-skin patients may also require starting laser sessions at lower energy levels than white-skin patients [40], Hypopigmentation is gone after three months.
- Recurrence may be due to residual melanocytes that have not been targeted or which did not contain sufficient melanin for eradication [37,38].

Table (3): Laser parameters

Parameter	Q-Switched Nd:YAG	Q-Switched KTP
Wavelength	1064 nm	532 nm
Pulse width	5-10 n-sec.	5-10 n-sec.
Fluence	(8.98-11.25) J/cm 2	(3.98-5.31) J/cm 2
Spot size	3 mm	3 mm
Number of pulses/sec.	3 Hz	3 Hz
Number of passes	1	1
Number of laser sessions	10-14	10-14
Pulse overlap	50%	50%
Target chromphores	Melanin, blue-black tattoo pigment	Melanin, hemoglobin
Mechanism of action	Selective photothermolysis	Selective photothermolysis
Absorption/ penetration characteristics	Deep into the dermis	Limited into the epidermis and upper dermis (33)

On the other hand, the recurrence may be due to the presence of deep nests and sheets of nevus cells extending into the reticular dermis or even the subcutaneous fat [39].

Conclusion

Patients of Nevus of Ota were treated with Q-switched (532 and 106 4nm). Concurrent use of the Q-switched (532 nm) in combination with the (1064 nm) Nd:YAG is more effective in pigment clearance than the Q-switch (1064 nm) alone for the treatment of nevus of Ota.

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Author Contribution
The study was done by Dr. Mohammad Ali under the supervision of Prof Dr. Shakur.

Conflict of Interest
Authors declare no conflict of interest.

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Medico-legal study of Violence against Females

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Abstract

Background Violence against female is an important and serious public health and medico-legal problem. It carries important risk factor related to ill health of female and its consequences on physical, social, mental and reproductive health of women.

Objectives To determine the type of fatal and non-fatal intentional injuries involving women, girls and young female kids and method used for such injuries and to reveal the magnitude of domestic violence of both physical and sexual types.

Methods A cross-sectional study was carried out within 6 months period on 82 medico-legal female cases with intentional violence. All females (living and postmortem cases) were studied. Information was collected from the same victim or her relative (in non-living cases) through an interview, police reports and their medical reports. Digital photography was done for some interesting cases while radiological survey followed by complete autopsy for all non-living cases.

Results The study showed that 57.3% were living subjects while 42.7% were postmortem victims. Regarding the living cases, their mean age was 20.61±9.37 years. Illiteracy constitutes 57.4% of them. All of them were referred as cases of violence directed to the genital area (sexual assault) but 72.3% were with no tear to the hymen. In post-mortem victims, the mean age was 28.6±12.84years. Housewives constituted 85.7% of them and 45.7% were having primary school level of education only. Burn was the main type of injury constituting 71.4%. In 65.71% were affected by sexual assault and in 69% of victims there were old hymen tears. In 80% death was due to suicide.

Conclusion Violence was more common among living female and highest in the third decade of life. Majority were either with low level of education or having primary school level of education. All of the living females were sexually assaulted while burn was the main type of injury among the postmortem group.

Key words Violence, Hymen, women, female.

Introduction

Violence against women is now well recognized human rights violation of worldwide significance. Women and girls are frequently victims of both physical and sexual violence by partners and acquaintance, as well as strangers [(1)].

Domestic violence can be defined as a set of systematical behavioral violence acts (physical, verbal, sexual or it may take the form of continual and habitual psychological, social or financial abuse) occurring within a household or between family members [(2)]. It is said that one in every four women will experience domestic violence in her lifetime [(3)]

An estimated 1.3 million women are victims of physical assault by an intimate partner each year [(4)]. A study conducted in the two Scandinavian capitals, Oslo and Copenhagen regarding asphyxial homicide, 73% of the victims was the women. The most common method of causing death was manual strangulation [(5)]. Among rape homicide in South Africa, more often mechanism

of death was strangulation, asphyxia, or blunt trauma, rather than gunshot [6].

Homicide victims are more likely married females usually killed by family members as shown in a study in United States of America [7].

Sexual violence is also a common and serious public health and medico-legal problem affecting million of people each year throughout the world [8]. Whilst sexual violence can take many forms, the most widespread severe form is contact sexual violence and particularly rape with oral, anal or vaginal penetration [9]. In western countries it is estimated that about 25% of women experience intimate partner violence over their life time (i.e., UK 25%, Russia 25%, and Estonia 25%), lower than in other continents (i.e., USA 28%, Chile 26%, Kenya 42%, Egypt 35%, India 45%, and Thailand 20%) [10]. Females who are 20-24 years of age are at the greatest risk of non-fatal intimate partner violence [11]. Most cases of domestic violence are never reported to the police [12].

The objectives of this study was to determine the type of fatal and non-fatal intentional injuries involving women, girls and young female kids and method used for such injuries and to reveal the magnitude of domestic violence of both physical and sexual types.

Methods

A cross sectional study was carried out on within 6 months period started from the first of November 2012 till the end of May 2013 on 82 medico-legal cases (living and dead) referred to the Main Medico-legal Institute in Baghdad as cases of violence in female (physical, sexual or domestic).

Examination of the living victims

Before starting examination, information were collected about the act from police reports, through an interview with the case under study, eye witnesses, close relatives and medical reports if available. These information included age, marital status, level of education, perpetrator relationship, previous history of such type of violence and habits such as smoking or drinking .

External examination was followed looking for any sign of trauma such as abrasion, contusion or different other types of wounds or previous scar. Searching for other evidences was done as well like blood or semen stains which might help the investigation.

Examination of the anogenital region was done looking for injuries to the labia and the inner thighs. The hymen was examined under good light source for the presence of recent or old tears.

Samples were taken for forensic biological testing. The cotton – wool swabs on sticks were used to take the following samples by touching gently on the mucosal surface of the interior of the vulva, labia and around the vaginal orifice and the margins and interior of the anus.

Examination of the post-mortem victims

Before autopsy examination the same information should be collected as in the living cases and external examination should be done for any type of trauma or trace evidence.

Swaps were taken from ano-genital area for detection of semen spots and send for biological exam. Five ml blood was withdrawn from femoral vein. Samples were collected in tubes and preserved with (1%) of sodium or potassium fluoride for detection of alcohol using Alcohol GC and other 10 ml of blood for toxicological screening tests, then stored in a refrigerator for future analysis using GC.

Digital photography was taken for some interesting cases.

Radiological examination was conducted for all cases reveal foreign bodies, bullets, bone injury, shells and their sites.

Complete autopsy examination was done for each cases to reveal the cause of death and record all external and internal injuries.

Results

The study revealed that among the total 82 cases, 47 cases accounting about 57.3% were living victims, while 35 cases accounting about

42.7% were dead victims.

Regarding the living victims, their mean age was 20.61±9.37 years with a range between 9-51 years. Thirty of them were within the age group 10-20 years old accounting for 63.8% while 6 only accounting for 12.8% were within the age group >30-40 years as shown in fig. 1.

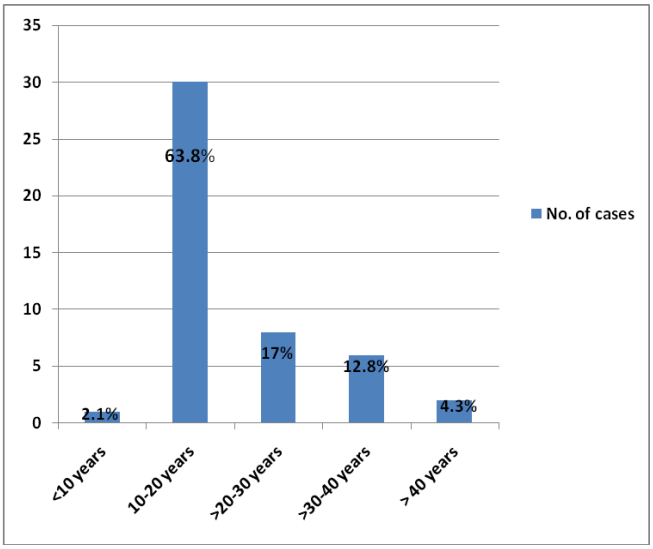

Fig. 1. Distribution of intentional violence in living victims according to age groups

Thirty four of them accounting for 72.3% of all living victims were unmarried females and the rest 13 (27.7%) were married.

As to the level of education, 27 of them (57.4%) were illiterate and those with primary school level were 11 (23.4%) while only 2 were with preparatory and 2 other were having undergraduate levels of education (4.3% for each) as listed in table 1.

Table 1. Distribution of intentional violence in living victims according to education

Education	No	%
Illiterate	27	57.4
Primary	11	23.4
Secondary	5	10.6
Preparatory	2	4.3
Undergraduate	2	4.3
Total	47	100.0

All of them were referred as cases of violence directed to the genital area (sexual assaults).

On examination of the hymen, 34 of them (72.3%) were with no tear to hymen and the rest 13 were having old hymen tear. None of them were with recent tear.

Thirty one were with single exposure to sexual assault (66%) and 9 were having twice exposure (19.1%) while the remaining 7 (14.9%) were having three times exposure to such violence (Table 2).

Table 2. Distribution of intentional violence in living victims according to the frequency of assault

Frequency	No	%
1 time	31	66.0
2 times	9	19.1
3 times	7	14.9
Total	47	100.0

In 45 of them (95.75%), the perpetrator was stranger and in only 2 cases the perpetrator was a relative to the victims.

In postmortem cases, their mean age was 28.6±12.84 years with a range between 15-69 years. The highest number of victims (12) were within the age group 20-30 years accounting for 34.3% of them followed by 11 cases (31.4%) who were below 20 years of age while only 1 (2.9%) was above 60 years of age as shown in table 3.

Table 3. Distribution of intentional violence in postmortem victims according to age groups

Interval (years)	No	%
<20	11	31.4
20-30	12	34.3
>30-40	5	14.3
>40-50	4	11.4
>50-60	2	5.7
>60	1	2.9
Total	35	100.0

Married females were the majority of postmortem cases as it is seen in 24 of them represented in 69% and 11 were unmarried

represented in 31% of them.
Housewives were the commonest occupation as it is seen in 30 cases represented in 85.7% of them, other three of them were employee and only one was student while the other was retired as shown in table 4.
Primary school level of education was the commonest educational level seen in 16 of the accounting for 45.7% of them followed by illiterates in 10 cases accounting for 28.6% with other lower levels of education as seen in table 5.

Table 4. Distribution of intentional violence in postmortem victims according to occupation

Occupation	No	%
house wife	30	85.7
employed	3	8.6
student	1	2.9
retired	1	2.9
Total	35	100.0

Table 5. Distribution of intentional violence in postmortem victims according to Education

Education	No	%
Illiterate	10	28.6
primary	16	45.7
secondary	5	14.3
preparatory	2	5.7
undergraduate	2	5.7
Total	35	100.0

Burn was the commonest type of intentional injury as it was seen in 25 victims represented in 71.4% of postmortem cases followed by bullet injury in 4 of them and fewer cases in other types of injury as shown in fig. 2.
Injury was seen in all over the body in 25 victims represented in 71.1% of them and those victims were cases of burn followed by the head as the second common anatomical site involved in intentional injuries as it was in 6 victims 17.1% of them as it is seen in fig 3. In 24 cases (69%) there were old hymen tears and in 11(31%) there were no hymen tears.

Among the total 35 cases, 23 cases accounting about 65.71% were sexual assault while the remainder 12 cases accounting about 34.29% were physical assault.

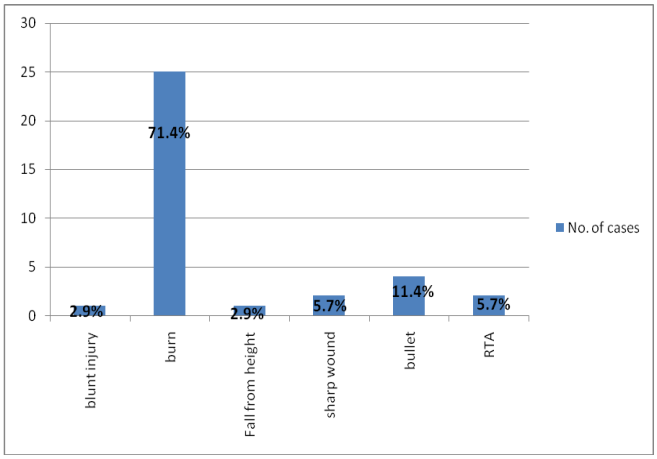

Fig. 2. Distribution of intentional violence in postmortem victims according to the type of injury

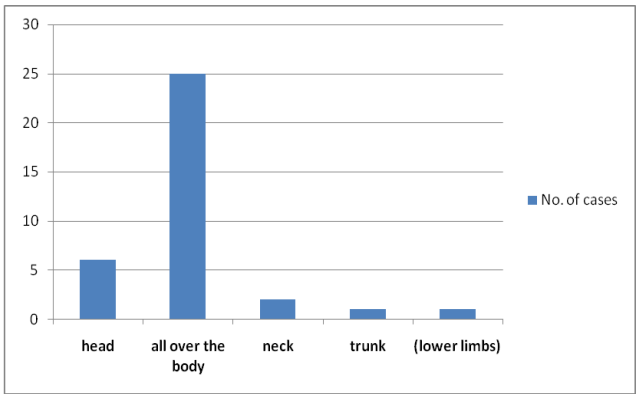

Fig. 3. Distribution of intentional violence in postmortem victims according to the anatomical region

Suicide was the commonest manner of death in post-mortem victims as it was reported in 23 of them accounting for 65.71% while homicide committed by a stranger was seen in 12 cases accounting for 34.29%.
None of the living and post-mortem victims were proved to be under the effect of alcohol or other drugs as their toxicological screening tests results were negative.

Discussion

Intentional violence against female especially women still considered a global problem. The

mean age of the living cases was 20.61±9.37 years. These results are almost similar to the results of a previous study done in Bangkok [14]. While it disagreed with the result of another study done in USA where most of the females were women 20-24 years old. This could be due to implantation of legislative law against any assault to young girls in USA [13].

Most of the living cases were unmarried while in another study the majority were married [16]. This might be due to large population size of this study and the lower age group affected in the current study.

Most of the living cases were illiterate due to lack of education as a result of low socio-economic status of most of them.

All of the assaults were reported as sexual in type and towards the genital area (rape) after kidnapping. This finding agreed with finding of another study [16].

On examination, most of them were found with intact hymen and the rest were found with old tear. This could be due to that most of them were referred on allegation or untrue assault in order to gain money or threatening him to marry her.

Almost all of them were attacked by a stranger perpetrator. This finding contradicts the finding of other study in which most of the perpetrator was known to the female [11]. This is explained by the fact that the perpetrator prefers to attack a stranger female than to attack a relative or a female knows him in order to escape punishment by the law.

Regarding post-mortem victims, their mean age was 28.6±12.84 years with a range between 15-69 years and the highest number was within the age group 20-30 years. These results were similar to the results found in other studies in Punjab, in New York city and Faisalabad [14,16].

Numbers of married victims were more than double the number of unmarried. This was because all of them were subjected to fatal intentional physical trauma. This result is similar to the result found in a previous study in Pakistan [16] .

Most of them were house wives. Being at home as house wife makes woman more vulnerable to domestic violence which might be inflected from her husband or by herself due to the stress and tension she might suffer during her work in a difficult socio-economic status.

Highest percentages of them were having primary level of education only; next comes the illiterate group. This is because of lower commitment of the community in general toward the female in Iraq to continue their education.

Burn was the highest type of injury among postmortem cases. All of them were suicidal in manner escaping from the feeling of being disgraced in their society. This result disagreed from the result of another study which found that burn cases were only minority from the total number where other forms of abuse were inflicted [14, 16].

Multiple anatomical regions were affected in most of the victims. Those were cases of burn. Burn was suicidal in manner therefore the injuries were severing, deep and massive due to the use kerosene.

Physical type of injury was the only type of intentional injury seen in postmortem cases and 69% of them were having old hymen tears representing the married group while the rest were with intact hymen.

The perpetrator was stranger in only 34.29% of victims. This was because most of the postmortem victims attempted suicide by burning them self while females have been most often victimized by someone they knew [11].

Lab. tests for alcohol and drugs in both living and the postmortem groups were negative. This fact was expected as they are prohibited in Islamic law.

In conclusion, violence was more common among living female and highest in the third decade of life. Majority were either with low level of education or having primary school level of education. All of the living females were sexually assaulted while burn was the main type of injury among the postmortem group.

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Author contribution
Dr. Al-Giboori has designed the study and co-writes the manuscript; Dr. Al-Saadi has collected and analyzed the data and write the manuscript.

Conflict of Interest
The Authors declare no conflict of interest.

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Awareness and Knowledge of Diabetic Ocular Diseases among Diabetic Patients at Aden Diabetic Center, Aden, Yemen

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Abstract

Background Awareness about diabetic eye complications and regular eye examinations plays an important role in avoiding blindness.

Objective To assess the level of awareness and knowledge regarding diabetic ocular diseases among diabetic patients attending Aden Diabetic Center at Al-Gamhoria Teaching Hospital in Aden.

Method This is a cross sectional study including a sample of 182 diabetic patients attending this center during the period from January – March 2013. To achieve the objectives of the study, a closed-ended questionnaire including different variables related to diabetic patients' awareness supplied to each patient. The authors filled in the questionnaire during interviews with the patients.

Result The results showed that 109 patients were females and 73 were males with a mean age 53 years and half of the patients were illiterates. Of the total 182 respondents, 157 (86.3%) had variable awareness and knowledge of ocular complications of diabetes mellitus. From those 157 respondents, 124 (79%) had awareness of blindness as an ocular diabetic complication. Awareness of diabetic complications affecting other organs was 93.6%. Awareness regarding the importance of controlling issues caused by diabetes mellitus and its impact on preventing eye complications as well as other body organs was 73.9%. About 36.8% of respondents never visited eye specialists. Medical staff together with the media was the main source of information about eye diseases.

Conclusion The majority of patients was aware of diabetes mellitus complications and had knowledge of cataract, glaucoma, and retinopathy as the most known ocular complications.

Key words Awareness, eyes, diabetes mellitus, complications.

List of Abbreviation: DM = Diabetes mellitus, IDF = International Diabetic Federation, DRP =diabetic retinopathy, EMR = Eastern Mediterranean Region, ADC = Aden Diabetic Center

Introduction

Diabetes mellitus (DM), particularly type II, is a major public health concern worldwide. In developing nations, the estimated increase in patients with DM could approximately be 150%, from 30 million in 2000, to 80 million in 2030 [(1)].

In 2005, the International Diabetic Federation (IDF) confirmed that diabetes is one of the most common non-communicable diseases globally, and it constitutes the fourth leading cause of death in most developed as well as many developing and nearly industrialized countries [(2)]. In 2007, the United Nations resolution adopted to mark DM as a significant global public health issue [(3)]. In Yemen, two studies showed that the prevalence of DM ranged between 4.6% [(4)] to 9.7% [(5)].

Diabetic eye disease refers to a group of eye problems that people with diabetes may face as a complication of diabetes. All diabetic retinopathy (DRP), cataract, and glaucoma can cause severe vision loss or even blindness [(6)]. Moreover, diabetics are 25 times more likely to become blind than non-diabetics due to DRP [(7)].

However, 90% of diabetes-related blindness is preventable through early detection, treatment, and appropriate follow-up care [8].

The most commonly reported barrier to screening by people with diabetes is that they did not know the need for eye examination. Since DRP remains asymptomatic in its early stages, another major barrier to achieving the first and regular eye examination is the belief that" nothing is wrong with my eye" [9].

In the United State of America, an American Diabetes Month considered more important in that it encourages people with diabetes to take steps to prevent complications of diabetes [10]. They realized that people with diabetes should have annual dilated eye examinations to identify early signs of DRP and other diabetic eye disease [10].

The International Agency for the Prevention of Blindness Eastern Mediterranean region (EMR) had motivated the professional bodies of the member countries to improve eye care for diabetics. For this purpose, the theme for the 'World Sight Day 2004' was Eye in Diabetes [11].

Optimum management of the problem requires an individual to be aware of the nature and consequences of the disease; the treatment and its complications [12]. Increasing the awareness will lead to an increase inthe understanding and accepting of the importance of routine eye examination for early detection and treatment, thereby decreasing visual impairment [13].

Realizing the importance of studying this problem as an important public health difficulty affecting the majority of population, comprehending consequences of this problem in Yemen, in addition to the scarce studies conducted on this problem, which are all considered as factors that motivated authors to address this problem. Results achieved by this study provide health system policy makers in Yemen with a real estimation of this problem in order to suggest plans for controlling methods for this problem in the future. Avoidance of visual impairment caused by diabetes requires regular eye examinations. Awareness about diabetes eye complications can play an important role in encouraging people to seek timely eye care [14].

The goal of the current study is to assess the level of awareness and knowledge of diabetic ocular diseases among diabetic patients who attended Aden Diabetic Center (ADC); and to equip targeted patients with knowledge about diabetes eye complications and the importance of periodic examination**.**

Methods

A cross-sectional study conducted during the period from January- March 2013 and targeted all diabetic patients presented for consultation in ADC at Al-Gamhoria Teaching Hospital, which is the only national center in Aden city that can give results representing diabetic patients from the whole city.

Non-randomize sampling method was applied. Only respondents from those who attended the center on the first two days of the week were selected during the study period.

Instrument for data collection

Data collection conducted using personal interview- questionnaire, which was available in English and Arabic languages. It was administered in Arabic language to the respondents and the authors filled the questionnaire on their behalf. The questionnaire included information on patient background data, education, knowledge about diabetes and its ocular complication, the duration and family history of diabetes and medical history of any eye diseases, surgery and drugs. Questions regarding awareness and knowledge of ocular diabetic diseases were answeredin the form "yes or no". The assessment was done as following: the answer to each question ranged from (0 Poor) to (3 Excellent), this scoring was divided into two parts by the median so the lowest part (Poor, Fair) was considered to be (Not aware) and the highest part (Excellent, Good) was considered to be (Aware). The questionnaire also included the awareness in relation to the duration since diagnosis, different types of eye complications, the sources where the

information obtained from and frequency of doctor visiting as well.

The data were processed and analyzed using Statistical Package for Social Sciences (SPSS) program version 15. A choice was made to treat awareness as a dichotomous variable.

Ethical consideration

Permission was taken from the director of the center, the purpose of the study was explained to the patients and data were collected, after obtaining verbal consent from the patients. The Research and Ethics Committee of the Faculty of Medicine and Health Sciences of Aden University have approved this study

Results

Table 1 shows the sociodemographic distribution of the sample. It was found that, out of the total 182 patients interviewed; 109 patients were females and 73 patients were males, the mean age of the patients were 53.2±11.8 years old. Concerning distribution within age groups, 22 patients were within age group less than 40 years; 102 of were between 40-59 years old and 58 of patients within 60 years old and more. Illiterates or having basic level of education were 110 patients, while 72 patients with secondary high school and university levels of education. The relation of these socio-demographical characteristics with the awareness of patients towards eye complication of DM was tested. There was no significant relationship between awareness and gender (Pearson $\chi 2$ 1df = 1.769, P = 0.183) and between awareness and age of the participants (Pearson $\chi 2$ 2df = 1.718, P = 0.424). While, there was significant relationship between awareness of the participants and level of education (Pearson $\chi 2$ 3df = 11.636 P = 0.009), with 0.05 level of significant.

Table 1. Socio-demographical characteristics of the study sample according to their awareness

Parameter		Yes	No	Total	*P* value
		Freq. (%)	Freq. (%)	Freq. (%)	
Gender	Male	66 (90.4)	7 (9.6)	73 (100.0)	0.183
	Female	91 (83.5)	18 (16.5)	109 (100.0)	
Age (years)	< 40	18 (81.8)	4 (18.2)	22 (100)	0.424
	40-59	91 (89.2)	11 (28.6)	102 (100)	
	60 & more	48 (82.8)	10 (17.2)	58 (100)	
Educational level	Illiterate	51 (75.0)	17 (25.0)	68 (100)	0.009
	Basic	39 (92.9)	3 (7.1)	42 (100)	
	Secondary	38 (92.7)	3 (7.3)	41 (100)	
	University	29 (93.5)	2 (6.5)	31 (100)	
	Total	157 (86.3)	25 (13.7)	182 (100)	

Table 2 shows the distribution of patients according to their educational level and awareness about the influence of DM complications on other body organs, and ability to avoid DM complications by the control of blood sugar. The study found that 116 (73.9%), out of 157 patients were aware that controlling DM may prevent complications (Pearson $\chi 2$ 1df = P = 0.005) and 147 (93.6%) from the 157 patients, were aware that complications of DM affects other body organs (Pearson $\chi 2$ 3df = P = 0.413).

Table 3 shows the distribution of patients according to their period of DM by the knowledge and awareness of ocular complication of DM. Of the total 182 participants, 58.3% of patients diagnosed their disease since < 10 years, while 41.8% diagnosed their disease since 10 years and more. The mean period of diagnosis of DM among the patients

was 8.5 ± 5.9 years. There is no significant relationship between knowledge and awareness of ocular complication of DM and duration of diseases (Pearson Chi square test 3df = 7.193 *P* = 0.066).

Table 2. Educational level and knowledge about prevention of diabetes mellitus complications that affect other organs

Educational level	Diabetes complication influence on other organs		Control of diabetes prevents the complication		Total
	Yes	No	Yes	No	
	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)
Illiteracy	48 (90.5)	5 (9.4)	32 (60.4)	21 (39.6)	53 (100)
Basic	37 (92.5)	3 (7.5)	32 (80.0)	8 (20.0)	40 (100)
Secondary school	34 (94.4)	2 (5.6)	27 (75.0)	9 (25.0)	36 (100)
University	28 (100)	0 (0.0)	25 (89.3)	3 (10.7)	28 (100)
Total	147 (93.6)	10 (6.4)	116 (73.9)	41 (26.1)	157 (100)
P value	0.413		0.005		

Table 3. Distribution of patients according to time since diagnosis of diabetes mellitus in relation to awareness and knowledge of its complication

Duration of diseases (years)	Knowledge and awareness of ocular complication of DM			*P* value
	Yes	No	Total	
	Freq. (%)	Freq. (%)	Freq. (%)	
< 5	40 (76.9)	12 (23.1)	52 (100)	0.066
5-9	46 (85.2)	8 (14.8)	54 (100)	
9-14	39 (92.9)	3 (7.1)	42 (100)	
15 & more	32 (94.1)	2 (5.9)	34 (100)	
Total	157 (86.3)	25 (13.7)	182 (100.0)	

Table 4 shows the distribution of patients according to their awareness about DM eye complications. Out of the total 157 of award respondents, 23.6 % knew that Cataract was one of DM eye complications, while 17.8 % and 57.3 % of the award respondents mentioned Glaucoma and Retinopathy respectively. Finally, 124 (79.0%) patients award that blindness was a complication of DM. It was found that there was a significant relationship between awareness and the participant knowledge about each eye disease.

Table 4. Distribution of patients according to awareness about different diabetic eye complications

Awareness of patients on eye complication	Yes	No	Total	*P* value
	Freq. (%)	Freq. (%)	Freq. (%)	
Cataract	37 (23.6)	120 (76.4)	157 (100.0)	0.007
Glaucoma	28 (17.8)	129 (82.2)	157 (100.0)	0.022
Retinopathy	90 (57.3)	67 (42.7)	157 (100.0)	0.000
Blindness	124 (79.0)	33 (21.0)	157 (100.0)	0.000

Table 5 shows the distribution of patients according to variables related to eyes. It was found that 78.6% of patients had eye complaints or problems (blurred vision, itching, and pain). Regarding to the frequency of visits to an eye doctor; it was found that 17 patients (9.3%) visited an eye doctor 1-2 times per year, and 8 patients (4.4%) visited an eye doctor once per 2 years, while 90 (49.5%) patients visited eye doctors occasionally and 67(36.8%) patients had never visited any.

By asking patients about their use of any eye treatment; 10(5.5%) patients used only eye drops, mostly were (antiglaucoma, antihistamine and lubricants), 13(7.1 %) patients out of 182 underwent eye operations (for cataract or glaucoma), and five (2.7%) patients had Laser as an eye treatment.

Table 5. Distribution of patients according to eye variables

Variable		Frequency	%
Presence of any Eye problem	Yes	143	78.6
	No	39	21.4
Frequency of visits to Eye doctors	1-2/year	17	9.3
	1/year	8	4.4
	Occasionally	90	49.5
	Never	67	36.8
Use any eye treatment	Eye drop	10	5.5
	Operation	13	7.1
	Laser	5	2.7
	No treatment	154	84.6
	Total	182	100

Table 6 shows the distribution of patients according to the sources of information about the influence of DM on any part of the body. It was found that only 7% of patients in the sample obtained the information from relatives and 28 (17.8 %) got information from their friends, while 68.2% of patients obtained information from medical staff (health teams), and 40.8% of patients got information from the mass media. The medical staff and the media were the main sources of information for the study sample.

Table 6. Distribution of patients according to the source of information about the influence of diabetes mellitus on the eyes and other parts of body

Information source	Frequency	%
Relatives	11	7.0
Friends	28	17.8
Medical staff	107	68.2
Media	64	40.4

Discussion

Awareness creation is a vital important step in the creation of a successful program to fight against any disease in the community [13]. Awareness is not the same as knowledge, hearing about a problem is awareness, whereas understanding the causes or treatment of a disease is knowledge [15].

As reported, eye manifestations are important health problems in the diabetic population. The eye complications in this study sample were 30.2%, 14.6 and 3%, for retinopathy, cataract and glaucoma respectively [16].

In the current study, almost 86.26% of 182 respondents were aware that diabetes could affect the eye, males were 66 (90.4%) out of 73 and females were 91, (83.5%) out of 109. Of the total 157-awared patients, 79% had the knowledge that diabetes may cause blindness.

Moreover, patients who knew ocular disorders such as cataract (presumed problem in the lens) were 23.6%, glaucoma (high pressure of the eye)

17.8% and knowledge was higher forretinopathy (such as retinal hemorrhage or /and detachment), 57.3%. Some studies discussed the same understanding of possible ocular effects of diabetes 86% [14] 84% [15] 72.9% [17]. Moreover, study reported greater knowledge of diabetes eye complications as 98% [18]. On the other hand, in other studies, reported low Knowledge of DM ocular effects 37.1% [6], 3.8% [19].

Also among those who reported that diabetes could affect the eyes, 93.6% knew that diabetes could affect the body organs (presumed kidney, heart, and tooth). Moreover, 73.9% of the total 157 respondents knew that controlled diabetes (good control of blood sugar) prevents diabetic complications. In contrast, a published study reported that little awareness of diabetes might develop complications affecting the eyes, kidneys and nerve 6.8% [20].

In the current study, no significant association of gender regarding awareness and knowledge of diabetes complications was found. The same finding was reported [14]. In contrast, another study showed that awareness of diabetic eye diseases among females was higher than in male [21].

Like other studies [15,22] that supported the proposition that education is important in creating awareness, in this study, the level of awareness of diabetes ocular complications was found to be significantly associated with respondents' educational level. Another reason to explain this high level of awareness among respondents was information about ocular and organ complications presented in a lecture at Diabetic Center, where interviews were performed. Thirty-two (60.4%) illiterates in this study knew about the association of good control of blood sugar with preventing disease complications, compared to 89.3% of highly educated respondents.

In the ADC, there is no screening protocol for diabetic ocular diseases or retinopathy. Of the total 182 respondents, 36.8%, have never visited ophthalmologists (eye clinic) since they had DM, presuming that they had a good sugar control; while 49.5% did not know the frequency of eye checkups. For that reason the information given to diabetic patients, regarding eyes, should be clear including; signs and symptoms of eye involvement as well as complications, and how to prevent such eye complications by regular visits to the eye clinic. Unfortunately, 9.3% and 4.4% had routine eye examination of a frequency of 1-2 visits per a year and once per 2 years respectively. They claimed satisfaction from changing their spectacles at the optics shops. This may explain low rates of positive attitudes for healthy eye care. In contrast, studies showed better knowledge of routine eye checkups among diabetic patients 67.2% [14] and 50.8% [15] in spite of good control of DM. In this study, all patients in the ADC were advised and encouraged to seek timely eye checkups by ophthalmologists for early diagnosis and management of any eye complications before loss of their vision.

The majority of respondents 68.2% reported that medical staff was the main source of their information about diabetes and its complications, followed by media 40.8%, friends, and diabetic family member. The same findings were reported in other studies where the medical staff was the source of information but with less significant role of media [22, 23]. This illustrated the influence of the medical staff on patients` trust.

There are some limitations in this study; respondents were limited to one place, small number of patients, and limited parameters were included in the questionnaire. In conclusion, there is significant relationship between educational level and patients' awareness about diabetic eye complication, cataract, glaucoma, and retinopathy are the most mentioned diabetic eye complication and health team and media are the main sources of information about DM complications among studied patients.

We recommend to emphasis on the knowledge toward diabetic eye complications through health education programs and to strengthening the role played by DM centers for better eye healthcare for all patients.

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Author contributions

Dr. Sawsan did the interview and data collection with Dr Azal, and conducted the writing of manuscript; Dr. Ahmed review the manuscript and Dr. Jamil participated in data collections and statistical analysis review of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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The Incidence of Breast Cancer in Examined Biopsies of Breast Masses in Al-Hussain Teaching Hospital in Kerbala

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Abstract

Background Breast cancer is the most common type of malignancy among females worldwide. Breast cancer is the second most commonly diagnosed cancer in women under 35 years with the most common hitological type being infiltrative ductal carcinoma.

Objective To determine the incidence of breast cancer in excised breast biopsy specimen in Al-Hussain Teaching Hospital in Kerbala governorate as well as determining the most prevalent histopathological type, pathological stage and grade at time of diagnosis.

Methods It was a statistical study in which a total of 200 excised biopsy and mastectomy specimens where enrolled in the study, biopsy processing and slide preparation from paraffin blocks was processed in the pathology laboratory in Al-Hussain teaching hospital in Kerbala from January 2011 – January 2012.

Results Out of 200 cases who were initially enrolled in the study, 140 (70%) were benign, and 60 (30%) were malignant. The highest incidence of breast cancer was found among 40-49 years age and ductal carcinoma is the most common histological type.

Conclusions Breast cancer accounts for about 30% from all excused biopsy materials in Al-Hussain Teaching Hospital in Kerbala. This study highlights some of the features of breast cancer seen in this region; younger age, intermediate stage at presentation, and higher incidence in housewives. In contrast to data from the West, where more than half of breast cancer patients are above 50 years of age and higher incidence among high socioeconomic group.

Key words Breast cancer, lesions, histopathology, incidence, percent.

List of abbreviation: BC = breast cancer, FNA = fine needle aspiration

Introduction

Breast cancer (BC) is the most common type of malignancy among females worldwide and about 1.38 million women are diagnosed with BC annually accounting for about a tenth (10.9%) of all new cancer cases and nearly a quarter (23%) of all female cancers [1,2]. BC is the second most commonly diagnosed cancer in women under 35 years with the most common histological type being infiltrative ductal carcinoma [3,4].

The incidence of BC differ between different socioeconomic groups with highest rates for the most affluent group [2,3]. In Iraq, BC is more common in housewives [4]. Its incidence has been increasing for many years in economically developed countries [5].

BC risk is strongly related to age, with 81% of cases occurring in women aged 50 years and over and nearly half of cases are diagnosed in the 50-69 age groups [6].

More than 50% of total BC diagnosed annually is found in premenopausal women, creating the need to initiate BC screening programs in this

population and one of these measures include breast self examination [7].

Retrospective demographic regional studies have shown that most patients with BC present for the first time at stages two to three [8,9]. Regarding education, 31.7% are illiterate and only 10% graduated from college so, it is lowest among college learning women [10].

Recent steep rise in incidence rates for women aged 60-69 years is almost certainly caused by the introduction of national BC screening programs for this age group. Although the incidence of BC is rising worldwide, but still the overall 5 years survival rates are over 80% [11].

The aim of the study is to determine the incidence of BC in excised breast biopsy specimen in Al-Hussain Teaching Hospital in Karbala governorate as well as determining the most prevalent histopathological type, pathological stage and grade at time of diagnosis.

Methods

This study includes a total of 200 excised biopsy and mastectomy specimens were enrolled in the study, these specimens were collected from Histopathological Laboratory in Al-Hussain Teaching Hospital, and biopsy processing and slide preparation from paraffin blocks was processed in Al-Hussain Teaching Hospital from January 2011 to January 2012. Slides were carefully examined and the histopathological type and grade were determined on all excised biopsy materials. Most cases were diagnosed by fine needle aspiration (FNA) before excisional biopsy and mastectomy.

We did staging for cases operated for carcinoma of breast by mastectomy (staging for mastectomy specimens only), which were either diagnosed by FNA or excisional biopsy, we exclude from staging cases with excisional biopsy only.

Tumor grade is the description of a tumor based on how abnormal the tumor cells and the tumor tissue look under a microscope. It is an indicator of how quickly a tumor is likely to grow and spread.

We use the Nottingham grading system (also called the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system) for breast cancer [12].

Staging describes the extent or severity of a patient's cancer. Knowing the stage of disease helps the doctor to plan the treatment and estimate the patient's prognosis.

TNM staging system was used and is based on the size (T), regional lymph nodes metastasis (N), and distant metastasis (M).

We did different radiographic examination including chest x-ray and abdominal ultrasound and liver function test to detect presence or absence of distant metastasis.

Histopathological Technique

- Fixation: in 4% formaldehyde in buffered isotonic saline.
- Dehydration: A graded series of mixtures of water and ethanol are use, 50%-70% to 100% ethanol for 2 hours each.
- Embedding: two changes of 100% paraffin in an oven at 58-60 °C.
- The first paraffin bath lasts for 2 hours; the second one is 3 hours.
- Tissue Embedding.
- Next; the tissue is oriented and embed in a paraffin block.
- Block is placed in ice water to solidify.
- Sectioning with a microtome, serial sections form a ribbon
- Mounting: The slides are placed on a warming tray and distilled water is added to float the paraffin sections and allow them to expand and straighten out
- Staining

1) Slides with paraffin sections on them must have the paraffin removed for staining.
2) Place slides in xylene for 10 minutes.
3) Next a second change of xylene for 10 minutes.
4) Slides are then rehydrated through a grades series of alcohols to distilled water.
5) The slides are then placed in hematoxylin for 3 to 5 minutes.
6) Then rinsed in water.

7) Slides stained with eosin for 1-2 minutes.
8) Then rinsed in water.
9) Next rehydrated in graded alcohol
10) Next cleared in xylene
11) A small drop of mounting medium was added to the slide and finally add a cover slip.

Results

Out of 200 cases who were initially enrolled in the study, 140 were benign (70%), and 60 were malignant 30%. Table 1 shows the age distribution for patients involved by BC, the highest incidence was found among 40-49 years age group and the lowest incidence was for the 20-29 years age group.

Table 1. Incidence of breast cancer according to age

Age group (years)	Number of cases	%
20-29	2	3.3
30-39	12	20
40-49	26	43
50-59	12	20
60-69	4	6.6
70-79	4	6.6
Total	60	100

The most common histopathological type of BC was ductal carcinoma which constitutes about 83.3% of the cases (50 case) followed by lobular carcinoma 10% (6 cases), medullary carcinoma 3.3 % (2 cases), tubular carcinoma 1.6% (1 case), and mucinous carcinoma 1.6% (1 case) as seen in table 2.

Table . Breast cancer distribution according to histological type

Histological type	No. of cases	%
Ductal carcinoma	50	83.3
Lobular carcinoma	6	10
Medullary carcinoma	2	3.3
Tubular carcinoma	1	1.6
Mucinous carcinoma	1	1.6
Total	60	100

Figures 1 through 6 showed different histopathological type of BC.

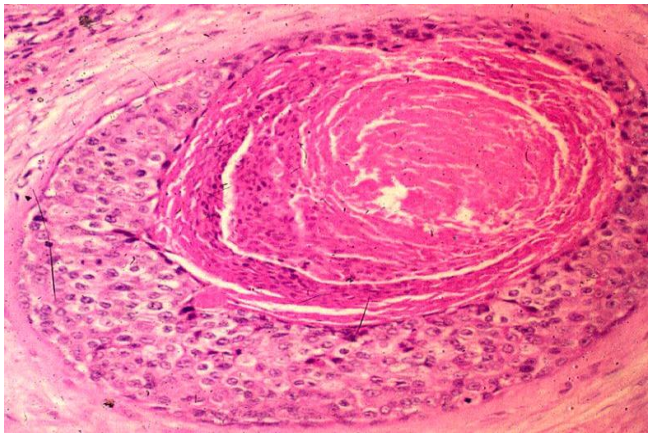

Fig. 1. Ductal carcinoma in situ (Comedocarcinoma)

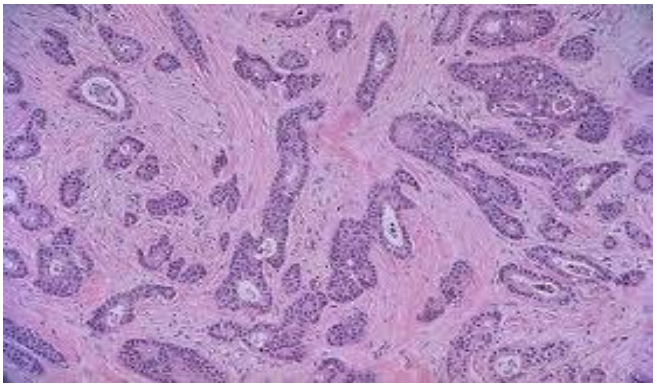

Fig. 2. Infiltrative ductal carcinoma

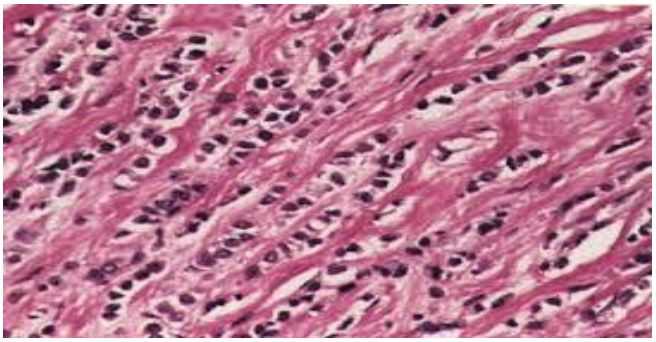

Fig. 3. Infiltrative lobular carcinoma

Staging was carried out on all malignant cases submitted for modified radical mastectomy most patients presented in stage II of the disease (20/40 cases, 50%) followed by stage III (25%) of the cases, 5% of cases were in situ ductal carcinoma, and 5% were stage IV of the disease, as shown in table 3.

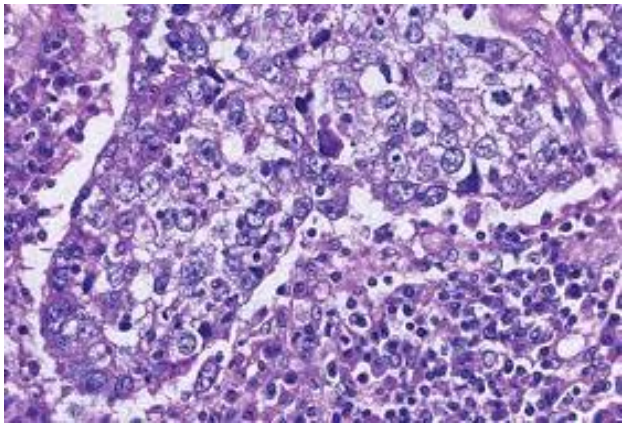

Fig. 4. Medullary carcinoma

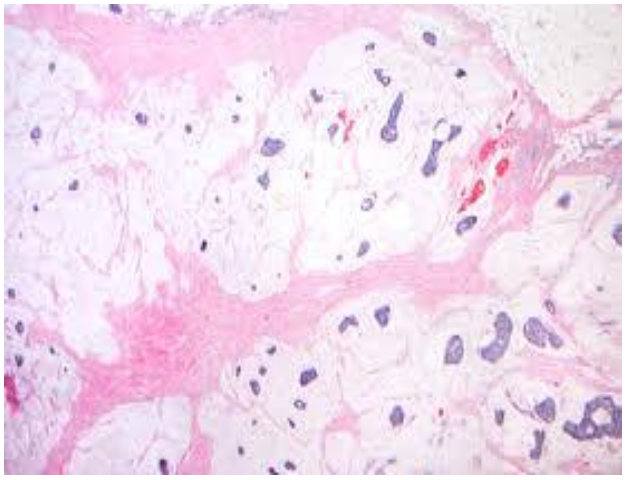

Fig. 5. Mucinous carcinoma

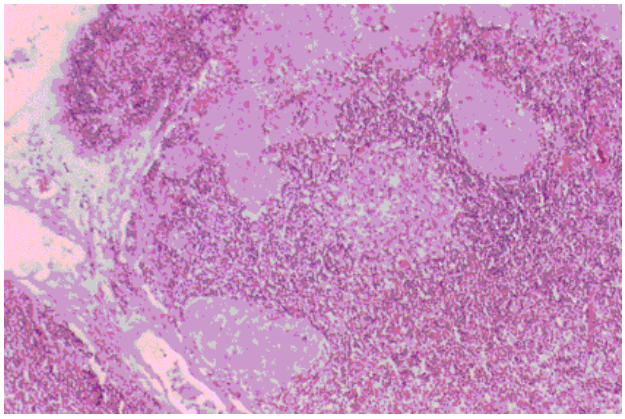

Fig. 6. Metastatic mammary carcinoma (Lymph Node)

Grading was done on all malignant cases (excisional biopsy and mastectomy specimen). The most predominant grade for infiltrative carcinoma of the breast was grade II (39/60 cases, 65%), because most tumors show prominent glandular structures and intermediate degree of mitotic activity, followed by grade III (12/60 cases, 20%), followed by grade I (9 cases, 15%) as illustrated in table 4.we can see the age distribution with regards to histopathological type, stage and grad as shown in tables 5 through 7 respectively.

Table 3. Number and percent of cases according to stage

Stage	No. of cases	%
0	2	5
I	6	15
II	20	50
III	10	25
IV	2	5
Total	40	100

Table 4. Number of cases according to histological grade

Grade	No. of cases	%
I	9	15
II	39	65
III	12	20
Total	60	100

By applying T-test there was no significant association found between age and histological type, age and disease stage, age and tumor grade with a *P* value of 0.653, 0.339, 0.168 respectively.

In our study, 66% of patients had low level of education, 34% of malignant cases were graduated from college.

Discussion

It was estimated that 332,000 new cases diagnosed in the countries of European Union in 2008 [(1)].

The etiology of breast cancer is uncertain and adequate primary prevention is not possible [(7)].

The changes that have been noticed in the incidence and the age of presentation of breast

carcinoma in Iraq could be attributed only to the usual risk factors [4].

We believe that stressful life events might be more significant trigger for the development of breast cancer.

Table 5. Age distribution according to histological type

Age (yrs)	Histopathologic type				
	DC	LC	MC	TC	MuC
20-29	2	0	0	0	0
30-39	8	2	1	1	0
40-49	23	2	1	0	0
50-59	11	0	0	0	1
60-69	3	1	0	0	0
70-79	3	1	0	0	0

DC = ductal carcinoma, LC = lobular carcinoma, MC = medullary carcinoma, TC = tubular carcinoma, MuC = mucinous carcinoma

This study was done over a very stressful condition in Iraq, characterized by war and the disasters that occurred after the year 2003.

A study in China suggested that psychological stress is associated with increased oxidatant production and oxidant damage was found in breast tissue [13].

Table 6. Age distribution according to stage

Age (yrs)	Stage				
	0	I	II	III	IV
20-29	0	0	1	0	0
30-39	0	1	4	2	1
40-49	1	3	9	3	0
50-59	1	2	5	3	-
60-69	0	0	1	1	0
70-79	0	0	0	1	1

For age, the same finding of patients with breast cancer in this study tends to be in younger age group. It occurred in (56.3%) of patients before the age of 45 years. This tendency is similar to other study done in Jordan [14].

Peak frequency was recorded equally in the age categories 40-49 years; similar peak age frequencies were recorded in other reports from our country [4].

In this study, the peak incidence according to age group was under age of 50 years unlike in the United States of America where women aged 50 years and older are the most commonly affected, this is because of the population pyramid in these Middle East countries and then higher rates among younger age groups [2].

Table 6. Age distribution according to grade

Age (yrs)	Grade		
	GI	GII	GIII
20-29	0	1	1
30-39	1	9	2
40-49	4	18	4
50-59	2	7	3
60-69	1	3	0
70-79	2	1	1

In our study, the most prevalent stage at time of diagnosis was stage II (50%), while in other study in Iraq, they found that 47% of them presented with advanced stage breast cancer; either stage III or IV in a study carried out in Erbil in 2004 [4]. The relative early diagnosis might be related to increased education and awareness about breast cancer or might be related to decrease aggressiveness of the tumor, this is not like other study, in which, most patients presented in late stages of the disease when seeking medical advice, which is again similar to the above studies in the region. It is not well understood whether this delay in presentation is due to cultural and social customs or due to more aggressiveness of the disease in this part of the world [15].

Although low percent of carcinoma in situ, which is nearly same finding in other study [3], the patients in our study were relatively presented at low stage at time of diagnosis, this highlights increased community awareness about breast cancer and the need for early detection and screening programs including periodical mammography and periodical physical and breast self-examination.

Also in our study, the most predominant stage at time of presentation was stage II, which

accounts for about 50% of cases, and 83% were invasive ductal carcinoma which was similar to study of others [16].

In our study, most patients were housewives who lived in intermediate and low socioeconomic state, while in Europe it was found that breast cancer incidence is higher in the most affluent groups in society [1].

In conclusion, BC accounts for about 30% from all examined biopsies of breast in Al-Hussain Teaching Hospital in Karbala, most malignant cases diagnosed between 40-49 years 43%. The most common histological malignant type is ductal carcinoma (83.3%), 50% of malignant cases comes at pathological stage II, 65% of malignant cases diagnosed at histological grade II, there was no significant association was found between age and histological type, age and disease stage, age and tumor grade, and in our study most patient were housewives who lived in intermediate and low socioeconomic state.

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Authors' contribution

Dr. Anees and Dr. Akram responsible for collection of cases and treatment and follow up of the patients, Dr. Fatin as a pathologist responsible for histopathological review of slides and reports, staging and grading of mastectomy specimen.

Conflict of Interest

No conflict of interest of any type in the work.

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