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Iraqi Journal of Medical Sciences

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CONTENTS

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Concept of Composite Lymphoma

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The term composite lymphoma (CL) was first proposed to denote the occurrence of more than one lymphoma type in a single patient; however, the present term is now restricted to the rare occurrence of 2 or more morphologically and immunophenotypically distinct lymphoma clones in a single anatomical site i.e. within a single organ or tissue Both distinct clone processes co-exist persistently and equally, i.e. a biclonal or oligoclonal origin. Rarely, a single original clone could deviate to form 2 distinct diseases**.** The term collision tumor is used to delineate the multidirectional pathways of malignant lymphomas**.** However, as morphologic cross-over among the B-cell NHL, T-cell NHL and HL is wide, confirmed immunohistochemical and/or molecular methods must be fulfilled for proof documentation of the concurrent composite disease entities [(1)].

CL may be confused with other lymphoma conditions, from which it must be differentiated:

1. Transformation and progression:

Lymphomas tend to evolve over time from small-cell to large-cell and from follicular to diffuse forms. Also the transformation of HL nodular lymphocytic predominance (NLP) into diffuse large B-cell lymphoma has been documented. Transformation of lymphoma over time is considered disease progression rather than composite lymphoma. In these situations, given the time, all malignant cells will eventually transform to the more aggressive disease as part of their natural history [(2)].

2. Discordant lymphoma:

Other rare conditions present different types of malignant lymphomas occurring in different sites of the body, e.g. nodal Hodgkin's lymphoma and intestinal MALT lymphoma. The two conditions may present clinically as concurrent or sequential disease [(2)].

3. Differentiation:

Occasionally, peripheral differentiation occurs in low grade lymphomas e.g. follicular lymphoma with marginal differentiation [(3)].

No single definite mechanism has been proposed to explain the pathogenesis of the different types of CL as the etiology is variable, complex and differs according to the types of lymphomas involved. Generally, the immunological status of patients is a crucial element that may predispose to CL. It may arise during the course of atypical lymphoproliferative lesions namely, Castleman disease states of immunosuppression, chemotherapy, or multiple viral infestations [(4)].

However, suggested theories for different combinations include the following:

i. Composite B-cell lymphoma could be due to:

A. Clonal selection:

A clone of malignant B cells within a tumor may be exposed to additional mutational accumulation and change into a more aggressive neoplasm, co-existing with the original clone. An example is Richter's syndrome of B-cell small lymphocytic lymphoma changing to diffuse large B-cell lymphoma with the persistent co-existence of both clones in the same tissue [(5)].

B. Genomic instability and congenital predisposition:

A state of immunoglobulin gene instability, that might be inherited, may predispose to multiple types of B-cell NHLs. This explains the positive family history in some cases of CL [(6)].

C. Common precursor cell:

Immature precursor cell may have a multi-deviant pathway which results in more than one type of B-cell lymphoma [(1)].

ii. B-cell NHL and HL:

The Reed-Sternberg cell in most cases of HL is a type of B lymphocyte. So, similarly, the co-existence of both diseases would be conceivable through a common precursor cell origin theory [(1)].

iii. T-cell lymphoma with HL or B-cell lymphoma:

Since there is difference in cell lineage, the development of T-cell lymphoma in the setting of B-cell NHL or HL raises the possibility of some cooperative process between T lymphocytes and B-lymphocytes that favored neoplasia. The presence of an infective agent, mostly a virus, could explain the theory of multiline age cooperative and reactive process [(6)]. Although Epstein Barr Virus (EBV) preferentially infects B cells, it may also infect T cells through the CD21 receptor, which is present on developing but not mature T cells. Although half of T-cell lymphomas show EBV-infected cells, EBER-positive cells are mostly B, null, with few T cells. Down regulation of surface markers could possibly be related to the viral infection process. EBV positive CL strongly expresses p53 protein, possibly with a background state of immunosuppression [(1)].

For documentation of CL, all morphologically consistent cases must be verified by the objective confirmation of the co-existence of 2 or more types of lymphomas, using one or more laboratory tests. Diagnostic tests could be applied on tissue sections, cell suspensions, or DNA extract. Results of tests done on DNA extracts are more accurate using the laser capture microdissection method [(3)].

Diagnostic tests include: (1) immunohistochemistry and protein expression profile; (2) flow cytometry; (3) immunoglobulin and T cell receptor gene rearrangement by PCR; (4) cytocytogenetics and FISH techniques for chromosomal translocations; (5) in-situ hybridization for detection of viral DNA; (6) DNA sequencing for clonality studies; and (7) cDNA microarray for gene expression profile .[(7)]

Synchronous occurrence of 2 or more types of NHLs is more common than the occurrence of NHL with HL. Moreover, composite B-cell NHL is more common than composite T-cell NHL. About 30-40% of cases with Richter's syndrome had a second B-cell lymphoma of a different origin. The combination could be restricted to lymphomas of germinal center origin (follicular and diffuse large B-cell NHL), non-germinal center cell origin (SLL, mantle, marginal NHL), post germinal center origin (LPL, plasmacytoma, immunoblastic NHL), or a mix of different compartmental origin [(1)].

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Role of DNA Integrity of Spermatozoa in Male Infertility

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Abstract

Background Classical semen analysis gives an approximate evaluation of the functional competence of spermatozoa, but not the quality of sperm DNA. With the advent of assisted reproductive technologies (ART), the concern over using damaged DNA has become apparent.

Objective To clarify the role of DNA integrity and maturity of ejaculated spermatozoa in male infertility.

Methods A randomly selected group of 50 nonazoospermic infertile patients with a history of infertility of at least 1 year duration were included in this study. Whereas control group consisted of semen samples obtained from healthy volunteers of proven fertility (n = 27). Two main assays were studied in ejaculated spermatozoa: The green Acridine Orange (AO) fluorescence test that measures DNA integrity and the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL)- based ApopTag® technology that assesses DNA fragmentation as a late apoptotic marker. Results were compared with the standard sperm characteristics (concentration, motility and morphology) between infertile patients as well as some patients' subgroups against control donors.

Results Significant low levels of green AO fluorescent spermatozoa were observed in all patients and patient's subgroups with asthenoteratozoospermia and oligoasthenoteratozoospermia (P = 0.00001). High percentages of TUNEL-positive spermatozoa were significantly noticed, against control donors, in all patients as well as asthenoteratozoospermic and oligoasthenoteratozoo-spermic infertile patients. Interestingly, normozoospermic patients had a significantly low percentage of green AO fluorescent spermatozoa and high levels of TUNEL-positive spermatozoa versus control donors (P = 0.0005, P = 0.0069, respectively).

Conclusion From this study, it can be concluded that male infertility is associated with high rates of DNA damage in the spermatozoa, and that sperm DNA damage analysis could reveal a buried deformity of sperm nuclear DNA in infertile men classified as idiopathic, having apparently normal standard sperm parameters.

Keywords DNA denaturation, DNA fragmentation, acridine orange, TUNEL.

Introduction

Traditionally, the diagnosis of male infertility is based on microscopic assessment of semen sample, including the concentration, motility, and morphology of the sperm, as well as other parameters, but the results of this conventional semen analysis are insufficient as a diagnostic tool in male infertility [(1)]. New markers are needed that might be better for accurate diagnosis of infertile from fertile men and that may predict pregnancy outcome and the risk of adverse reproductive events, where conventional sperm parameters fall short. There is now some evidence to suggest that markers of sperm DNA integrity may be better measures of male fertility potential than conventional measures [(2)]. Intact human sperm DNA is an essential prerequisite for successful fertilization and embryo development.

Immature spermatozoa from infertile men have been shown to contain various nuclear alterations, including abnormal chromatin structure, microdeletions, chromosomal rearrangements, aneuploidy and DNA strand breaks [3].

It is well known that DNA damage in spermatozoa occurs during late spermatogenesis as a consequence of endogenous factors present in the testis/epididymis, or due to exogenous factors present after ejaculation [1]. Potential mechanisms for generating DNA damage in sperm have been proposed, and include incomplete chromatin packaging [4], abortive apoptosis [5], and oxidative stress by reactive oxygen species (ROS) [6]. Unlike other cells, spermatozoa are more vulnerable to DNA damage because they do not have the capacity for DNA repair [1]. Sperm DNA damage can be measured directly by assessment of oxidation and fragmentation using Terminal deoxynucleotidyl Transferase dUTP nick end labeling "TUNEL" assay, liquid chromatography to measure DNA oxidation levels, and by using single-cell gel electrophoresis assay or "Comet" assay [2]. DNA damage can also be assessed indirectly by means of sperm chromatin integrity assays and by evaluation of nuclear protein levels using DNA stains for detection of denatured or single-stranded DNA (e.g., acridine orange) as well as nuclear protein stains (e.g., aniline or toludine blue) for detection of histones [2].

The study of sperm DNA damage is particularly relevant in an era where advanced forms of assisted reproductive technologies are frequently used. The objectives of this study are to evaluate sperm DNA integrity in semen samples from infertile men compared to control donors; by calculating the percentage of mature spermatozoa that enclose native double-stranded and normal DNA using the acridine orange (AO) fluorescence test. In another approach, DNA fragmentation and presence of single- and double-stranded DNA breaks were measured by the TUNEL-based In Situ Apoptosis Detection Kit. Then, the relationship of the above studied markers and their correlation, if any, with conventional sperm parameters (sperm concentration, motility, and morphology) were inspected.

Methods

This study included a randomly selected group of nonazoospermic Iraqi infertile patients (n = 50) attending the Higher Institute of Infertility Treatment and Assisted Reproductive Technology/ Al-Nahrain University/ Baghdad, with a history of infertility of at least 1 year duration between June and December 2008. Their age ranged from 20–50 years with a mean of 32.58 ± 6.3 years and they all had a normal physical evaluation. Their female partners had no history of untreated female–factor infertility. Written consent for use of the spermatozoa for research was obtained from the patients according to guidelines established by the local research ethics committee of Al-Nahrain College of Medicine. Freshly ejaculated semen samples were obtained by masturbation into a wide-mouthed sterile specimen container after 3–5 days of sexual abstinence. Controls consisted of samples obtained from healthy donors of proven fertility (i.e., fathered a child within the last 12 months) (n = 27). Their age ranged from 22–46 years with a mean of (32.81 ± 6.47 years). Normal control donors had an ejaculate volume of at least 2 mL and a sperm concentration of at least 20 × 10^6/ mL, of which at least 50% were motile and 30% had normal sperm morphology according to the World Health Organization classification [7]. Infertile men with azoospermia were excluded from the study.

Ejaculates were allowed to liquefy at 37^{o}C for 30 minutes before analysis. Semen profile was assessed by light microscopy according to the procedure proposed by the WHO. Following liquefaction, semen specimens were evaluated for sperm motility, concentration and morphology and scored according to WHO guidelines [7].

The AO Fluorescence of Human Spermatozoa:
Acridine Orange staining was performed according to the method of Tejada *et al* [8]. Slides were read by two independent observers blindly counting on the same day of staining, who gave results that generally agreed within 10% discrepancy. The nuclei of at least 300 spermatozoa from each individual were examined and scored as fluorescing green, red or yellow. Spermatozoa displaying green fluorescence were recorded as mature, whereas sperm heads displaying red, orange or yellow fluorescence were considered as immature; as well as those displaying green and red color simultaneously [8]. The threshold of green AO fluorescence as 50% was adopted from Hoshi *et al*. (1996) and the values <50% were considered as positive in the test [9,10].

Spermatozoa TUNEL assay:
DNA fragmentation in sperm nuclei was investigated using the TUNEL assay, which is the basis of the ApopTag® technology (ApopTag® Peroxidase In Situ Apoptosis Detection Kit, CHEMICON® International, Inc., USA & Canada). The reagents provided in the Kit are designed to label the free (3'-OH) DNA termini in situ with chemically labeled and unlabeled nucleotides. Staining was detected by brightfield microscopy (OLYMPUS® model BX41TF, Olympus Optical Co., LTD., Tokyo, Japan). At least 200 spermatozoa were counted for each sample and the percentage of spermatozoa with fragmented DNA (TUNEL-positive) was determined. Photographic records were obtained using (OLYMPUS®, Camedia C- 60 zoom) digital compact camera.

Statistical analysis:
The results were expressed as (mean ± SD). Results of the standard semen characteristics in addition to the results of the main tests employed (AO and TUNEL assays) were compared between infertile patients and fertile controls; as well as between some patients' subgroups and control donors; using unpaired Student's t- test for two samples of unequal variance. The different types of relationships and correlations accomplished in this work (between results of main tests and the basic sperm parameters) were examined using bivariate Pearson's correlation coefficient (two-tailed) test. All hypothesis testing was two-sided with a probability value of < 0.05 deemed as significant. Analyses were conducted with Microsoft Excel/ Microsoft Office XP 1985-2001 and Statistica/ version 6.0 (USA) statistical package.

Results

According to the WHO criteria of semen variables, subjects included in this study exhibited isolated asthenozoospermia (5 subjects); oligoasthenozoospermia (4 subjects); asthenoteratozoospermia (12 subjects); oligoasthenoteratozoospermia (18 subjects); normozoospermia (10 subjects) and finally; oligozoospermia (1 subject).

Conventional Semen Analysis:
Table (1) shows the results of the classical semen analysis parameters performed by light microscopy, according to the WHO criteria, for the 50 infertile men and 27 control volunteers. Compared to controls, infertile patients had significantly lower sperm concentration (25.74 ± 25.3 vs. 129.76 ± 96.18; $P = 0.00001$), reduced progressive motility (31.1 ± 20.4 vs. 62.59 ± 12.74; $P = 0.00001$) and poorer sperm morphology (32.1 ± 12.37 vs. 45.30 ± 5.62; $P = 0.00001$) (Table 1).

AO Fluorescent Test:
As compared to healthy donors, the percentage of green AO fluorescent sperms (enclosing native normal double-stranded DNA) was significantly lower in all infertile patients (39.52 ± 16.09 vs. 68.15 ± 8.52; $P = 0.00001$) (Table 1).

Figure 1 shows representative patterns of AO staining of spermatozoa. Normal sperm heads revealed a visible green color (Figure 1A), which can be differentiated from the red color of some immature sperm cells. Other sperm heads had colors that range from orange to yellow, or mixture of red and green (Figure 1B).

Table 1. Comparison of conventional semen characteristics and the results of AO, and TUNEL assays between infertile patients and healthy fertile volunteers

Spermatozoa	Patients (n = 50)	Control donors (n = 27)
Concentration **(M/ml)** ***Progressive motility (%)***	25.74 ± 25.3*	129.76 ± 96.18
(grades a and b)	31.1 ± 20.4*	62.59 ± 12.74
Normal morphology *** (%)**	32.1 ± 12.37*	45.30 ± 5.62
Green AO fluorescence **(%)**	39.52 ± 16.09*	68.15 ± 8.52
TUNEL positive cells (DNA fragmentation rate) **(%)**	44.56 ± 9.41*	8.37 24.47

* = <0.00001, According to WHO criteria1999.

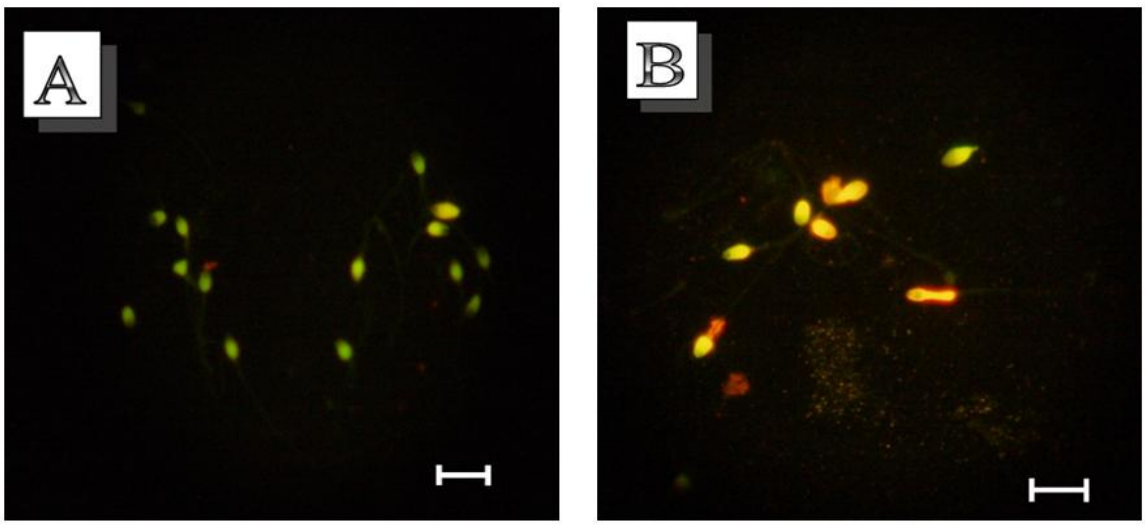

Figure 1. *A*: Representative patterns of sperm cells stained with the Acridine Orange fluorescence stain, demonstrates normal mature spermatozoa with native double-stranded DNA from fertile control donors. *B*: The green tinge which is quite discernible from the yellow-orange tinge of immature spermatozoa from infertile patients. Scale bar = 10 µ

Concerning the three major patient's subgroups, significant low levels of green AO fluorescent sperms were seen in asthenoteratozoospermic and oligoasthenoteratozoospermic patients with more lower percentages in the latter group (51.85± 22.50 and 41.49± 24.43; *P* = 0.0309 and 0.0003; respectively, vs. control donors) (Table 2). Interestingly, normozoospermic patients had also a significantly low percentage of green AO fluorescent sperms (52.08 ± 9.97; *P* = 0.0005) compared to control donors (Table 2).

TUNEL Assay: According to table 1, the percentage of TUNEL-positive cells with fragmented DNA was significantly more in infertile patients compared to controls (44.56 ± 9.41 vs. 24.47 ± 8.37; *P* = 0.00001). Characteristic patterns of TUNEL staining of spermatozoa is shown in figure 2, with apoptotic spermatozoa stained with brown color (Figure 2A), while faint violet was the stain of normal non apoptotic cells (Figure 2B).

As for the three major patient's subgroups, high percentages of TUNEL-positive spermatozoa was noticed against control donors in asthenoteratozoospermic, in addition to oligoasthenoteratozoo-spermic infertile patients (41.77±6.90; *P* = 0.00001; and 41.21±

14.84; *P* = 0.0002; respectively). Again for the normozoospermic infertile patients (idiopathic/ unexplained infertility); significant high levels of TUNEL-positive spermatozoa were observed vs. control donors (32.72 ± 6.97; *P* = 0.0069) (Table 2).

Table 2. Comparison of the results of the standard semen parameters, AO, and TUNEL assays between three major subgroups of infertile patients and healthy fertile volunteers

Standard Semen Parameters	Normozoo-spermia (n = 10)	Asthenoteratozoo-spermia (n = 12)	Oligoasthenoteratoz-oospermia (n = 18)
***Concentrat.* (M/ml)**	94.17 ± 46.70^{ns}	49.22 ± 11. 02†	38.89± 25.09†
Progressive motility* (%) *grades (a & b)	64.00 ± 10.75^{ns}	31.67± 25.61†	37.22± 30.93†
***Normal morphology* * (%)**	46.14 ± 11.07^{ns}	20.95± 7.76‡	31.61± 14.99†
Green AO fluorescent (%)	52.08 ± 9.97†	51.85± 22.50*	41.49± 24.43†
TUNEL positive cells (%)	32.72 ± 6.97**	41.77±6.90‡	41.21± 14.84†

* = p < 0.5, ** = P < 0.1, † = P < 0.0005, ‡ = P < 0.00001, * According to WHO criteria 1999, Probability against control donors.

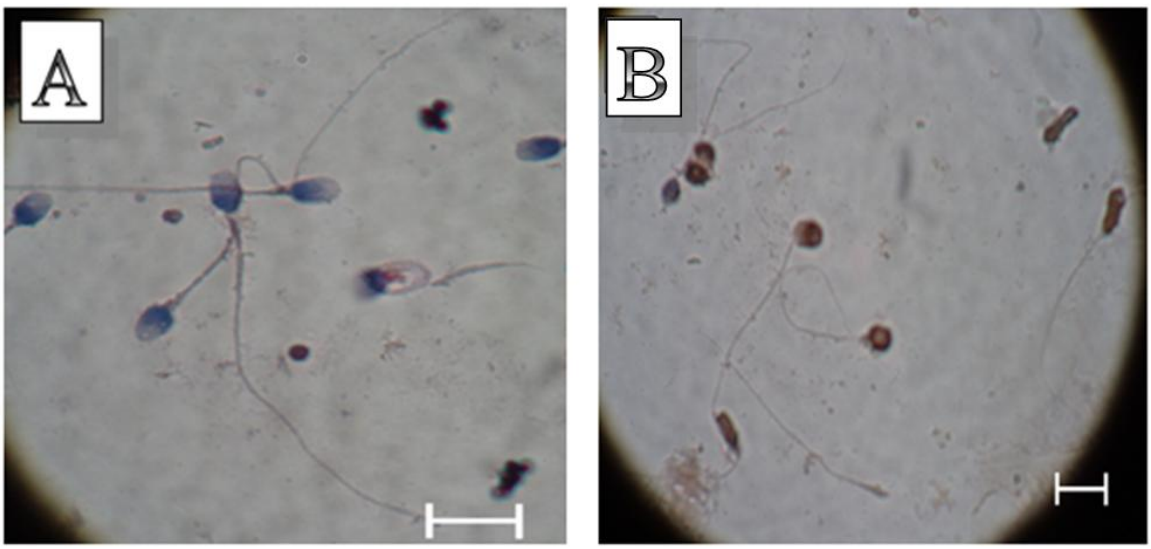

Figure 2. *A*: non apoptotic sperm cells processed using TUNEL assay with non fragmented DNA from fertile controls. Sperm heads are stained with faint violet of the counter stain Hematoxylin. *B*: TUNEL-positive apoptotic sperm cells with fragmented DNA from infertile patients stained with brown color in the head region. Scale bar = 10 µ

Correlations between Standard Semen Characteristics and the Results of AO and TUNEL Assays:

In all infertile patients, there was no significant correlation between standard semen characteristics and the results of AO and TUNEL assays. Nevertheless, there was a significant inverse correlation between the percentage of spermatozoa with normal native double-stranded DNA (green AO fluorescence) and DNA fragmentation (TUNEL-positive) (r = – 0.567, *P* = 0.0001).

Discussion

Sperm DNA contributes one half of the genomic material to the offspring and the integrity of sperm DNA is of crucial importance

for balanced transmission of genetic information to future generations [11].

The results of this study illustrate that infertile patients and their main subgroups had significantly lower percentage of mature spermatozoa that enclose normal native double-stranded DNA i.e. higher percentage of immature spermatozoa with denatured DNA, as compared to healthy donors (Tables 1 and 2). These findings are consistent with those of several other workers who had found that infertile men had a significantly higher mean percentage of sperm with DNA denaturation than fertile men [13,12]. Sperm DNA integrity can be assayed indirectly, based on the principle that damaged DNA denatures much faster than undamaged DNA when subjected to stresses such as heat and pH changes [11]. It has been reported that cells showing a higher DNA fluorochrome stainability compared to normal spermatozoa represent sperm cells which failed to tightly condense the chromatin during spermiogenesis; cells in which histones have not been replaced by protamines during spermiogenesis; or cells which contain endogenous DNA nicks, explaining associated chromatin packaging anomalies with infertility [14-16]. The human sperm DNA integrity as assessed microscopically by AO stain, although still controversial, has been widely used for evaluation of male infertility and pregnancy outcome in ART [11].

In this study, spermatozoa DNA quality was further examined using the TUNEL assay that measures percentage of spermatozoa with DNA fragmentation. Infertile patients, whether originally or after dividing them into major subgroups, had significantly more percentage of spermatozoa with fragmented DNA compared to controls (Tables 1 and 2). These data are coherent with those of other investigators [17,18]. Presence of DNA fragmentation as a late apoptotic marker in spermatozoa from infertile patients may indicate an apoptotic mechanism that might be interrupted 'aborted' at some stage of spermatogenesis causing the seminiferous tubule release of sperms with apoptotic markers, which is coherent with a previous study [5].

Data presented in this study on the interrelations between the studied parameters, shows a significant inverse correlation between the results of TUNEL assay and DNA integrity in AO fluorescence assay ($r = -0.5667$, $P = 0.0001$), compliant with previous studies [15]. Thus, a cause– effect relationship exists between apoptosis and DNA damage (assessed indirectly by AO test). However, no significant relationship was demonstrated in this work between DNA integrity and basic sperm parameters (data not shown), probably because of the small population of the studied groups. Nevertheless, reports on correlation of nuclear integrity with semen parameters have been somewhat inconsistent [11]. In different study populations, using different assays to measure DNA damage, some investigators found associations between some semen parameters and sperm DNA integrity whereas several others did not find associations [19]. Other researchers did not observe a close relationship between sperm DNA integrity and sperm morphology, fertilization rate, embryo development or pregnancy outcome for ICSI [1].

Interestingly, normozoospermic infertile patients (idiopathic) showed a significantly lower percentages of green AO fluorescent sperm cells (enclosing native normal double-stranded DNA), i.e a significantly higher levels of denatured DNA ($P = 0.0005$) compared to controls. Moreover, patients with normozoospermia demonstrate significantly higher percentage of spermatozoa with fragmented DNA (TUNEL-positive) as compared to control donors (Table 2). In at least 30% of cases classified as unexplained or idiopathic, repeated standard semen analyses of the male partner of an infertile couple reveal normal results [20]. Men with normal spermograms may still be infertile; the cause could be related to abnormal sperm DNA [19]. Varghese *et al*., found that approximately 30% of the patients with normal sperm parameters had DNA

normality varying from 1% to 70%. They stated that these high variations in DNA normality among normozoospermic patients may account for the incidence of unexplained infertility [11]. Therefore, it is possible that sperm DNA analysis may be better at discriminating between infertile and fertile men than standard analysis. This is in agreement with other studies which have suggested that sperm DNA damage analysis is an independent test of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters [21,22].

From the present study, it can be concluded that male infertility is associated with high rates of DNA damage in the spermatozoa, and that sperm DNA damage analysis may reveal a hidden abnormality of sperm nuclear DNA in infertile men classified as idiopathic, based on apparently normal standard sperm parameters. These findings are of great concern, particularly, in an era where advanced forms of assisted reproductive technologies are frequently used, bypassing the natural barriers to fertilization, with the inevitable risk that the use of DNA-damaged spermatozoa in ART will compromise the health of the progeny.

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Duration and Some Determinants of Interbirth Intervals in a Sample of Women from Baghdad/ Iraq

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Abstract

Background Understanding the practice of birth spacing and factors which influence women's interbirth interval (IBI) is critical for countries like Iraq with high fertility levels.

Objectives This study aimed at measuring the duration of the interval between births and determining some of the factors favored optimal spacing intervals of Iraqi women.

Methods A cross-sectional study was conducted among ever married and having at least 2 live births 472 women during March 2008 in Al-Kadhimyia Teaching hospital. Data were obtained by interview questionnaire, included birth intervals, demographics, and fertility variables. Actual birth interval was measured and data were analyzed using a logistic regression model.

Results The mean birth interval was 31.16 ± 21.56 ranged 9–228 months, and 65.6% of interbirth intervals were of less than 36 months. The multivariate regression revealed that older woman, a woman's husband higher education and having male children were the significant predictors of longer interbirth interval.

Conclusions Relatively short interbirth interval found in the present study may help national health program conveying the message of optimum birth interval, with expanding education and employment opportunities for women can act as a motive for child spacing.

Key words Pregnancy spacing interval, contributing factors, Iraqi women.

Introduction

Interbirth interval (IBI) is the length of time between two successive live births including the period of postpartum amenorrhea, the menstruating interval, and the following period of gestation [(1)]. Birth spacing (the practice of timing the period between births) has been identified as an important life saving measure for mothers and children. Previously, health professionals have advocated for a two year birth interval. However, groundbreaking new research showed that there is substantially more health benefit gained from lengthening the birth interval beyond the previously recommended two years to a three to five year birth interval. The new research showed there is an optimal interval for birth spacing- a period associated with the lowest risks for adverse health outcomes-and that optimal interval is three to five years [(2)].

Moreover, natural fertility depends on the duration of effective reproductive span and length of birth interval. Analysis of those factors influencing the span and those affecting the length of birth interval has proven useful, since in many cases they appear to vary quite

substantially across populations [3]. Thus, spacing of births through a deliberately prolonged interval between births and a delay in child bearing following marriage could be logical alternative strategies for fertility control [3,4].

There is a paucity of studies concerned with birth interval among Iraqi women, specifically lacking of adequate information on interbirth interval duration and its determinants, also little is known about the perception of Iraqi women regarding optimum birth spacing or their awareness of the advantages and disadvantages of long and short birth intervals. Such information would help planners and policymakers in developing strategies to encourage longer intervals between consecutive births that may ultimately decrease the number of children each woman has with subsequent beneficial effects on population and on the health status of the mother and her children.

Therefore, understanding the practice of birth interval and its determinants is helpful to design evidence based strategies for interventions. The objective of this study was to identify the duration and some determinants of interbirth interval among a sample of women attending Al-Kadhimyia Teaching Hospital from Baghdad, Iraq.

Methods

This cross-sectional study was conducted among female attendees to Al-Kadhimyia Teaching Hospital (third largest referral hospital in the capital Baghdad) during March 2008. All women eligible to participate in this study were interviewed after obtaining their informed consents.

Eligibility criteria were being ever married once only and having at least 2 live births. Women with history of infertility (primary or secondary) and those who were married more than once were excluded to avoid heterogeneity of interbirth intervals for the same woman.

Sample size was estimated based on the prevalence of contraception use reported by the Iraq Multiple Indicator Cluster Survey (MICS3), 2006 [5]. Among Baghdad population, the proportion of contraception users (p) was 0.53 and for non-users (q) was 0.47. The chosen degree of precision (d) was 0.045 at the 95% confidence interval. The total sample size was 472 women.

Data were collected using a specially designed questionnaire adapted from different literatures to obtain information regarding the participants' and their husbands socio-demographic profile (age, education, employment status), reproductive history and attitudes (age of child bearing, parity, history of abortion, and pregnancy outcomes including number, survival status and gender of children), and their perception of ideal age of child bearing, the ideal number of children for a family and the ideal interbirth interval.

The interbirth interval was calculated as the time (in months) between two consecutive birth dates of live births, and birth dates was used (rather than approximate dates of conception) for calculating the interbirth interval because this information was nearly 100 percent complete. Two types of intervals were omitted from our analysis: the interval between marriage and the first birth was excluded from the analysis because it is not an inter-birth interval, and the open interval between the last birth and the interview due to the problem of censoring, thus, only closed intervals were considered in this study.

Two data files were created: one for women interviewed and another for “interbirth

intervals". The absolute mean of the interbirth interval for all children each woman had was estimated as well as the mean interbirth interval for each participant.

Data entry and analysis were performed using SPSS for windows version 16.0 (SPSS Inc., Chicago, IL, USA, 2007). Analysis of variance (ANOVA) and Student's t- test was used to compare the differences in means whenever applicable. Estimates of crude risk ratio with 95% confidence interval were computed to measure the association between each determinant and less than 36 months IBI. Multiple logistic regression analysis was conducted for the adjustment of confounding variables to evaluate the association between interbirth interval and its determinants. Statistical significance of results was judged at the 5% level.

Results

This study included 472 ever-married women aged 16-60 years (mean ± standard deviation 36.74 ± 11.12 years). The mean age of first child bearing of the participants was 20.37 ± 4.80 years, as 51.1% (n = 238) were married before 20 years of age (almost always Iraqi women conceive after marriage). The mean age of their husbands was 41.89 ± 12.11 years. The mean years of education for the participants was 7.72 ± 4.91years (ranged 0-24) and for their husbands was 9.69 ± 4.96 years (ranged 0-22), with 83.3% (n= 388) of the studied women were housewives and 53.9% (n = 251) of their husbands were self-employed.

The women had on average 5 pregnancies (mean ± standard deviation 4.73 ± 2.52) and 4 deliveries (mean ± standard deviation 4.08 ± 2.07), and more than one third of the women (38.2%) reported having abortion.

The 472 enrolled women contributed to 1435 interbirth intervals with an absolute duration mean ± standard deviation of 31.16 ± 21.56 months (95% C.I. for the mean: 30.05–32.28 months) ranged between 9–228 months and only less than one quarter of them (n= 342) with interbirth interval of more than 36 months. Nearly three quarters of the participants (72.6%) had 1-3 interbirth intervals and only 3 women had eleven IBI (Figures 1 and 2).

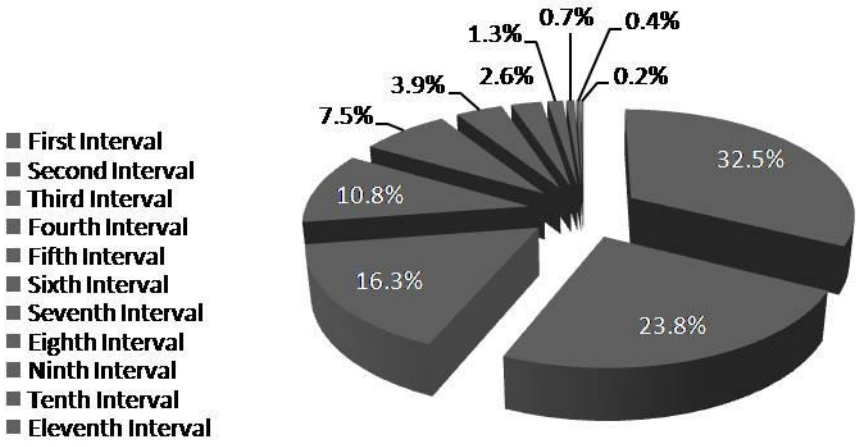

Figure 1. Distribution of 1435 IBI among the participated women

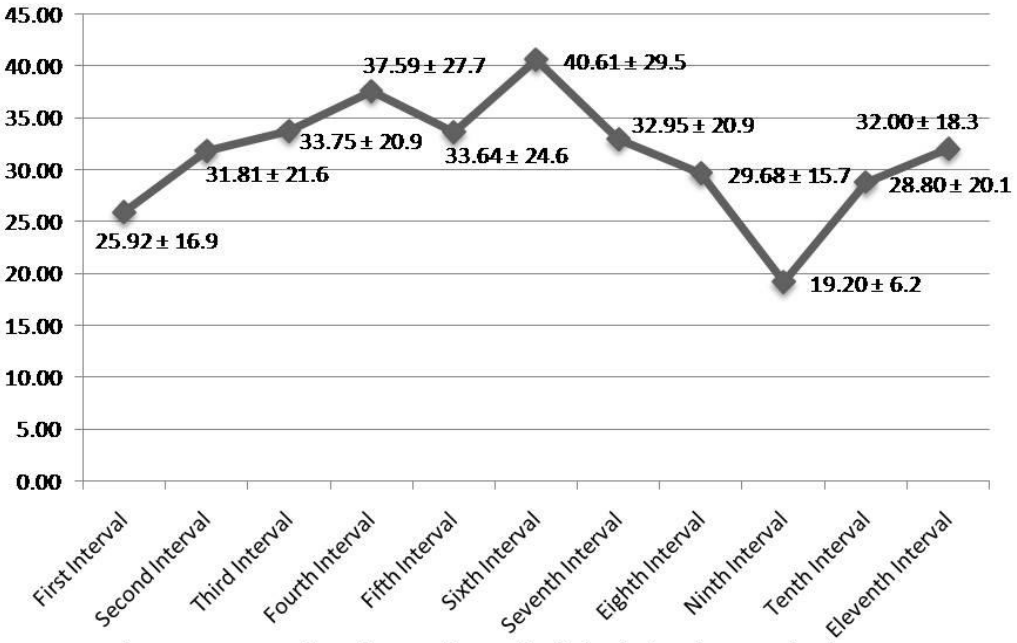

Figure 2. Mean duration and standard deviation in months for differet IBI

Women aged 35 years and older (constituted two thirds of the sample) were significantly spacing longer, with less risk for having IBI less than 36 months [risk ratio 0.87 (95% C.I.: 0.82–0.92)], however, both young and old mothers had mean IBI less than 36 months. On the other hand, the means IBI were nearly equal for women who conceived for the first time before or after the age of 20 years. Results also showed that older husbands (more than 40 years old) had significantly longer IBI than younger fathers with less risk of having IBI less than 36 months [risk ratio 0.88 (95% C.I.: 0.83–0.93)] (Table 1).

The study revealed that the educational level of both parents made significant difference in the duration of IBI being shorter for both illiterate mothers and fathers compared to highly educated parents [risk ratio 1.12 (95% C.I.: 1.01–1.24) for illiterate mothers] and [risk ratio 1.19 (95% C.I.: 1.07–1.31) for illiterate fathers] to have IBI more than 36 months. Nevertheless housewives had a mean IBI of 30.84 months compared to a mean of 33.29 months for governmental working participants with no statistically significant difference, while professional husbands had significantly longer IBI with 20% more probability to have IBI more than 36 months compared to governmental employee [risk ratio 1.20 (95% C.I.: 1.03–1.41)] (Table 1).

Four reproductive variables were considered in this study; analyzing the results of the 472 participants revealed that women having parity of 2 children had significantly shorter IBI as well as for women having equal number of male and female children compared to those having males more than females (male: female ratio >1) with about 20% more risk to have IBI less than 36 months [risk ratio 1.21 (95% C.I.: 1.08–1.36)] and [risk ratio 1.22 (95% C.I.: 1.06–1.42)] for both factors respectively, while neither having a history of abortion nor having a dead child made a difference in the duration of IBI (Table 2).

Table 1. The mean IBI of the sample according to some demographic factors.

Variable		No. (n=1435)	%	Mean IBI (months)	SD	(Significance)
Maternal Age (Yrs)	< 35	435	30.3	26.71	15.6	t = 5.19
	35 and more	1000	69.7	33.09	23.5	(P = 0.00001)
Age at Child Bearing (Yrs)	< 20	781	54.4	31.17	23.1	t = 0.01
	20 and more	654	45.6	31.16	19.6	(P = 0.99)
Husband's Age (Yrs)[a]	< 40	420	31.5	27.34	17.8	t = 4.33
	40 and more	913	68.5	32.86	23.2	(P = 0.00002)
Maternal Educational Level	Illiterate	338	23.6	28.02	18.2	F = 6.46 (P = 0.0001)
	Primary (1-6Yrs)	501	34.9	30.32	19.0	
	Secondary (7-12Yrs)	420	29.3	34.69	26.8	
	Higher (> 12Yrs)	176	12.2	31.17	19.4	
Husband's Educational Level[b]	Illiterate	167	12.2	28.32	19.4	F = 4.24 (P = 0.005)
	Primary (1-6Yrs)	364	26.6	29.40	19.7	
	Secondary (7-12Yrs)	536	39.3	31.48	21.9	
	Higher (> 12Yrs)	300	21.9	34.55	24.5	
Maternal Occupation	Housewife	1246	86.8	30.84	20.8	t = 1.46
	Governmental Employee	189	13.2	33.29	26.0	(P = 0.15)
Husband's Occupation	Non-employed	199	13.9	31.97	20.7	F = 2.71 (P = 0.04)
	Self-employed	782	54.5	30.76	20.7	
	Governmental Employee	360	25.0	30.09	21.4	
	Professional	94	6.6	36.91	29.4	

[a] 102 missing values

[b] 68 missing values

Table 2. The mean IBI of the sample according to some reproductive factors.

Variable		No. (n=472)	%	Mean IBI (months)	SD	(Significance)
Parity	2	124	26.3	27.02	16.9	F = 7.91 (P = 0.000)
	3-5	239	50.6	33.42	14.9	
	≥ 6	109	23.1	30.63	9.5	
Sex of Offspring's	Males Only	60	12.7	31.71	20.5	F = 3.65 (P = 0.006)
	Females Only	46	9.7	28.35	13.9	
	Male : Female Ratio = 1	100	21.2	27.16	13.5	
	Male : Female Ratio < 1	126	26.7	31.67	12.2	
	Male : Female Ratio > 1	140	29.7	34.00	14.5	
History of Abortion	Positive	181	38.3	32.13	13.2	t = 1.19
	Negative	291	61.7	30.47	15.6	(P = 0.23)
Survival Status of Children	Dead Child	43	9.1	28.22	10.8	t = 1.33
	Alive Child	429	90.9	31.37	15.1	(P = 0.18)

Multivariate logistic regression revealed that older woman, a woman with higher educated husband, and having males children only, as opposed to equal number of male and female children, were the significant predictors of longer interbirth interval. While shorter interbirth interval was independently predicted by older child bearing age, a woman with governmental employee husband, and the presence of a dead child in the family (Table 3).

Table 3. Multivariate logistic regression of the predictors of interbirth interval

Predictors		Coefficient	Hazard ratio	95% CI	P-value
Maternal Age (Yrs)	<35[a]				
	35 and more	- 1.187	0.305	0.136–0.684	0.0041
Age at Child Bearing (Yrs)	<20[a]				
	20 and more	0.732	2.080	1.185–3.649	0.011
Husband's Age (Yrs)	<40[a]				
	40 and more	-0.731	0.481	0.219–1.061	0.070
Maternal Educational Level	Illiterate[a]				
	Primary (1-6Yrs)	-0.529	0.589	0.234–1.481	0.261
	Secondary (7-12Yrs)	-0.867	0.420	0.161–1.094	0.076
	Higher (> 12Yrs)	-0.397	0.672	0.197–2.299	0.527
Husband's Educational Level	Illiterate[a]				
	Primary (1-6Yrs)	-0.678	0.507	0.169–1.526	0.227
	Secondary (7-12Yrs)	-0.750	0.472	0.158–1.410	0.179
	Higher (> 12Yrs)	-1.469	0.230	0.068–0.785	0.019
Maternal Occupation	Housewife[a]				
	Governmental Employee	-0.210	0.810	0.361–1.818	0.610
Husband's Occupation	Non-employed[a]				
	Self-employed	0.531	1.701	0.705–4.103	0.237
	Governmental Employee	1.091	2.976	1.142–7.758	0.026
	Professional	0.389	1.475	0.446–4.878	0.524
Parity	2[a]				
	3-5	-0.092	0.912	0.403–2.063	0.825
	≥ 6	0.606	1.833	0.635–5.290	0.263
Sex of Offspring's	Male : Female Ratio = 1[a]				
	Females Only	-0.264	0.768	0.269–2.192	0.621
	Males Only	-1.177	0.308	0.126–0.757	0.010
	Male : Female Ratio < 1	-0.408	0.665	0.289–1.532	0.338
	Male : Female Ratio > 1	-0.399	0.671	0.295–1.527	0.342
History of Abortion	Negative[a]				
	Positive	-0.363	0.696	0.414–1.168	0.170
Survival Status of Children	Alive Child[a]				
	Dead Child	1.810	6.112	1.668–22.401	0.006
Constant		2.992	19.922		0.00001

[a] Reference category

Finally, a comparison of the real parity, the real age of child bearing, and the mean IBI practiced by each woman was done with their knowledge regarding the ideal values of these variables. Results showed that no statistical significant difference found between the actual values and their knowledge about the ideal values, moreover, the results showed that 324 (78.6%) of the 412 respondents, reported that IBI should be 36 months or less (Table 4).

Table 4. The differences of the mean IBI between their knowledge about the ideal spacing norms and the actual values

Variable		No.	Mean IBI (months)	SD	Significance
Age of child bearing	Real values	472	20.37	4.8	t = 0.22
	Ideal values[a]	421	20.43	3.1	P = 0.83
Number of children	Real values	472	4.01	2.1	t = 0.83
	Ideal values[b]	441	3.90	1.9	P = 0.41
IBI	Real values	472	31.10	14.7	t = 1.14
	Ideal values[c]	412	32.23	14.6	P = 0.25

[a] 51 missing values, [b] 31 missing values, [c] 60 missing values

Discussion

For countries like Iraq with total fertility rate of 4.3% and the current use of contraception of currently married women or their husbands of these women is 49.8% [(5)] understanding practice of birth spacing and factors which influence women's interbirth interval is essential.

The mean interbirth interval was found to be 31.16 ± 21.56 months with a median of two years among the study population. This mean is close to mean duration reported from Al-Oyaynah in Saudi Arabia 31.2 ± 10.1 months [(6)], from Ethiopia 33 ± 16.7 months[1] and from Al-Khobar in Saudi Arabia 33.5 ± 17.8 months [(7)], shorter from that reported from Jordan 40.36 ± 0.8 months [(8)], Armenia 41.12±31.15 months [(9)], India 48.6 months [(10)], and Iran 61.0±25.7 months [(11)], but longer than the mean reported from Al-Taif in Saudi Arabia 28.56±14.88 months [(12)]. However, a median of two years found in the present analysis is 8 months shorter from the 32 months reported for the median birth interval in developing countries based on the Population Reports analysis of 55 countries with Demographic Health Survey (DHS) data [(13)].

Four explanations can be justified for this relatively short IBI; the first is that the open interval between the last birth and the interview date was excluded from this analysis as open intervals tend to be longer than closed intervals [(14)], and to support this fact, the mean duration of this excluded interval was found to be (87.71±79.21 months) making the mean IBI if they were included to be (44.46±49.03months), the second explanation is the poor knowledge of the participants regarding the ideal IBI as our results showed that the participants believed that the ideal IBI is about 32 months which was very close to what they actually practice, the third explanation is that Iraqi women like other women in developing countries use long-term contraceptive methods for limiting births more commonly than using short-term methods for spacing [(13)], and the last reason appears to be the decline of traditional practices that contribute to longer birth intervals such as prolonged breastfeeding as the results of a recent study interviewed 2008 mothers from Baghdad /Iraq 1281 (63.8%) were using bottle feeding for their children whom age ranged between 0 and 24 months [(15)].

The study also found that 65.6% of IBI were of less than 36 months, and this is one of the high percents reported and close to the rates

reported by the Population Reports from 9 out of the 55 countries of Demographic Health Survey (DHS) data analysis, including: Zambia 64%, Eritrea 65%, Central African Rep. 66%, Chad 66%, Haiti 66%, Mozambique 66%, Paraguay 66%, Philippine 66%, and Rwanda 66% [13].

A variety of factors influence a woman's birth spacing, some of which are rooted in social and cultural norms, others in the reproductive histories and behaviors of individual women, utilization of reproductive health services and other background factors [14].

Eleven explanatory variables for birth spacing have been selected for evaluation in this paper. These variables are parents' age, age at child bearing, parents' education, parents' occupation, parity, history of abortion, survival status of the children, and the sex of the children.

Many studies found that older mothers tend to have longer interbirth intervals. This could be due to two reasons: older women are later in their childbearing process and are likely to have achieved their desired family size and hence likely to have long subsequent spacing; they are also likely to be less fertile leading to long spacing [1,4,6-10,14,16,17]. Our findings go in consistent with these studies and a significant negative impact of maternal age on short IBI was found. Also older women at child bearing age tend to have shorter IBI to catch up time to achieve the family size desired before losing fecundity and this may explain why age at child bearing had a significant positive correlation with short IBI on the regression analysis in spite of the absence of a sizeable difference in IBI between those under 20 and those in their 20s and older at child bearing [1,3,10].

Albeit the present findings showed that paternal age had significant impact on the duration of IBI, but it failed short of statistical significance in the regression analysis. However, older fathers like older mothers tend to have longer IBI because they have achieved their desired family size and try to persuade their wives to use long acting birth spacing methods.

Education is considered to be one of the most important socio economic factors having an indirect influence on birth interval length through its impact on one or more of the bio-behavioral variables. Sometimes better educated women compress child bearing into fewer years to participate in non child bearing activities and hence have shorter birth intervals than less educated [1,14]. While in 38 of 51 countries with DHS data, women with no education were more likely than educated women to have shorter intervals [16]. Also several other studies found that less educated women had shorter birth intervals than more educated ones [1,4,8,10]. In the current study, not only maternal education, but both parents higher education was significantly associated with longer IBI, a finding reported also by studies from Saudi Arabia [7,12]. Higher educational attainment improves a person's status, gives more decision-making power, lives in urban regions, and provides better employment opportunities, beside the use of contraception resulting in an increase in the spaces between births [6,12,16]. However, the occupation of the husbands and not that of the participants contributed for longer IBI in this study as professionals (physicians, engineers, school teachers, and lawyers) had about 7 months longer IBI than governmental employees (clerks and officers).

In most countries women with low parity have shorter birth intervals than women with more children, but in a few countries the reverse is

true [16]. Results of the current study showed U-shaped trend with significantly shorter IBI for women with 2 children or more than 5 children and longer intervals for those who have 3-5 children as the majority of Iraqi women prefer to give birth to their first children in quick succession resulting in significantly shorter early birth intervals, then birth intervals increased steadily with the increase in the number of surviving children, however, more fecund women who conceive easily and quickly are also those who are more likely to have more children with shorter IBI. Similar observations have been reported [14].

On the other hand, our results showed that child death had a positive impact on short IBI in the regression analysis; yet, it failed to show significant difference in the duration of IBI. This can be explained either by planning of the parents for a new pregnancy to replace a lost child, or due to the death of a child cuts short nursing durations which results in earlier resumption of menses and ovulation. This goes in agreement with findings from 55 countries surveyed by DHS between 1990 and 2001 [16], and studies from different areas [1,3,4,.7,10,14].

Among Iraqis preference for sons dominates, and couples who prefer son tend to have their next child soon after the birth of a daughter. Lack of sons among this study participants reduced the interbirth interval by an average of 6 months, after which IBI became considerably longer once women reached the desired balance of sons and daughter, thus, the sex of children was found to be another determinant factor for short birth spacing in the present regression analysis This finding is reported also in studies conducted in different places [1,3,7,8,10,12,17].

One last striking finding from our data showed that the participants had poor knowledge about the optimal birth spacing interval as only 44.7% of them (if we add up those reporting 36 months as ideal IBI) were knowledgeable mothers about birth spacing compared to 49.4% of Jordanian women reported an ideal spacing of 3 or more years [8], 60% knowledgeable mothers from Mozambique [14], and 66.6% of Saudi women preferred ≥ 3 years intervals [7].

Iraq is a country with poor reproductive health situation (high infant mortality 42 per 1000 live-births, child mortality 59 per 1000 children and maternal mortality 84 per 100000 live-births) [5]. This provides a rationale for focusing on Optimal Birth Spacing Initiative (OBSI) to be one of several interventions undertaken to promote the health and survival of women, infants and children, particularly few countries have policies and norms on birth spacing, and in Iraq as in many developing countries the need for birth spacing services is not being met.

This research is significant because no review specifically looking at interbirth interval and its determinants for Iraqi women was identified and it may provide a baseline as well as scientific endeavor to the future researchers working on this crucial area of human research.

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Goserelin acetate for recurrent endometriosis

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Abstract

Background Endometriosis is a chronic and recurrent disease characterized by the presence and proliferation of functional endometrial glands and stroma outside the uterine cavity. GnRH analogues are currently one of the most widely used medical therapies for endometriosis.

Objective This study assesses the role of zoladex (goserelin acetate depot) for patients with recurrent endometriosis after surgical treatment.

Methods A descriptive follow up study of 20 women with recurrent endometriosis after surgery were arranged to receive Goserelin acetate (Zoladex) one month depot administered subcutaneously monthly for a period of six months followed by one year follow up in AL- Karama Teaching Hospital -Wasit Governorate/ Iraq from May 2008 until May 2010.

Results Ninety percent of patients showed symptomatic improvement, ultrasound improvement achieved in 80%, recurrence rate of 20% within one year after stopping treatment and none of the patients required surgical interference during the period of the study.

Conclusions Our results support the beneficial role of Goserelin acetate (3.6 mg) month depot administered subcutaneously in the treatment of recurrent endometriosis after surgery.

Keywords Endometriosis, GnRH analogues, Goserelin acetate

Introduction

Endometriosis is a chronic and recurrent disease characterized by the presence and proliferation of functional endometrial glands and stroma outside the uterine cavity .It is estimated to occur in up to 10% of women of reproductive age [(1)]. Although endometriosis is seen primarily among women of reproductive age, this disease also can affect post-menopausal women and adolescents especially adolescents with uterine abnormalities, in particular, women with Müllerian anomalies resulting in outflow obstruction (increasing retrograde menstrual flow), as well as women with prolonged menstruation and shorter cycles (27 days or less) [(1)]. However, retrograde menstruation can be observed in up to 90% of women, suggesting the involvement of additional factors in the implantation and growth of endometriotic lesions in women [(2)].

Susceptibility to endometriosis is thought to depend on the complex interaction of genetic, immunologic, hormonal and environmental factors [(3)]. Endometriosis appears to be a multifactorial genetic disorder, in which allelic variants of many genes (including cancer susceptibility genes and genes coding for cytochrome P450 enzymes, nuclear receptors and immunologic mediators) can predispose women to develop endometriosis, depending on environmental conditions [(3)]. The growth of endometriotic lesions is also estrogen dependent, with lesions becoming inactive and gradually undergoing regression during states of ovarian down-regulation, such as amenorrhoea or menopause [(4)].

Pelvic pain associated with endometriosis generally is cyclical, the pain may become continuous as the disease worsens. The most common symptoms of endometriosis are dysmenorrhoea, dyspareunia and chronic non-menstrual pain. If endometriotic lesions affect the bladder or rectum, pain may also occur during micturition or defecation [5].

Infertility is a problem for many women with this disorder, the mechanisms of endometriosis-associated infertility still are not completely understood, generally, it is agreed that the most advanced stages of endometriosis are strongly correlated with infertility, particularly if pelvic adhesions distort normal pelvic anatomy and impair tubo-ovarian function [6].

Endometriosis also negatively impacts women's quality of life. A decreased quality of life may result not only from the symptoms of pelvic pain and infertility but also from the effects of various medical and surgical treatments [7].

Diagnosis of endometriosis can be difficult, given the non-specific nature of many of its symptoms, the common occurrence of pelvic pain in women without endometriosis and the considerable overlap with other conditions (e.g. pelvic inflammatory disease or irritable bowel syndrome) For this reason, a diagnosis can be confirmed only by a surgical procedure (generally laparoscopy) to excise and histologically evaluate disease implants [5,8].

In addition to relieving pain, the goals of treatment for patients with endometriosis are to prevent or delay disease progression by reducing endometriotic implants through surgical treatment or medically induced atrophy of the implants [8]. Neither medical nor surgical treatments have been proven to improve fertility rates and because of the chronic nature of this disease, long-term or repeated courses of medical therapy are required to control these symptoms [9].

Currently available medical therapies for endometriosis act by attempting to mimic periods during which a woman does not menstruate: menopause (GnRH analogues), amenorrhoea (chronic anovulation with danazol) or pregnancy [oral contraceptives (Ocs) or progestins] [5].

GnRH analogues are currently one of the most widely used medical therapies for endometriosis. These agents induce medical menopause by down-regulating hypothalamic-pituitary GnRH receptors, thus causing decreased gonadotropin secretion, suppression of ovulation and reduced serum estrogen levels. Several GnRH analogues used for the treatment of endometriosis include nafarelin, buserelin, histrelin, goserelin, triptorelin and leuprolide [10].

Methods

A descriptive follow up study performed in AL-Karama Teaching Hospital -Wasit Governorate/ Iraq from May 2008 until May 2010. The study included 20 women already diagnosed with endometriosis (endometrioma) in whom the diagnosis was confirmed by histopathological study after surgical exploration and presented with recurrence after surgery.

Recurrence defined as recurrence of symptoms (dysmenorrhea, dysparunea, pelvic pain or indurations on examination and complex ovarian cyst identified by ultrasonic examination (Semen's ultrasound machine) within a year of follow up. Exclusion criteria included patients who received any hormonal treatment for endometriosis postoperatively or lost their follow up during the period of treatment and follow up. All patients gave signed informed consent before inclusion in this study and arranged to receive Goserelin acetate (Zoladex) depot injection (3.6 mg) subcutaneously in the lower abdomen every 28 days for 6 cycles [11] and each patient had at least 1 year follow up after treatment. Follow up included monthly abdominal and pelvic examination and ultrasound examination.

Results

Twenty patients with recurrent endometriosis included in this study, their age ranged

between 30 and 42 years (mean 34), and parity (0-1). Symptomatic improvement where noticed in 18 patients (90%); 12 of them (60%) after the second injection. However the improvement on abdominal and pelvic examinations were noticed in 15 patients (75%) during the same period and raised to 85% (17 patients) after finishing the course of treatment (Table 1).

Table 1. The distribution of patients according to symptomatic improvement

Improvement	Number	Percentage
Improved	18	90%
Not improved	2	10%
Total	20	100%

Ultrasound examination showed complete resolution of endometrioma in 16 patients (80%) and improvement in ultrasonic features in the remaining 4 patients (20%); (Table 2).

Table 2. The distribution of patients according to radiological changes

Radiological changes	Number	Percentage
Total resolution	16	80%
Improvement	4	20%
Total	20	100%

Recurrence after stopping the treatment was recorded in 2 patients (10%) within the first 6 months and 2 patients (10 %) in the second half of the year (Table 3).

Table 3. The distribution of patients according to recurrence

The recurrence	Number	Percentage
Recurrent endometriosis	4	20 %
No recurrence	16	80%
Total	20	100 %

Side effects were recorded during the period of treatment including hot flushes in 12 patients (60%), sweating in 5 patients (40%), and bone pain in 4 patients (20%), a reduction in breast size in 4 patients (20%); (Table 4).

Table 4. Side effects recorded during the period of treatment

Side effect	Number	Percentage
Hot flashes	12	60%
Sweating	5	40%
Bone pain	4	20%
Decrease in breast size	4	20%

Pregnancy was recorded in 3 patients (15%) after finishing the treatment; however only one of them succeeded to finish the pregnancy and result in a live birth rate of (5%).

Discussion

Endometriosis is a common debilitating disease occurring in 1–5% of premenopausal women with a prevalence of 38.5% in infertile women and 5.2% in fertile women [12]. The medical management of endometriosis depends on the stage of the disease, the severity of symptoms, the age of the patient, and the future fertility intentions. The most widely utilized treatment modalities are expectant management, surgery, induction of a pseudopregnancy state with hormonal therapy, and induction of a pseudomenopausal state [13]. Six months of GnRH agonist therapy immediately following surgery reduces the rate of symptom recurrence, and increases the length of time before symptoms recur. It is also more effective in managing endometriosis-related pain after surgery than using oral contraceptives in the same way. The benefits may be particularly relevant for women with active peritoneal disease [11, 14].

In this study 20 women with recurrent endometriosis after surgery were arranged to receive Goserelin acetate depot injection each 28 days for six months, symptomatic improvement achieved in 90%, and radiological improvement in 80%, 20 % recurrence and 15% pregnancy rate. In the study of Reichel and Schweppe [15] the total subjective score and total pelvic symptom score showed a reduction by 86% and 93%, respectively, 54% of the patients showed a reduction of implants and adhesions by at least 50% or more, and 31.5% had a complete resolution of visible deposits. The mean reduction of implants and adhesions was 50%, 72% respectively. Twenty of 64 (31.3%) previously infertile patients successfully conceived within 12 months after discontinuation of the therapy [15]. Shaw [16]; concluded that the monthly administered 3.6-mg depot preparation of goserelin was highly effective at inducing resolution of endometriotic implants and relieving the symptoms of endometriosis with prevention of their return during 24 weeks follow-up in the majority of patients. However, results were not significantly different from those achieved with danazol 600 mg/d [16].

While in the study of Soysal et al [17] six months of treatment with anastrozole and goserelin as compared to goserelin alone increased the pain-free interval and decreased symptom and recurrence rates in patients following surgery for severe endometriosis. Furthermore, menopausal quality of life at 2 years after medical therapy remained unaffected [17].

However, adverse effects reported by women receiving GnRH agonist treatment are those of secondary hypogonadism (hot flushes, sweating, vaginal dryness, etc..). Furthermore, this treatment induces a dramatic decrease in bone mineral density (BMD) higher than in the early months of natural menopause, reaching 4-5% at the lumbar spine in 6 months, this bone loss has been found to be reversible in most studies. These adverse effects limit the duration of treatment in diseases that are chronic or recurrent by nature [18].

Side effects were recorded during the period of treatment including hot flushes in (60%), sweating (40%), bone pain in 4 patients (25%), decrease in breast size in (20%) otherwise no other serious side effects occurred. In the study of Fernandez et al [14], the incidence of adverse events was 97.4% with a mean number of 8.3 adverse events per patient; the main reported side effects were hot flushes and headaches, the adverse events resulted in discontinuation of the study for 10 patients, among the main side effects were one case of sciatalgia, one case of asthenia, and two psychotic depression events in the same patient [14].

Conclusions

Our results support the beneficial role of Goserelin acetate 3.6 mg depot injection each 28 days for 6 months in the treatment of recurrent endometriosis after surgery.

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Microspectrophotometric Quantification of the Skeletal Muscle Glycogen Contents with Aging

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Abstract

Background Skeletal muscle fibers contain about 2% of its weight is glycogen, this glycogen used to keep the muscle functioning if it fails to receive sufficient oxygen. PAS stain is useful in detecting cytoplasmic accumulation of glycogen. Glycogen had been studied in skeletal muscles under various state of muscle activities and nutritional states but glycogen quantification with aging is not clearly defined till now.

Objectives Quantification of the mean glycogen concentration in skeletal muscles fibers stained with PAS stain in various age groups by microspectrophotometry.

Methods The tibialis anterior muscle of 20 Albino male rats (rattus rattus norvegious) of neonate, 3, 6, 9, 12, and 18- months were selected. Paraffin blocks were performed, sectioned and stained with PAS stain.
Analysis of the PAS stained sections by microspectrophotometry at 510 nm wave length. For the test group, mean absorbance, standard deviation, maximum, minimum, and mode values were estimated and compared with the control groups.

Results A significant difference in PAS absorbtion between test and control groups, and among different age groups, being increased with age.

Conclusion The variation in PAS absorbtion with aging indicates that the glycogen content in skeletal muscle increase with aging, this could be due to the influence of age on skeletal muscle glucose transport and glycogen metabolism.

Key words Skeletal muscle, PAS, Glycogen, Microspectrophotometry

Introduction

Muscle glycogen is an important fuel during exercise and its depletion is used as an indicator of fiber recruitment pattern during various types of exercises [(1)].

Although data related to muscle glycogen are interpreted as showing it is homogenous when quantified biochemically [(2)]. It is now well recognized that glycogen exists as individual particles located in distinct subcellular locations [(3-5)]. It's found in the sarcoplasm in form of coarse granules that seen in Electron microscope [(6-7)].

Glycogen in skeletal muscle can be demonstrated in paraffin sections by the periodic acid-Schiff (PAS) reaction [(8)], but conflicting reports exist concerning its validity as a quantitative test for polysaccharides [(9)].

A linear correlation between photometrically measured absorptions of PAS-stained tissue (paraffin embedded endometrium) and the

chemically measured glycogen content was reported and high correlation has been reported between glycogen concentration measured by microphotometry of single fibers of PAS stained cryostat sections and concentrations determined by biochemical analysis of human muscles [(10)].

In 1979 Halkjaer and Ingemann [(11)] described identical concentration of PAS (colour) in histological sections from the same human muscle irrespective of the type of preparation either cryostat or paraffin-embedded sections. The histochemical assessment of the glycogen content in various types of muscle fibers is usually carried out by subjective rating of the intensity of the PAS staining as described for animals [(12)], the subjective rating has been extensively used for human muscles [(13)].

Periodic acid-Schiff (PAS) stain when preceded with α-amylase treatment this will digest glycogen into smaller units that would be washed away during tissue processing and by comparing a slide stained by PAS technique with and without diastase digestion could reveal the amount of glycogen present in tissue [(11)].

However the glycogen concentration in skeletal muscle aging has not been thoroughly investigated. This study aim to demonstrate the effect of aging on glycogen concentration in PAS stained skeletal muscle sections by using microspectrophotometry.

Methods

- ***Animal sampling:***

A sample of twenty albino rats (Rattus norvegieus) male was selected, with different age: neonate (25 day), 3, 6, 9, 12, 18 month. Scarified animals killed with chloroform. Tibialis anterior muscle was selected for the study it taken out and divided into two halves and fixed in Bouins fixative.

- ***General histological preparation:***

Paraffin blocks were prepared and sectioned at 10µm thickness by Reichert-jung 3030-Biocutmicrotome.

- ***PAS –stain: (11).***

The paraffin sections were deparaffinized by xylene, rehydrated in descending concentration of ethanol alcohol 99.9%, 96%, 70% for 3minutes in each concentration, sections oxidized in 1% periodic acid for 5minutes, then treated with Schiff reagent for 10 minutes at 38°C, dehydrated in ascending ethanol alcohol 70%, 96% and 99.9% (3 minutes) for each concentration, cleared in xylene and mounted in Eukitt.

Control sections were preincubated in1% amylase (30 minutes, at 37°) before oxidation in periodic acid.

- ***Quantification of PAS staining intensity***:

Using microspectrophotometry, PAS staining intensity was expressed as absorbance and measured by spot measurement with a circular measuring diaphragm using Reichert-jung microspectro-photometer. Serial sections of each age group were scanned and 50 values recorded for each age group.

The wave length of the transmitted light was 510 nm selected as (Isobestic wave length), corresponding to the maximum absorption of the PAS-positive material [(11)].

The absorbance of each section measured by sum of absorbances of its fibers (cells), calculation of the mean of absorbance, standard deviation for both test and control groups.

In test group the maximum, minimum values of PAS stain absorption and mode (most frequent absorbance value) are calculated for each age group.

For the test group analysis of varience done by ANOVA (single factor) test to analyze the varience among the different age groups. Tukey's test which is usually refered to as HSD (honestly significant difference) used as a multiple comparison test that make use of a single value against which all differences in means are compared this value called HSD is given by:

HSD=qα, k, N-k√MSE/n

Where α is a chosen level of significant, k: number of means in the experiment, N: the total number of observation in the experiment, n: the number of observation in the treatment, MSE: is the error mean square from the ANOVA table, q: obtained by entering appendix table K with α, k, and N-k [14].

Technical setting of reichert-jung microspectrophotometry [15]:

It contains three units:

1. Voltage stabilizer unit by reichert-jung this should kept at 12 voltages through out the process of measurement.
2. Power unit.
3. control unit: data entry needed at this unit as:

A. Mode: extinction (optical density).
B. Isobestic wave length:510nm
C. Display rate :5 sec^{-1}
D. Damping rate: 10000 msec
E. Objective lenss:10X
F. Magnichanger of polyvar microscope:1.25X
G. Measuring diaphragm 10µm (numeric value 4) selected from the polyvar microscope.

Measurements of the control group:

Control group include sections from same age groups that treated with 1% amylase digestion prior to the step of Schiff reagent in the staining procedure, these slides used to detect the percentage of glycogen that lost by amylase digestion in each age group compared to test group. Student t-test (two samples with unequal varience) used to compare the difference in the mean absorption of skeletal muscle for the PAS stain between the test and control groups.

Results

PAS stain the skeletal muscle sarcoplasm homogenously pink in color not showing the myonuclei since in this procedure the Haematoxylin stain not used as a counter stain. It shows the connective tissue compartments of the muscle tissue: epimysium, permysium, and endomysium with the neurovascular bundle in the epimysium (Figures 1-3).

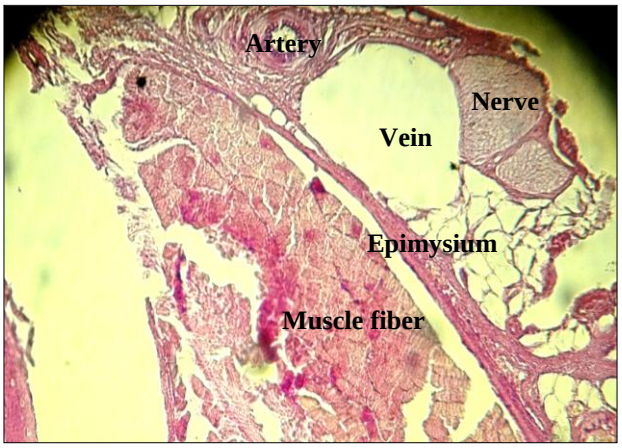

Figure 1. Section in neonate tibialis anterior shows the connective tissue covering (the epimysium) and neurovascular bundle (artery, vein and nerve). (40X)

The muscle fibers show various shades of pink color in same age group in single section indicating various rates of PAS absorbtion in different fiber groups (Figure 2).

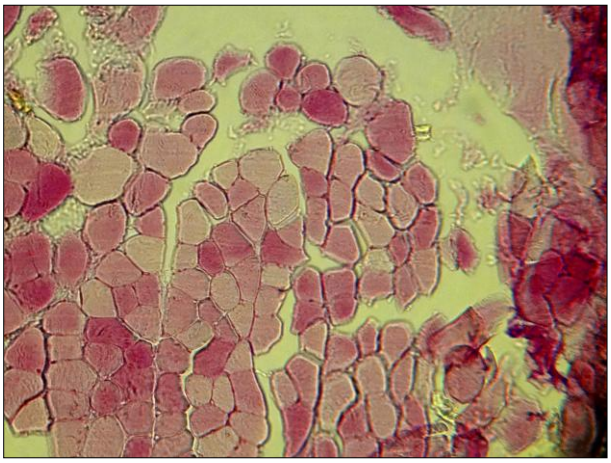

Figure 2. Section in tibialis anterior of 12-month showing fibers with homogenously stained sarcoplasm without myonuclei with various intensities of PAS stain in different fibers. (400X)

The absorbance of PAS stained material was determined by spot measurements and area scanning by systematic scanning of a fifty areas in each age group with the aid of illuminated

measuring diaphragm of the microspectro-photometry, the mean absorbance is considered the best representative for whole age group absorbance.

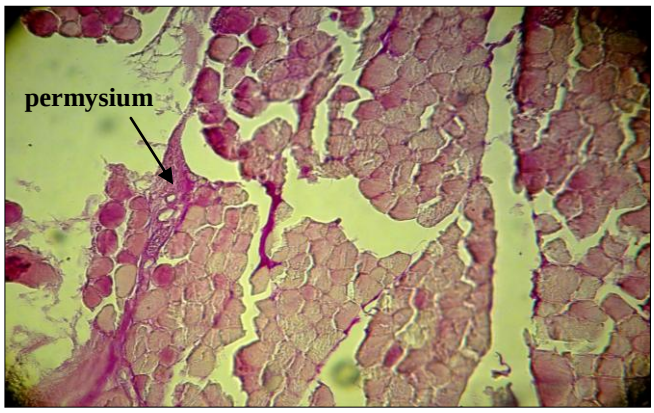

Figure 3. Section in tibialis anterior of 6-month showing the permysium of the skeletal muscle and muscle fibers without myonuclei. (400X)

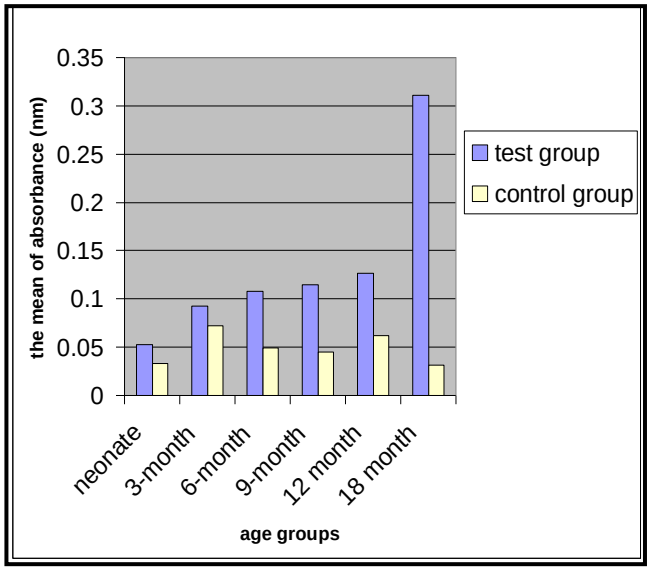

Figure 4. The mean absorbance of PAS stain (nm) measured by microspectrophotometry in test and control group for the six age groups.

The mean absorbance of control groups that treated with 1% amylase showed marked reduction from the mean of absorbance of test groups in all age groups, as shown in table 1 and figure 4.

Student t-test show statistically significant P – value: since t statistic > t critical table 3 indicating that a significant difference in PAS absorption found between test and control groups of the different age groups.

The timing and tissue thickness were fixed throughout the procedure; and all slides were stained in single container of Schiff reagent.

The mean absorbance of test groups increased with aging being in its lowest value in neonate age group and increased gradually reaching its highest value in 18- month age group (Figure 4 and table 1).

Maximum value of absorbance of PAS stain in a single spot were seen in 18- month age group, followed by 3-month age group, then 12-month age group, then 9-month age group, and lowest in neonate age group (Table 2).

Minimum value of PAS stain absorbance in single spot was recorded in 9-month age group, then in neonate, 3-month, 6-month, 12-month and 18-month consequently (Table 2).

The mode (most frequent observation) of absorbance of PAS in single spot show similar values in neonate and 18-month age group, and close values in 9-month and 6-month age groups (Table 2).

The analysis of varience among the age groups of the test showing a significant difference in PAS absorbance among the different age group, since F (calculated) **> F critical Table (4).**

Tukey's test used to demonstrate which age groups contribute to the significant overall p - value. In table 5, all age groups show a significant HSD value, in other words all groups contributed to the significant p-value.

Table 1. The mean absorbance of PAS-stain ± standard deviation measured in control and test samples in each age group.

Age groups	Mean value of maximum absorbance of test and control groups in fifty areas in each age group±SD (nanometer)	
	Test	Control
Neonate	0.05236±0.073119	0.033±0.02372
3-month	0.09296±0.049865	0.07208±0.097552046
6-month	0.10786±0.140265202	0.04926±0.033557
9-month	0.1144±0.146127	0.0448±0.02629
12-month	0.1265±0.181438	0.06198±0.017689
18-month	0.31056±0.086654	0.03178±0.017197

Table 2. The maximum, minimum and mode of PAS absorbance values in a single spot in each age group. (Nanometer)

PAS absorbance	Neonate	3-month	6-month	9-month	12-month	18-month
Maximum	0.54	0.198	0.86	0.77	0.99	0.455
Minimum	0.006	0.01	0.019	0.005	0.032	0.088
Mode	0.033	0.08	0.095	0.093	0.06	0.333

Table 3. t- Test for the comparison between the mean absorbance of the test and control groups.

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	Variable 2
Mean	0.134107	0.048816667
Variance	0.008129	0.00025424
Observations	6	6
Hypothesized Mean Difference	0	
df	5	
t Stat	2.281709	
P (T<=t) one-tail	0.035693	
t Critical one-tail	2.015048	
P (T<=t) two-tail	0.071385	
t Critical two-tail	2.570582	

Table 4. ANOVA single factor test for the different age groups

SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Neonate	**50**	2.618	0.05236	0.005346		
3-month	**50**	4.648	0.09296	0.002486		
6-month	**50**	5.393	0.10786	0.019674		
9-month	**50**	5.72	0.1144	0.021353		
12-month	**50**	6.325	0.1265	0.03292		
18-month	**50**	15.528	0.31056	0.007509		

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2.032322	5	0.406464	27.31351	1.09E-22	2.244703
Within Groups	4.375144	294	0.014881			
Total	6.407467	299				

Table 5. Demonstrate the significant HSD for the PAS absorption for each age group versus other groups.

Compared age groups	Difference in means	df	HSD	Significance
Neonate/3month	0.0406			significant
Neonate/6 month	0.0555			significant
Neonate/9 month	0.06204			significant
Neonate/12 month	0.074			significant
Neonate/18 month	0.2585			significant
3 month/6 month	0.0149			significant
3month/9 month	0.02144			significant
3 month/12 month	0.0339	5	1.3596	Significant
3 month/18 month	0.2176			Significant
6 month/9 month	0.00654			Significant
6 month/12 month	0.01864			Significant
6 month/18 month	0.2027			Significant
9month/12 month	0.0121			Significant
9 month/18 month	0.019616			Significant
12 month/18 month	0.18406			Significant

Discussion

PAS stain oxidize the hydroxyl (-OH) group that found in glycogen to aldehyde group (-CHO) by the periodic acid, this aldehyde group will react with the Schiff reagent to form the red-purple product, so PAS reaction is useful in detecting intracellular glycogen in skeletal muscle fibers. Haematoxylin stain is usually used as counter stain but not in this work [16].

The validity of PAS stain for assaying glycogen muscle contents was confirmed by a good correlation between the biochemical determined concentration in a whole muscle biopsy and the

mean concentration calculated from the area fractions for each principal fiber, and a high correlation was found between area scanning and spot measurements [10].

The absolute requirements for the use of PAS stain as a mean of obtaining quantitative expression of glycogen concentration must ensure that only glycogen in the tissue is stained and that's all glycogen or a constant fraction of the glycogen is present [17-18] .

These requirements have been fulfilled since most of the PAS positive material inside the skeletal muscle fibers is glycogen that removed selectively with diastase digestion (α-amylase) digest glycogen into smaller units that would be washed away during processing and by comparing a slide stained by PAS technique with and without diastase digestion could reveal the amount of glycogen [11].

The differences in the mean absorbtion of PAS stain between the test and control samples shown in table 1 and table 3 caused by incubation of control sections in 1% amylase caused a reduction in the absorbance, which indicate that glycogen alone is responsible for the intracellular absorbance of PAS stained material [11].

Thickness of sections were fixed at 10µm and the timing of staining in periodic acid also fixed although no statistical differences was found between the PAS staining intensity (absorbance/thickness) in relation to either to change in thickness or the time of oxidation in periodic acid . This indicates that the oxidation in periodic acid for glycosyl of glycogen has reached a constant level during the first 5 minutes [11].

The differences in the activity of different Schiff reagents is another important factor which might seriously affect the PAS staining intensity this may be due to variation in the manufacture of the reagent or the way of its storage, so its important there for sections that to be compared are stained in same histochemical bath [11].

The variation of PAS stain absorption with aging could be due to the influence of age on skeletal muscle glucose transport and glycogen metabolism. There is an age-related alteration in skeletal muscle carbohydrate metabolism; muscle glycogenolysis is accelerated in old male rats compared with young animals, perhaps secondary to the age-related reduction in muscle oxidative capacity and blood flow [19].

In a study of perioral muscle specimens, lipid pigment (Lipofuschin) granules were present in 68.5% of the cases. These granules were PAS positive, they also stained with other stains :stained black or brown with the Masson-Fontana procedure, black with Sudan black and strong purple-pink with Ziehl-Neelsen staining; yellow auto fluorescence was emitted in ultraviolet light. Statistical analyses indicated a direct correlation between increase in quantity and distribution of the pigment and increase in age in both males and females [20].

Variation in minimum, maximum, and mode values of the test group in different age groups may be due to the type of muscle fiber on the spot on which the measuring diaphragm placed. In this study tibialis anterior muscle was selected since its mixed type of muscle with equal proportion of type I and type II fibers since the mean glycogen concentration was found different in different types of fibers , its higher in type II fibers than in type I fibers in resting muscles [21].

Training enhances muscle oxidative capacity and promotes muscle glycogen sparing during exercise by young and old rats, exercise training increases the muscle glycogen levels of older people. After one bout of exercise, muscle sensitivity for insulin-stimulated glucose transport is improved in young and old rats. These findings indicate that several age-related changes in muscle carbohydrate metabolism can be minimized by acute or chronic exercise [22].

Muscle glycogen recovery is the process through which the muscles of the body are replenished

with carbohydrate sources that have been depleted through the energy expended in exercise. Most glycogen is entirely consumed from muscle stores within 15 minutes to 30 minutes from the commencement of the exercise; the athlete may exhaust all of the stored glycogen reserves with 10 minutes of muscle effort. The glycogen must be recovered and the supplies restored at the conclusion of the activity. The primary danger associated with this depletion is damage to cells and muscle structures; it may trigger the breakdown of cell structures to create an alternative energy supply. Muscle glycogen depletion also places significant stress on the overall function of the immune system, such depletion, if not corrected, carries with it risks to the structure and the function of the body. Muscle glycogen depletion is most effectively counteracted through diet; athletes who understand the demands placed on their muscle glycogen stores will plan how they shall achieve glycogen recovery through the foods ingested before, during, and after their workouts and their competitive events [23-24].

On other hands Calorie restriction's (CR) effects on age-associated changes in glycogen-metabolizing enzymes were studied in rat tibialis anterior (TA) muscles in old (24 months) compared to young (6 months) rats maintained ad libitum on a standard diet. Age-associated impairments in Glycogen synthesis (GS) protein and activation-phosphorylation were shown in TA, but glycogen phosphorylation (GP) was inactivated in TA with age. CR did not alter GS or GP activity/protein levels in young rats. CR hindered age-related decreases in GS activity/protein. Thus, the predominant age-associated impairments on skeletal muscle and CR can attenuate the loss of GS activity/activation and stimulate glycogen accumulation [25].

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Prevalence of Enuresis in Sample of Iraqi Children

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Abstract

Background Enuresis, which is frequently diagnosed amongst school age children, is an important psychosocial problem for both parents and children.

Objective In the present study we aimed to determine the prevalence and associated risk factors of enuresis in sample of Iraqi children and to identify common methods for its management.

Methods Across sectional epidemiological study was performed among primary school age children living in Tikrit and Beji cities during the period from the October 2008 to the June 2009. A self-administered questionnaire was prepared for this study and distributed to the parents of 1.150 school age children whom aged 6-12 years.

Results Of the 1.150 questionnaires distributed, 1000 (87%) were completed. The overall prevalence of nocturnal and diurnal enuresis were 22% (n = 220) and 1.9% (n =25) respectively. Although male gender, low age, history of enuresis among parents, low educational level of the parents, deep sleep, increased number of siblings, increased numbers of people sleeping in the child's room, history of enuresis among siblings, poor school performance and history of recurrent urinary tract infections (UTI) were significantly associated with enuresis, but not with the severe enuresis. The percentage of children with enuresis seen by physician for treatment was 17.2%. The most preferred treatment option for enuresis was medications (59.5%), whereas alarm treatment was the least preferred (2.4%).

Conclusion The results with enuresis prevalence and associated risk factors were comparable to other epidemiological studies from various countries, but it is higher in our country. Furthermore we demonstrated that families in the Tikrit do not pay sufficient attention to enuresis and most of enuretic children do not receive professional treatment.

Key Words Enuresis, family characteristics, prevalence

Introduction

Nocturnal enuresis (NE) also known as nighttime incontinence or bed-wetting refers to involuntary voiding only at night beyond the age at which most children have stopped [(1)]. Nocturnal enuresis is a very common clinical problem in children, especially in boys. Despite the fact that this condition is usually labeled benign, it often leads to considerable emotional distress and concern in affected children and their parents [(1)]. Approximately 15% a spontaneous resolution rate of about 15% per year; therefore, by the age 15, only about 1% of adolescents have a problem with NE [(2)]. The etiology of enuresis is not completely understood. This condition probably has a multifactorial etiology. Most studies have consistently found that the risk factors for enuresis are male gender, low age, and family history of enuresis, divorced parents and deep sleep [(3-7)].

The aim in this study was to determine the prevalence and associated risk factors of

enuresis in Tikrit children and to identify common methods of its management.

Methods

A prospective cross sectional epidemiological study was performed among primary school children living in Tikrit, Beji during the period from the October 2008 to the June 2009. A self-administered questionnaire was prepared for this study and distributed to the parents of 1.150 school age children whom aged 6-12 years. The study consisted of five schools selected randomly. To minimize any embarrassment to the children, parents were accessed directly to obtain the information.

The questionnaire consisted of two parts. The first part was designed to investigate associated risk factors of enuresis, and the second part was planned to determine type and prevalence of enuresis and to identify common methods of its management. The questions in the first part asked about sex, age, education level of parents, other enuretic children in the family, presence of other people sleeping in the child's room, sleeping habit, number of siblings, school performance, history of urinary tract infection (UTI) and upper respiratory tract infections (URTI).

The second part of the questionnaire was completed only by the parents of the enuretic children. The questionnaire in this part asked about the frequency of bed-wetting at night and/or in daytime, wetting after a continuous dry period of 6 > months and any history medical treatment of enuresis.

Enuresis was defined as an episode of bed-wetting occurring at least once a month. Primary enuresis was defined as bed-wetting in subjects who have never been dry for an extended period. Furthermore secondary enuresis was defined as the onset of wetting after a continuous dry period of 6 > months and diurnal enuresis was defined as daytime wetting when the child awakes.

All the data was analyzed with SPSS software for windows (Chicago, IL, USA). Univariate Chi-square test and multivariate logistic regression test was used for the statistical analysis and P value < 0.05 was considered as statistically significant.

Results

Of the 1,150 questionnaires distributed, 1000 (87%) were returned from the parents. The main age of the children included in the study was 8.8 ±1.3 years. The overall prevalence of nocturnal and diurnal enuresis were 22% (n = 220) and 2.5% (n = 25), respectively. Nocturnal enuresis was primary in particularly more prevalent in boys than in girls, but diurnal enuresis did not reveal a gender bias (Table 1).

Table 1. Prevalence of enuresis

Type	Boys		Girls		Total	
	No.	%	No.	%	No.	%
Nocturnal enuresis	120	24.6	100	9.5	220	22
Diurnal enuresis	13	2.6	12	2.3	25	2.5

N = Total number. % = percent

Furthermore the prevalence of enuresis decreased with age. Of the 6- year-old children 30.8% still wetted their beds, while none of those aged 12 years did so. The prevalence of enuresis in males and females according to age group are shown in (Table 2).

Several parental factors that are related to enuresis like history of enuresis and low educational level of the parents were significantly higher in children with enuresis when compared to non-enuretics (Table 3).

Table 2. The frequency of nocturnal enuresis in relation to age and gender

Age (years)	Boys		Girls		Total	
	n/N	% *	n/N	% *	n/N	% *
6	4/8	3.3	-/5	-	4/13	1.8
7	20/71	16.7	16/75	16	36/146	16.3
8	41/135	34.1	31/164	31	72/299	32.7
9	31/136	25.8	34/134	34	65/270	29.5
10	16/70	13.3	14/69	14	30/139	13.6
11	8/44	6.6	5/57	4.8	13/101	5.9
12	-/24	-	-/8	-	-/32	-
Total	120/488	24.6	100/512	19.5		

n = number of enuretic children; N = total number in each age group; * = percentage in enuretic children.

Table 3. Parental factors that were related to enuresis

Factors		Enuretics		Non-enuretics		p Value
		No.	%	No.	%	
Family work	**Mother**	26	11.8	174	15.7	NS
	Father	201	91.3	1001	93.7	NS
Parent Death	**Mother**	1	0.4	6	0.5	NS
	Father	-	-	2	0.2	NS
Mother Education Level	**Primary school or less**	194	88.1	755	68.3	< 0.001
	High school or more	39	17.7	350	31.7	
Father Education Level	**Primary school or less**	169	76.8	570	51.6	< 0.001
	High school or more	61	27.7	535	48.4	
Family history of enuresis	**Mother or father enuretic**	72	32.7	122	11	< 0.001
	Mother & father enuretic	33	15	30	2.7	
	Mother & father non-enuretic	129	58.6	871	87.1	

NS = not significan

The rate of male gender, low age, deep sleep (state of sleep from which it is difficult to a waken. It is represented by slower brain waves called delta activity on EEG. It is a type of NREM sleep and is called stage 3 on sleep studies also known as delta sleep, slow-wave sleep. Example; when my son is in deep sleep, he is nearly impossible to a waken, poor school performance, history of enuresis in siblings, increased number of siblings, increased room sharing with other siblings and recurrent UTI were significantly higher in enuretics when compared to non-enuretic children (Table 4).

The severities of enuresis for four categories of frequency (every night, 4-6 times per week, 1-3 times per week and 1-2 times per month) were 33.3%, 10.7%, 25.6% and 30.3%, respectively. On the other hand the factors that were significantly related to enuresis were not related to severe enuresis (every night) ($p > 0.05$).

The percentage of children with enuresis seen by physician for treatment was 17.2%. The treatment modalities offered to these children were medications (59.5%), waking to void (26.2%), wait for maturity (7.1%) (Maturation of urinary sphincter occurs in 75% of children by age 5 year, and 90% by age of 8 year without any treatment), fluid restriction (4.8%) and alarm treatment (2.4%). The enuresis

alarm is one of the best and most widely used therapies against bed wetting, (not in our country).

Table 4. The associated factors related to children for enuresis

Factors		Enuretics		Non-enuretics		p Value
		No.	%	No.	%	
Deep sleeper		134	60.9	343	31	<0.001
Number of siblings	None	17	7.7	154	13.9	0.003
	Single sibling	93	42.3	520	47.1	
	2 or more	105	47.7	431	39	
Room sharing	None	40	19.5	298	27	0.009
	1 person	120	54.5	556	50.3	
	2 person	60	27.2	251	22.7	
History of enuresis in the siblings		115	52.2	248	22.4	< 0.001
School performance	Good	80	36.3	550	49.8	< 0.001
	Moderate	95	43.1	445	40.3	
	Fail	50	22.7	110	10	
Recurrent UTI		40	18.1	143	12.9	0.037
Recurrent URTI		51	23.1	233	21.1	NS

UTI = urinary tract infection; URTI = upper respiratory tract infection; NS= not significant.

It consists of a detector placed in the child's underpants or under the sheets and a device that gives off a strong sound signal whenever there is urine on the detector. In this way the sleep of the child is gradually changed so that he or she notices when the bladder is about to be emptied. The alarm treatment is completely harmless and will - if it is used correctly - cure most, but not all, bed wetting children. Unfortunately this alarm was not available in our country but can be getting from out side (other countries), as in my research some family was getting it from other countries.

The explanation of these risk factors is in male some percent of enuresis even after 18 years, but in female extremely rare, in low age because of maturation of urinary sphincter, in poor school performance either due to behavioral disorder or due to neurological problems, in recurrent UTI may be due to increase of frequency, urgency or may be due to congenital anomalies of urinary tract.

Discussion

Enuresis is one of the common disorders in pediatric population. In most countries the prevalence of enuresis among 6-11 years old children is reported as 1.4-28% [3-5,7]. Likewise in the present study we obtained the prevalence of enuresis in children at 6-12 years of age as 22%. In previous studies reported from different Iraqi provinces, the prevalence of enuresis was reported as 5-11.7%, which was lower than our study's prevalence [6].

Previous studies demonstrated that the prevalence of enuresis tended to decrease with increasing age, and it was more common in boys rather than girls. Similarly in the present study 30.8% of the children were wetting their beds at age 6 whereas none of them were wetting their beds at age 12. However a small number of children in the age group 6 and 12 (n = 13 and 32 respectively) was the limitation of our study. Furthermore the prevalence of enuresis in boys and girls were 24.6% and 19.5% respectively.

In another study which was conducted by Lee et al [4] reported the prevalence of enuresis at age 7 as 20.4% and this rate decreased to 5.6% by age 12 [4]. The parental factors that were significantly related to the prevalence of enuresis in our study were history of enuresis and the low educational level of the parents. In this study the rate of history of enuresis in the parents 32.7% in the enuretic children whereas this rate was only 11% in the non-enuretic children. Furthermore previous studies reported the prevalence of family history in the enuretic children as 22-48% [3,7, 8]. Twin studies also support a genetic basis for enuresis. The concordance rate is much higher in monozygotic twins (36%) [9]. Danish researchers were the first to report an unidentified enuresis gene (ENUR1) in chromosome region 13q [10]. Later studies have also shown linkage to chromosome 12q and chromosome 22 [11,12]. Corresponding with previous studies, in our study the factors that are significantly related to enuresis were male gender, low age, deep sleep, poor school performance, history of enuresis in the siblings, increased number of siblings, room sharing and recurrent UTI. However URTI of the children were not related with enuresis. On the other hand further reported factors that significantly related to enuresis were divorced parents, low birth rate, growth retardation, constipation, bronchial asthma, allergy and liquid intake before go to sleep [3,5,13,14].

We defined severe enuresis as bed wetting every night (33.3%) which did not related to any of factors stated above. However, Chang reported that deep sleep is significantly related with bed wetting more than three times a week [5]. Watanabe and Kawauchi showed that the arousal center was activated to turn deep sleep into light sleep when the bladder was distended [15]. They also found that a disturbance in this arousal system might result in sustained deep sleep and hence cause enuresis.

In the present study only 17.2% of the children were seen by a physician and previous series reported as 11-34% [4,6,7]. These low rates demonstrate that most of the children with enuresis were not treated. On the contrary in the present study most of the children (61.9%) were treated with professional methods provided by physicians. While the use of alarm treatment was significantly lower when compared to medications (2.4% vs. 59.5%). However at present the use of alarm for enuresis treatment is the preferred treatment modality because of high success rate and low relapses [16].

Conclusions

The results with enuresis prevalence and significant risk factors were male gender, low age, history of enuresis among parents, low educational level of the parents, deep sleep, poor school performance and history of recurrent UTI, and were comparable to other epidemiological studies from various countries. We documented that most of the children with enuresis were not treated and the families in Tikrit do not have adequate attention about enuresis and most of the enuretic children do not receive professional treatment.

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Chlamydia Trachomatis and Recurrent Spontaneous Abortion in Iraqi Pregnant Women

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Abstract

Background Certain infectious agents have been identified more frequently in cultures from women who have had a spontaneous pregnancy loss; these include *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Chlamydia*.

Objective The aim of the study was to evaluate the frequency of *Chlamydia trachomatis* infection among women who experienced recurrent spontaneous abortion.

Methods A total of 119 women, age ranged from 23.9−28.5 years were enrolled in the current study and were classified into: Group A- Recurrent spontaneous abortion (RSA): n= 62 women, with a mean age of (28.5±0.68); Group B- non- recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of (26.4±0.85) and group C- Control (successful pregnancy): n= 23 women, with a mean age of (23.9±0.88). From each patient and control blood and urine samples were collected. Urinalysis test strips including Leukocytes esterase in urine was done, and estimation of IgM levels against ***Chlamydia trachomatis*** in sera of patients was done using ELISA method.

Results Based on ELISA screening assay, results showed a significant difference in the level of circulating *C.trachomatis* specific IgM antibody between group A and group C ($p< 0.05$) as well as between group B and group C ($p< 0.01$). Also highly significant positive correlation (r=0.401, $p<0.001$) between *C.trachomatis* acute infection and urine level of leukocyte esterase.

Conclusion *C.trachomatis* infection is an important causative agent of miscarriages in women. *C.trachomatis* infection diagnostic procedures should be considered in screening tests during pregnancy.

Key words *Chlamydia trachomatis*, RSA, ELISA, Leukocytes esterase

Introduction

The increased risks of viral and intracellular bacterial infections suggest that there is reduced Th1 cell activity against pathogens during pregnancy because of the Th1 cytokines are important for continuing pregnancy, the shift away from Th1 cells is consistent with this increased risk of maternal infection due to intracellular organisms, the more sever risk to the fetus [(1)]. Although sporadic pregnancy loss has been associated with such organ-isms as *Ureaplasma urealyticum, Mycoplasma hominis, Chlmydia trachomatis*, TORCH (*Toxoplasma gondii,* rubella, human cytomegalovirus and herpes) there is no convincing association with repeated miscarriage. The mere presence of an organism at the time of the loss can not be assumed to be proof of cause [(2,3)].

Bacterial vaginosis, which refers to an imbalance in the polymicrobial vaginal flora, is more commonly associated with mid-trimester losses [(4,5)]. Lower genital tract infection with *Chlamydia trachomatis* is currently the most commonly diagnosed sexually transmitted disease, *Chlmydia trachomatis* infection is an important causative agent of miscarriages in women [(6,7)]. However there are also investigations that were unable to

prove any relationship. More recently it has been shown that only women with evidence of recent infection were at a higher risk of developing premature rupture of membranes and preterm labor [4]. Others postulated that an immune response to an epitope shared by a Chlamydial and a fetal antigen is responsible for recurrent miscarriage [8].

Hence this study was designed to study was the frequency of *Chlamydia trachomatis* (C.t.) infection among women who experienced recurrent spontaneous abortion.

Methods

One hundred and nineteen women attending the Obstetrics and Gynecology department of Al-Kadhimyia Teaching Hospital in Baghdad between December 2004 and August 2005 were the subject of this study. They comprised 62 pregnant ladies all of whom gave a history of previous 3-6 consecutive abortions. (Recurrent spontaneous abortion; RSA) (groupA); non-RSA(first and second abortion)(groupB) included 34 pregnant ladies ,and 23 pregnant ladies(full term) had at least two previous normal pregnancies as a control group(groupC).

Sample collection

Blood: Five ml of venous blood was collected from each patient and control group. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. The tube was centrifuged for 10 minutes at 4^{o}C at 450 x g for serum collection. The serum was then aspirated by using a Pasteur pipette and dispensed into sterile glass tubes (1 ml in each) and stored at -20 ^{o}C until used.

Urine: A mid stream urine specimen was collected in a sterile container; External and preineal area were cleaned, washed thoroughly and dried before collecting the specimens. These samples were used for strip test. These urinalysis test strips including Leukocytes esterase are simple, easy to use reagent strips for the detection of key diagnostic chemical markers in human urine

Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of *Chlamydia trachomatis* /IgM (NovaTec Immundiagnostica Gmb H. Germany), the test was done according to the manufacture instructions.

Statistical analysis: - The ANOVA analysis program **was used.**

Results

As shown in table 1, the current study investigated the possible existence of acute *C. trachomatis* infections among the three patient's groups based on IgM antibody detection assay. Accordingly, group A gave 16.1% positive reactive and group B showed 29.4% positive finding while group C gave 100% negative reaction.

Table 1. Prevalence of acute infection *C. trachomatis* in studied groups

Variable	Result	Groups						Total	Chi-Square *P* value
		A		B		C			
		No.	%	No.	%	No.	%		
C.trachomatis **(IgM)**	Negative	50	80.6	24	70.6	23	100	97	0.034*
	Equivocal	2	3.2	0		0		2	
	Positive	10	16.1	10	29.4	0		11	
Total		62		34		23			

*=significant difference (p<0.05)

Interestingly, the current study showed a highly significant positive correlation (r=0.401, *p*<0.001) between *C.trachomatis* acute infection and urine level of leukocyte esterase, as shown in Figure 1.

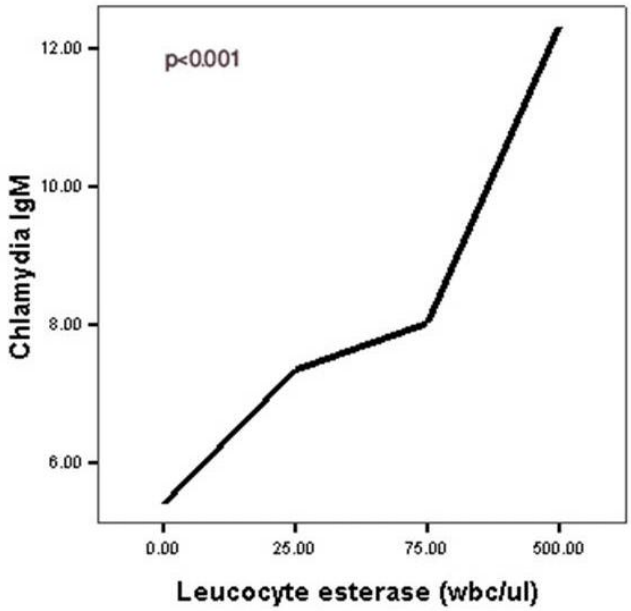

Figure 1. Correlation between *Chlamydia trachomatis* and leukocyte esterase

The ANOVA test analysis in table 2 shows significant difference (*p*<0.05) in the mean of *C.trachomatis* infection between group A (RSA) and group C (successful pregnancy), and a highly significant difference (*p*<0.001) between group B (non-RSA) and group C. In addition, the data showed marginally significant difference (*P*<0.05, *p*<0.1) between the mean value of *C.trachomatis* infection in group A and B (6.4±0.4 and 7.8± 0.6, respectively).

Table 2. Comparison of acute *C.trachomatis* infection in studied groups

Variable	Group	No.	Mean ± SE	F test *P* value	Significance between groups	
					Group	*P* value
***C.trachomatis* (IgM)**	**A**	62	6.4±0.4	<0.01	A & B	0.055[a]
	B	34	0.6±7.8		A & C	0.030*
	C	23	0.6±2.9		B & C	0.000**

[a]= marginally significant difference (0.05>*p*>0.1); *=significant difference (*p*<0.05); **= highly significant difference (*p*<0.01); SE= standard error.

On the other hands acute *C.trachomatis* infections showed no significance difference (*p*>0.05) in the mean value of infection in first and second trimester abortion, but statistically significant difference (*p*<0.05) in the mean value was found between first trimester abortion (6.7±0.5) and control (4.6±0.6) and highly significant difference (*p*<0.001) between acute infection in second trimester abortion (7.2±0.5) and control (full term), as shown in table 3.

Table 3. Comparison between *C.trachomatis* infection in first, second trimester abortion and control

Variable	Group	No. (119)	Mean ± SE	F test *P* value	Significance between groups	
					Group	*P* value
***C.trachomatis* (IgM)**	1st	53	6.7±0.5	<0.05	1st -2nd	0.485
	2nd	43	7.2±0.5		1^{st}-C	0.016*
	C	23	4.6±0.6		2^{nd}-C	0.000**

*=significant difference (p<0.05); **= highly significant difference (p<0.01); SE= standard error; 1st= first trimester abortion; 2nd=second trimester abortion.

Discussion

Acute C.trachomatis infections showed no significance difference (p>0.05) in the mean value of infection in first and second trimester abortion. However, a statistically significant difference (p<0.05) in the mean value was obtained when compared between first trimester abortion and control, and highly significant difference (p<0.001) between second trimester abortion and control. This result agreed with the study done by Oakesshott and colleagues [4], that showed chlamydial infection associated with second trimester abortion.

It has been shown no significant correlation (p>0.05) between gestational age and acute infection with *C.trachomatis*. This result might indicate that in this study gestational age was not a risk factor in *C.trachomatis* infection. In the present study, there was a significant difference (p<0.05), in the serum level of *C.trachomatis* specific IgM among the three investigated groups. The prevalence of positive acute infection of *C.trachomatis* was 10/62 (16.1%) in group A (RSA) and 10/34 (29.4%) in group B (non-RSA). These results agreed with studies stated by [8,9] who showed a significantly high titers of chlamydial antibodies found in the sera of women with habitual abortion.

Also, it was found a significant difference (p<0.05) in the mean of *C.trachomatis* infection between group A (RSA) and group C (successful pregnancy), and highly significant difference (p<0.001) between group B (non-RSA) and group C. In addition, the data showed marginally significant difference (0.05<p<0.1) between the mean value of *C.trachomatis* infection in group A and B (6.4±0.4 and 7.8± 0.6, respectively). Qublan [6] postulated that an immune response to an epitope shared by a Chlamydia and a fetal antigen is responsible for recurrent miscarriage. There were, however, no data available to confirm the role of intervention in improving the outcome of pregnancy. Interestingly, the current study showed a highly significant positive correlation (r=0.401, p<0.001) between *C.trachomatis* acute infection and urine level of leukocyte esterase as shown in figure 1. This result agreed with study of O'Brien et al [10], which utilized leukocyte esterase dipstick to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* urethritis in asymptomatic adolescent male detainees, they further explained that detection of leukocyte esterase as 100% sensitive, 83 % specific, and 54 % predictive for the presence of either organism.

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Evaluation of Whitnall Sling Procedure for Moderate to Severe Congenital Blepharoptosis with Fair to Poor Levator Function

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Abstract

Background Blepharoptosis has a significant impact on patient functional status and may cause poor visual development in childhood and correction of congenital ptosis is one of the difficult challenges the ophthalmologist faces**.** Several surgical techniques were used for correction. The selection of one technique over another depends on several factors including the experience and comfort level of the surgeon with various techniques, the severity of ptosis and the degree of levator function.

Objective To Evaluate Whitnall sling procedure for moderate to severe congenital blepharoptosis with fair to poor levator function as a good choice to those whom frontalis sling procedure was there only option.

Methods Twenty five patients with different types of congenital blepharoptosis of moderate to severe degree and poor to fair levator function were received in Alwasity Hospital for Reconstructive Surgery and treated with Whitnall sling procedure. The results were evaluated according to the following criteria; Good: where the ptotic lid lies within 1 mm of normal lid position (1 mm below superior limbus) in primary gaze position. Moderate: postoperative lid position drooped more than 1 mm of normal lid position but maintained above the pupil. Poor: post operative lid position drooped to obscure the visual axis in primary gaze position.

Results 20 cases (80%) were having good results, 5 cases had moderate results (20%). There were no poor results. There was no need for additional tarsectomy in our cases.

Conclusion Whitnall sling procedure is a good choice for cases lying in the overlap zone between it and the frontalis sling procedure whenever the surgical indications and procedure of choice were concerned.

Keywords blepharoptosis, congenital lid ptosis, whitnal sling

Introduction

Blepharoptosis means vertical narrowing of palpebral fissure secondary to drooping of upper eye lid to a lower than normal position (which is defined as upper lid position 1 mm below the superior limbus) [1, 2]. It is considered as congenital when it's present at birth or diagnosed with in the first year of life [2]. It can occur as an isolated neuromuscular disorder (simple congenital ptosis) or be part of larger spectrum of local (peri ocular) or general birth defects (syndromic) [3]. The incidence of simple congenital ptosis is about 0.18 % [2]. It is unilateral in about 75% of cases [4]. It may be associated with the development of visual disturbances such as myopia, astigmatism, anisometropia, amblyopia and strabismus [2]. The possibility of amblyopia and associated refractive

defects make early detection and surgical treatment necessary when indicated and should be repaired as soon as possible when amblyopia could be provoked [5]. In congenital blepharoptosis, amblyopia is detected in about 20% of patients and is usually secondary to convergent strabismus, high astigmatism or anisometropia [4]. Deprivational amblyopia that is solely due to occlusion of papillary axis is rare and estimated to represent about 3% of amblyopias [4]. The ideal age for repair in unilateral cases that are not associated with the risk of deprivational amblyopia is 4-5 years [4]. Bilateral cases that are associated with bad head posturing habits should be addressed earlier [2].

Methods

Twenty five patients with different types of congenital ptosis who were presented to Al-Wasity hospital for plastic and reconstructive surgery have been evaluated for severity and levator function. The patients' data are summarized in tables 1-5.

Table 1. Age of incidence and types of ptosis

Type of ptosis	Age of incidence in years				
	0-3y	4-5y	6-10y	11-15y	>15y
Simple cong.ptosis	2	8	8	2	1
Blepharo phimosis				-	1
Marcus gun syndrome				1	2

Table 2. Laterality according to the type of ptosis

Type of ptosis	No. of cases	Unilateral	Bilateral
Simple congenital ptosis	21	18	3
Blepharophimosis	1	-	1
Marcus-gunn syndrome	3	3	-

Table 3. Associated anomalies according to the type of ptosis

Type of anomaly	Diagnosis	No. of cases
Strabismus (sup.rectus)	Marcus-gunn syndrome	1
Amblyopia		0
Ophthalmoplegia		0

Table 4: severity of ptosis according to types

Diagnosis	Moderate	Severe
Simple congenital ptosis	13 eyes	11 eyes
Blepharo phimosis	1 eye	1 eye
Marcus-gunn syndrome	3 eyes	-

Table 5. Levator function according to type of ptosis

Type of ptosis	Poor	Fair	Good	Excellent
Simple congenital ptosis	11 eyes	16 eyes		
Blepharophimosis	2 eyes			
Marcus gunn syndrome				

Classification of severity of blepharoptosis was as follows (after Levine) [4]: Mild = 2 mm; Moderate = 3 mm; Severe = 4 mm and more

Method of evaluation of severity of ptosis:

For unilateral blepharoptosis, the difference between the two palpebral fissures was considered as the severity of ptosis. For bilateral ptosis, MRD1 distance was depended where the normal MRD1 is considered as 4 - 4.5 mm [9].

Classification of levator functions (after Levine): Excellent = 12-15 mm; Good = 8-11 mm; Fair = 5-7 mm; Poor = 4 mm or less

Method of evaluation of levator function:

The distance from maximum down gaze to maximum up gaze is measured in millimeter with the eye brow fixed by thumb pressure.

Before surgery, all the necessary ocular and general examinations were undertaken. Whitnall sling procedure was adopted in all patients after thorough discussion with the patient's family. the following technique was used; all patients were operated upon under G.A, the palpebral crease was marked bilaterally preoperatively, after infiltration with 1:100 000 adrenaline and waiting for 7 minutes, skin incision is done on the premarked crease followed by incision of orbicularis oris muscle and undermining below the muscle to expose the orbital septum superiorly and tarsal plate inferiorly.

The orbital septum is then incised the whole length with scissors and after retraction of orbital fat identification of whitnall ligament is done. On the inferior side, the levator aponeurosis fibers are sharply separated from the anterior surface of tarsal plate and upward gentle dissection off the transparent conjunctiva is done gradually including the muller muscle with the aponeurosis. Laterally the lateral horn followed to the lateral orbital margin between the orbital and palpebral lobe of lacrimal gland and separated. Medially the medial horn is followed and separated from the medial orbital margin.

Now the levator aponeurosis attached to its muscle which can be tested for good excursion is advanced to be fixed at the level of whitnall ligament (which is included in the fixation) to the anterior surface of tarsal plate about 2 mm from superior margin starting with medial limbus stitch followed by lateral limbus stitch and then two additional medial and lateral stitches are added, 6/0 nylon is usually used. The level of the upper eye lid is checked after each stitch. The level is chosen to be with superior limbus, not above not below. After insetting of the muscle, the excess aponeurosis is trimmed leaving about 3mm cuff for any later adjustment needed. The orbicularis oculi muscle is fixed to the aponeurosis with two absorbable stitches and the skin is closed with subdermal interrupted stitches without external sutures, sterile strip is added.

Results

The following criteria were used for evaluation of results (after Wong) [1]; Good: post operative lid position is maintained within 1 mm of normal lid position (1 mm below superior limbus) in the primary gaze position. Moderate: Post operative lid position drooped more than 1mm below normal lid position. Poor: Post operative lid position drooped to obscure the visual axis in the primary gaze position. The follow up period ranged from 3 weeks to one year with an

average of 6 months. The results and complications are summarized in Table 6 and Table 7 consecutively.

Table 6: Results

Results	Good	Moderate	Poor
Simple congenital ptosis	21 eyes	3 eyes	-
Blepharophimosis	-	2 eyes	-
Marcus gunn syndrome	2 eyes	1 eye	-

Table 7. Complications

Condition	No. of eyes	Action
Over correction	1	Revision after one week
Conjunctival prolapse	1	Revision after 3 weeks
Crease asymmetry	1	Left
Absence of crease	1	Revision after one month
Lateral drooping	1	Revision after 6 months

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Discussion

Blepharoptosis has a significant impact on patient functional status and may cause poor visual development in childhood [(6)] and correction of congenital ptosis is one of the difficult challenges the ophthalmologist faces [(2)].

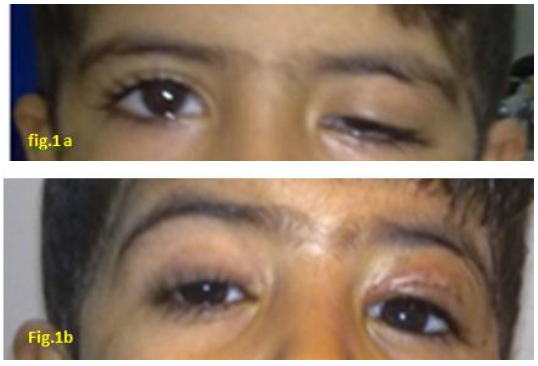

Figure 1 (a) a 5 year old boy with simple unilateral ptosis of severe degree with poor levator functions. (b) 6 weeks after whitnall sling operation.

In our series, there are three important aspects that worth discussion, these are the indication or choice of surgical technique, the technique itself and the evaluation of results. The selection of one technique over another depends on several factors including the experience and comfort level of the surgeon with various techniques, the severity of ptosis and the degree of levator function [(2)].

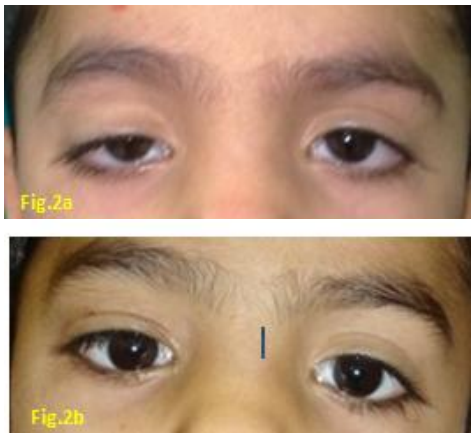

Figure 2 (a) Right sided severe congenital ptosis. (b) 6 weeks after whitnall sling surgery.

Allard and Durairag had mentioned that for children with < 3mm of levator function, surgical options include frontalis sling, frontalis muscle flap and whitnall sling procedures [(2)]. Considered that brow suspension is good only in bilateral ptosis, so he advocated conversion of unilateral

ptosis into bilateral by levator muscle excision on the normal side and bilateral fascia lata suspension [7]. Anderson considered that whitnall sling procedure is recommended for severe ptosis with levator function of 3-5 mm [2].

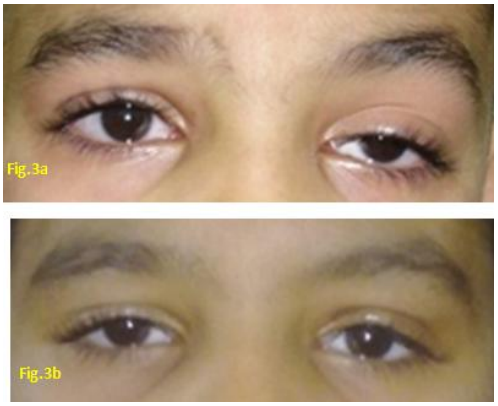

Figure 3. (a) Unilateral severe congenital ptosis. (b) 3 weeks after whitnall sling operation.

Lee has found in a retrospective study that whitnall sling (maximum levator aponeurosis resection) procedure was used for patients with severe ptosis associated with poor levator function interchangeably with frontalis suspension procedure [6].

Figure 4. Moderate left congenital ptosis with fair levator function. (b) 6 months after whitnall sling operation.

Durairaj had found that whitnall sling procedure and frontalis suspension procedure were used alternatively in case of failure of one of them [2]. Anderson in his original study on whitnall sling procedure had advocated its use in severe unilateral ptosis with levator function of 3-5 mm and opposite fissure height of 9mm or less [8].

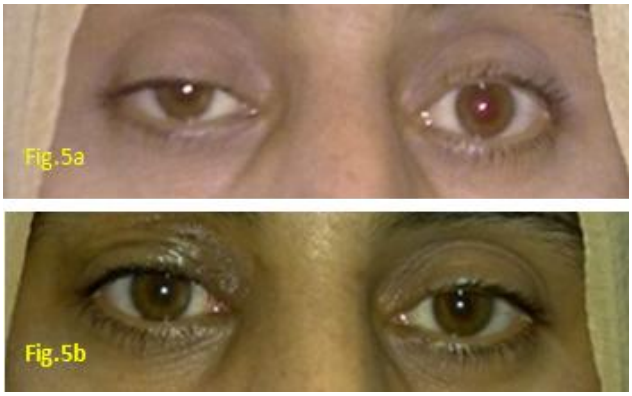

Figure 5. (a) Moderate unilateral congenital ptosis (marcus-Gunn) syndrome. (b) 6 Weeks after whitnall sling operation.

When the technique is concerned, there are few points worth mentioning, 1st is levator horns preservation, the second is the preservation of Muller muscle and the third is adjustment techniques and the last is the addition of any additional procedures like tarsectomy or skin resection. Steven Dresner had mentioned that whitnall procedure is maximum levator aponeurosis advancement where the levator muscle whitnall ligament is sewn to tarsal plate without cutting the lateral horns of levator aponeurosis [9].

Custer had stressed that levator horns act as check ligaments limiting posterior excursion of the levator muscle and when dehisced it leads to over correction after simple reattachment of dehisced levator aponeurosis [10]. In fact we have found that resection of levator horns (when needed) and preserving the Whitnall sling is necessary to prevent under correction as intact levator horns decrease the levator excursion while cutting the whitnall sling will decrease the excursion. When the muller muscle is concerned, we elevate it in combination with levator aponeurosis as the latter is usually under formed

in moderate to severe congenital ptosis and this agree with Custer opinion.

Figure 6. (a) 3 year old girl with bilateral severe congenital ptosis. (b) one year after whitnall sling operation for the left eye and levator advancement for the right eye. The right eye was revised later with whitnall sling surgery (c).

For the adjustment, there are many techniques used for levator adjustment when performing the procedure under general anesthesia, of them are the predetermined 3-7 mm shortening of levator muscle for each 1mm ptosis degree depending on the amount of levator function [(4)]. The McCord gapping technique where a 3mm is added to the degree of ptosis for the amount the palpebral fissure that should be kept open on the table postoperatively or the shortening according to the difference of levator function in mm between the normal and abnormal side multiplied by a factor of 1.2 [(9)].

We have found that as long as we are going to do maximum levator resection, there is no point of predetermined measured shortening of levator aponeurosis and found that preoperative setting of lid level at the level of superior limbus or slightly (1 mm) higher in very poor levator function with fine tuning through cutting the levator horns or changing the level of fixation of whitnall ligament to the anterior tarsus is of benefit in adjustment. When all these measures fail to reach the goal, superior tarsectomy may become necessary and this may agree with Levine where he omitted cases of poor levator function from his table for the predetermined amount of levator resection [(4)].

Figure 7. (a) 25 year old female with moderate congenital left sided ptosis with previos failed Vassanella- servat operation.(b) one month after whitnall sling operation.

Holds et al in a series of 25 patients with severe unilateral ptosis with poor levator function used 4-5 mm external resection of superior tarsus with maximum aponeuroctomy (whitnall sling) and found that 68% (17 out of 25 patients) achieved lid height within 1mm of contralateral lid but all patients developed mild to moderate degree keratopathy that ultimately resolved in most patients [(11)].

Nissman had used whitnall sling procedure with superior tarsectomy in a case of compressive 3rd nerve palsy with complete blepharoptosis and zero levator function with successful results, but he did not mention about any complication [(12)].

This point may make us reluctant to do superior tarsectomy routinely in our cases.

Figure 8. (a) 22 year old female with bilateral severe congenital ptosis and poor levator function; she has had failed previous frontalis sling operation with suture material. (b) one month after whitnall sling operation bilaterally.

The last point to be discussed is the evaluation of the results and the follow up period, three stations for evaluation of results were adopted, the first is at the 7th post operative day where any decision for intervention for over or under correction is to be taken and this is agreed upon by almost all authors. the second station is 6 weeks postoperatively which is considered as the stable end point [6] where the evaluation of results as good, moderate or poor were taken and the final evaluation were done 6 months post operatively where any change of results were evaluated or any final revision was to be taken.

Anderson in a series of 69 patients who underwent whitnall sling procedure without tarsectomy, 30% of patients which were considered as satisfactory (lid height within 2 mm of contralateral lid) became unacceptable and required reoperation so he recommended the augmentation of Whitnall sling procedure with tarsectomy in most cases especially those with poor levator function [2]. This finding is not accordant with our results where we needed to intervene only in 2 patients (8%) after 6 months for problems diagnosed at the 1st 6 weeks but the intervention was considered as unnecessary by the parents at that early time. Our conclusion is that whitnall sling procedure is safe, easy, and practical and can be considered as an extensile technique for cases with moderate to severe ptosis with poor to fair levator function but surgeon should not fit all cases to this procedure.

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Depression in a Group of Patients with Chronic Renal Failure Attending Heamodialysis Unit in Teaching Hospital in Baghdad

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Abstract

Background Depression is the most common psychopathological condition among patients with chronic renal disease yet it is still under recognized and underestimated. Depression adversely affects the already disturbed quality of life of patients with chronic renal disease.

Objectives To determine the rate of depression in a sample of chronic renal failure patients attending haemodialysis unit in teaching hospital in Baghdad and examine the sociodemographic variables of such patients.

Methods This is a cross-sectional study done at Al-Kadhmyia Teaching Hospital, during a period of 2 months from 4th of January 2009 to 4th of March 2009. The total of 50 random cases with chronic renal failure coming for haemodialysis were interviewed by semi structured schedule for psychiatric diagnosis. Results were reviewed by simple descriptive and inferential statistical measures.

Results The rate of depression in this sample of patients with chronic renal failure was 40%.There was no statistically significant relation between any of the demographic variables and depression.

Conclusion The rate of depression in this study is high. Results were compared with other studies in light of circumstances of this study.

Key words Depression, chronic renal failure, haemodialysis

Introduction

Depression is the most common psychopathological condition among patients with chronic renal disease, yet it is still under-recognized and misdiagnosed [1]. Depression reduces quality of life and has a negative clinical impact upon sufferers with chronic illness, including chronic renal disease [1]. End-stage renal disease (ESRD) has a significant impact upon the lives of sufferers; the experience of multiple losses, including kidney function, family role, work role, sexual function, time and mobility, have significant impact on the lives of patients [1,2]. Further stressors, including medication effects [3], dietary constraints, fear of death and dependency upon treatment [4], may affect quality of life and exacerbate feelings of loss of control [2,3]. While prevalent, depression is still often unrecognized [4], reflecting a lack of routine psychological evaluation among this patient population [4]. The consequences of missing depression among dialysis patients may be considerable. Comorbid depressive illnesses amplify the impact of chronic illnesses, and increase functional disability and the use of health care services [5].

Since its earliest known descriptions dating back to the Old Testament, depression has been observed as a disruption of normal lifestyle. Major depressive disorder is one of two serious mood disorders (the other is bipolar disorder or manic depressive disorder) that affect every aspect of life. Because there is no mania or elevated mood in major depressive disorder, it is called "unipolar"

depression. Changes in mood are a natural, normal part of life. People usually recognize, and are comfortable with a change in mood. People with depression, however, often cannot explain the reason for becoming depressed, though they describe it as emotionally painful and saddening. The predominant symptoms of depression are a general loss of interest and energy, and an inability to experience pleasure [6]. A person with depression typically withdraws from or becomes impaired in social interactions. Apathy toward work, school, relationships, responsibility, and eventually toward important goals, negatively affects the person and the family. The economic cost is significant in terms of lost hours, reduced productivity, and health care [6].The diagnostic and statistical manual, fourth revision of American Psychiatric Association (DSM-IV) provides four disclaimers to the clinical diagnosis of depression in chronic medical disease. It is necessary to describe depression as a mood disorder, which differs from depression associated with other psychiatric or medical conditions. If any of the following four situations apply to a person with chronic medical disease, then depression is present [7].

1. It cannot be established that an organic factor initiated and maintained the disturbance.
2. The disturbance is not a normal reaction to the death of a loved one (uncomplicated bereavement).
3. At no time during the disturbance have there been delusions or hallucinations for as long as 2 weeks in the absence of prominent mood symptoms (i.e., before the mood symptoms developed or after they have remitted).
4. Not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder NOS (not otherwise specified)

Chronic renal failure (CRF) refers to an irreversible deterioration in renal function which classically develops over a period of years. Initially, it is manifest only as a biochemical abnormality. Eventually, loss of the excretory, metabolic and endocrine functions of the kidney leads to the development of the clinical symptoms and signs of renal failure, which are referred to as uremia. When death is likely without renal replacement therapy, it is called end-stage renal failure (ESRF). The social and economic consequences of CRF are considerable. In the UK, over 37 000 patients (632 per million) are kept alive by renal replacement therapy and approaching 110 new patients per million of the adult population are accepted for long-term dialysis treatment each year. Of these, 50% are aged over 65 [7]. The incidence of CRF is much higher in some countries due to differences in regional and racial incidences of disease, as well as differences in medical practice. For example, in the USA, incident rates are over 300 per million populations, with nearly half of these patients having a primary diagnosis of diabetes mellitus [8].

DSM IV does not claim any direct biological causal relation between chronic medical disease including chronic renal failure and depression but understand depression in this population as a consequence of the psychological reaction to the losses accompanied [6].

Objectives

1- To determine the rate of depression among patients with chronic renal failure attending haemodialysis unit.
2- To review the sociodemographic variables in patients with CRF and depression.

Methods

This is a cross-sectional study which was performed in Al-Kadhimyia Teaching Hospital, during a period of 2 months from 4$^{th\ of}$ January 2009 to 4th of March 2009.

A total of 50 cases of chronic renal failure, as diagnosed by physician, were selected from patients in haemodialysis unit. Sampling was by randome-randome approach of selection of cases. All cases were interviewed face to face using a semistructured interview schedule

according to the DSM IV. Formal consent by the patient to participate in study was performed. Depression was diagnosed according to DSM IV criteria of depression in patients with chronic medical disease.

General Sociodemographic factors were examined such as age, address, educational level, and economic circumstances. The exclusion criteria were those with age less than 18 years, other primary physical disorder, and other primary psychiatric disorder.

Results

Of the 50 patients studied 27 were males (54%). The age mean was 55 years (SD 10.6).Depression occurred in rate of 40 % of the patients. Regarding symptoms of depression, 72% of total sample reported feelings of sadness, 24% reported diurnal variation of mood, 48% reported lack of interest, 10% reported worthlessness, 16% reported hopelessness, no patients reported helplessness, 6% reported guilty feelings, 32% reported thoughts of death wishes, 4% reported suicidal thinking and 2% reported suicidal attempt. 72% of total sample reported decrease in appetite. 70% reported decrease in weight and. 58% reported decrease in sleep (Table 1).

Table 1. Symptoms of depression

Symptoms	percent
Sadness	72%
Lack of interest	48%
Worthlessness	10%
Hopelessness	16%
Helplessness	0%
Guilty feelings	6%
Death wishes	32%
Suicidal thinking	4%
Suicidal attempt	2%
Diurnal variation	24%
Decreased appetite	72%
Decreased weight	70%
Decreased sleep	52%

Of the 27 male patients who participated in the study 10 had depression (37%) while 10 out of 23 females had the disorder (43%). There was no association between depression and difference in gender (chi square 0.215, df 1, sig. 0.6) (Table 2).

Table 2. Gender and depression chi square

0.215 df 1 Sig 0.6			
Gender	Depression		Total
	Yes	No	
Male	10	17	27
Female	10	13	23
Total	20	30	50

Most patients were in age range of 51-60.There was no association between age and depression (chi square 7.334, df 7, sig. 0.394) (Table 3).

Table 3. Age and depression

Chi square 7.334 df 7 sig 0.395			
Age	Depression		Total
	Yes	No	
18-30	0	3	3
30-40	4	2	6
40-50	5	4	9
50-60	2	6	8
60-70	3	8	11
70-80	3	3	6
80-90	3	3	6
90-100	0	1	1
Total	20	30	50

Most of patients had primary school education. There was no association between education and depression (chi square 7.074, df 3, sig.0.07) (Table 4).

Most patients had intermediate economic status.There was no association between depression and economic status (chi square 0.09, df 1, sig.0.764) (Table 5).

Table 4. Education level and Depression

chi square 7.074, df 3, sig. 0.07			
Education Level	Depression		Total
	Yes	No	
Illiterate	3	9	12
primary school	13	10	23
Intermediate school	0	5	5
College	4	6	10
Total	20	30	50

Table 5. Economic status depression chi square

0.09 Df 1 sig. 0.714			
Economic state	Depression		Total
	Yes	No	
Low	4	5	9
Intermediate	16	25	41
High	0	0	0
Total	20	30	50

Discussion

The findings of this study have similarities and differences with other studies as it is estimated that 20-30% of patients with end stage renal disease has depression, although research has tended to focus on the hemodialysis (HD) population and overlooked patients receiving peritoneal dialysis (PD). Among the medically ill, depression is a common concern accounting for half of the identified psychopathology [1,8-10].

On the other hand there are controversial figures in regard to the exact prevalence of depression in this population. In fact there is a wide variation regarding prevalence of depression among the medically ill ranging from 15%-61% depending on tools used for diagnoses and definition criteria [11]. Rating scales such as Beck Depression Inventory (BDI) and Hamilton Anxiety and Depression Scale (HADS) reveal high rates of depression in comparison to diagnostic interviews [12-14].

Although self-report screening measures, including the BDI, have been validated against well-established psychiatric methods in dialysis patients [8, 10, 20], the most accurate way of screening for depression (without professional evaluation) remains to be a difficult task [1,2,15], and is predominantly influenced by the issue of criterion contamination. Disagreements still remain regarding the preferred depression screening tool for the dialysis population. Using an existing or modified depression tool (BDI for example) for the dialysis population is beneficial.

A more ideal, yet problematic, task would be to develop a specific depression assessment tool for the dialysis population. Currently, this work is unlikely to succeed until we improve our understanding of the issues surrounding depression assessment in patients with a chronic illness. The current study used semi structured interview according to DSM IV performed by psychiatrist yet the rate of depression found here is high and comparable to results found through screening tools mentioned above (BDI, HADS).

It should be kept in mind that this study has some limitations that sample is relatively small and duration of study was short according to the circumstances of the study.

Special care is needed to avoid underestimation of depression in this population of patients on the light of impact of depression at both psychological and somatic level [5]. Depression increase burden on patients and carers in addition to direct risk to health by decrease in appetite and direct risk of death by suicide. It is important to mention here that 2 patients in this study attempted suicide.

This study did not find any association between depression and any of the examined demographic criteria such as gender, age, education, economic status. This can be explained by the fact that biological factor represented by the presence of renal disease act as common psychological and possible psychosocial stressful risk factor that contributes to depression in this population in comparison to depression in medically healthy population.

The current definition criteria for depression in chronic medical disease according to DSM IV exclude the direct biological contribution of such illness to depression or any direct biological causal relation between chronic medical including chronic renal failure and depression [6].

In another word this study assumes that the diagnosis of renal failure which is biological factor in this population acted as possible common stress or possible common psychological factor (and its possible accompanying social factors) in all age groups, both genders ,all the involved educational groups and all the involved economic categories resulting to depression

Conclusions

Depression is a prevalent and costly burdening to chronic renal failure patients impacting on a psychological and somatic level. Under-recognition of depression in this population is a major concern, particularly given the evidence of its impact on co morbidity and mortality.

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Anatomical Variation of the Appendix in Relation with Appendectomy Decision

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Abstract

Background The signs and symptoms of acute appendicitis vary according to the site of the appendix; and absence of tenderness in the right iliac fossa dose not exclude appendicitis like in postileal, subhepatic and pelvic appendix. Even Alvarado scale zero is not excluding the diagnosis of acute appendicitis.

Objective To study the incidence of delayed appendectomy and its relation to the anatomical variation of the appendix and its morbidity.

Methods A prospective study for patients whom underwent appendectomy for acute appendicitis during the period from-June 2009 to June-2010. The appendix of all the patients was submitted to histopathological examination and was proved to be acutely inflamed. The patients were divided into two groups according to the time interval from the onset of the first symptom to the time of appendectomy. In group A, this interval was more than 72 hours; while in group B it was less than 72 hours.

Results Group A includes 35 patients; while group B include 201 patients. The anatomical site of the appendix in group A was very significant in delayed decision of appendectomy in postileal appendix (P=0.0001), subhepatic appendix (P=0.0004), and significant in retrocecal appendix (P=0.017); but it is not significant in pelvic appendix (P=0.88), paracecal appendix (P=0.83) and preileal appendix (P=0.95). Patients in group A had longer hospital stay due to complications 35 (100%) generalized peritonitis and 3 (8.57%) patients were died due to septic shock which is significant (P=<o.o1).

Conclusion The classical visceral-somatic sequence of pain is not mandatory for diagnosis of appendicitis. In postileal and subhepatic appendicitis there is neither pain nor tenderness in the right iliac fossa (due to its anatomical position); and when the decision of appendectomy is delayed, there were generalized peritonitis and patients died due to septic shock.

Key words Appendectomy decision, Anatomical variation, Appendix

Introduction

The appendix first becomes visible in the 8th week of embryologic development as a protuberance off the terminal portion of the caecum. During both antenatal and postnatal development, the growth rate of the caecum exceeds that of the appendix, displacing the appendix medially towards the ileocaecal valve. The relationship of the base of the appendix to the caecum remains constant, whereas the tip can be found in a retrocaecal, pelvic, subcaecal, preileal, or right pericolic position (Figure 1). These anatomic considerations have significant clinical importance in the context of acute appendicitis [(1)].

During childhood, continued growth of the caecum commonly rotates the appendix into a retrocaecal position (figure 1). In approximately 25%, rotation of the appendix does not occur; resulting in a pelvic, subcaecal or paracaecal position. Occasionally the tip of the appendix becomes extraperitoneal, lying

behind the caecum or ascending colon. Rarely the caecum does not migrate during development to its normal position in the right lower quadrant of the abdomen. In these circumstances, the appendix can be found near the gall bladder or, in the case of intestinal malrotation, in the left iliac fossa, causing diagnostic difficulty if appendicitis develops. Acute appendicitis is the most common cause of acute abdomen and appendicitis is the most frequently performed urgent abdominal operation [2].

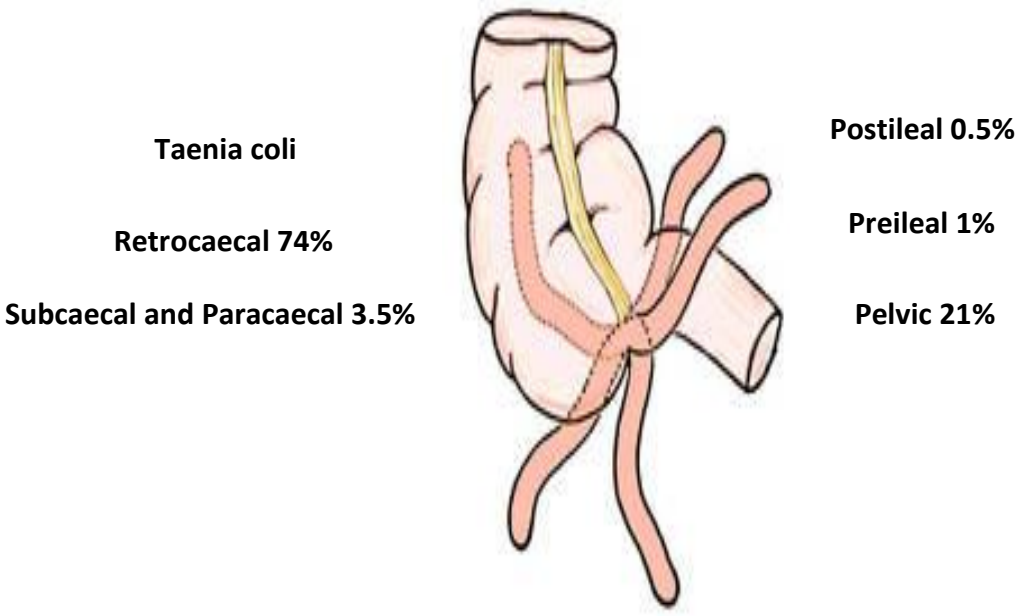

Figure 1. The various anatomical positions of the appendix .[2]

The credit for performance of the first appendectomy goes to Claudius Amyand, a surgeon at St. George's Hospital in London in 1736; he operated on an 11-year-old boy with a scrotal hernia and a fecal fistula. He successfully removed the appendix and repairs the hernia [3]. The Shattuck Professor of Pathological Anatomy from Harvard University, Boston, who presented a paper in 1886 describing the natural history and progression of the disease. He also recognized the vital importance of early diagnosis and immediate surgical intervention. The adoption of his conclusions by surgeons in North America in the following 15 years led to a decrease in the mortality of acute appendicitis from 50% to 15 % [4].

Notwithstanding advances in modern radiographic imaging and diagnostic laboratory investigations, the diagnosis of appendicitis remains essentially clinical, requiring a mixture of observation, clinical acumen and surgical science.

Peritonitis occurs as a result of free migration of bacteria through an ischemic appendicular wall, the frank perforation of a gangrenous appendix or the delayed perforation of an appendix abscess [2].

The differential diagnosis of acute appendicitis depends upon 4 major factors: the anatomic location of the inflamed appendix; the stage of the process (i.e., simple or ruptured); the patient's age; and the patient's sex. [5]

The classical features of acute appendicitis begin with poorly localized colicky abdominal pain due to mid-gut visceral discomfort in response to appendicular inflammation and obstruction. The pain is first noticed in the peri-umbilical region. With progressive inflammation of the appendix, the parietal peritoneum in the right iliac fossa becomes irritated, producing more intense, constant and

localized somatic pain that begins to predominant (pain that has shifted and changed in character).The classical visceral-somatic sequence of pain is present in only about half of those patients subsequently proven to have acute appendicitis. Atypical presentations include pain that is predominantly somatic or visceral and poorly localized according to the anatomical site of the appendix.

An inflamed appendix in the pelvis may never produce somatic pain involving the anterior abdominal wall (due to its anatomical position), but may instead cause suprapubic discomfort and tenesmus, and the tenderness may be elicited only on rectal examination which is the basis for the recommendation that a rectal examination should be performed on every patient who presents with acute lower abdominal pain [(2)]. Occasionally early diarrhea results from an inflamed appendix being in contact with the rectum. When the appendix lies entirely within the pelvis, there is usually complete absence of abdominal rigidity. And often tenderness over McBurney's point is also lacing. An inflamed appendix in contact with the bladder may cause frequency of micturition. Presence of pus cells in the general urine examination, urinary tract infection; and diarrhea not exclude diagnosis of appendicitis because of irritation to the urinary bladder and to the rectum by the inflamed appendix (according to its anatomical site) can produce these misleading symptoms [(2)].

In postileal appendicitis; the inflamed appendix lies behind the terminal ileum. It presents the greatest difficulty in diagnosis because the pain may not shift, diarrhea is a feature and marked retching may occure. Tenderness, if any, is ill defined, although it may be present immediately to the right of the umbilicus [(2)].

In subhepatic appendicitis the patient may present with abdominal pain and malaise without tenderness in the right iliac fossa but the white blood cell (WBC) count may be elevated and this is a useful and simple investigation for diagnosis of acute inflammatory disease [(2)].

The different anatomical locations of the inflamed appendix lead to vague signs and symptoms and wide differential diagnosis of acute appendicitis according to its anatomical site which may cause confusion and delayed decision of appendectomy; so acute appendicitis should always be kept in mind to avoid serious complications and mortality by early decision of appendectomy [(2)].

Methods

A prospective study of the patients whom underwent appendectomy for acute appendicitis in Al-Kadhimyia teaching hospital for one year during the period from June-2009 to June-2010 was included in this study. The appendix of all the patients was submitted to histopathological examination and was proved to be acutely inflamed, while all the non inflamed appendices were ignored from this study and not included. The patients were of the first symptom to the time of appendectomy. In group A, this interval was more than 72 hours; while in group B it was less than 72 hours.

Group A, include 35 patients; 14 male and 21 female (male to female ratio was 2:3) their age range from 4-65 years with mean age (28±87 years). While group B include 201 patients; 102 male and 99 female (male to female ratio nearly 1:1) their age ranges from 3-65 years with mean age (29 ±13 year). Table 1 show the age and sex distribution of patients in both groups.

The factors underlying diagnostic delay, and possible relations between diagnostic delay and the wide differential diagnosis due to different anatomical sites of the appendix and the course of the disease was also investigated.

Data were analyzed using SPSS 16 (Statistical Package for Social Sciences) and Microsoft office Excel 2007. Numerical variables were presented as mean ± SE, while discrete variables were presented as number and percentage. Chi-square test was used to

compare discrete variables. P-value less than 0.05 were considered significant.

Table 1. Age and sex distribution of the patients in group A and group B and its significance

Age group	Group A (total 35)		Group B (total=201)		P value
	male	female	male	female	
3-9 years	3	4	12	13	0.6
10-19 years	2	5	33	16	0.1
20-29 years	3	2	21	16	0.4
30-39 years	3	2	13	14	0.1
40-49 years	0	4	15	14	0.1
50-59 years	2	3	7	6	0.39
60-69 years	1	1	1	3	0.37
Total	14	21	102	99	

Results

The total number of the patients was 236 patients with mean age (25±48years). In group A, there were 35 patients and include 14 male and 21 female patients(male to female ratio 2:3); while in the group B there were 201 patients; and include 102 male and 99 female patients (male to female ratio nearly 1:1). Table 1 shows the age and sex distribution of patients in group A and group B.

The anatomical site of the appendix in both groups was as follows:

Postileal appendix: in group A there were 2 male and 4 female patients; while in group B there was only one male patient which is very significant (P= 0.0001).

Subhepatic appendix: in group A there were 2 male and 2 female patients; while in group B there was only one male patient which was also very significant (P=0.0004).

Retrocacal appendix: in group A there were 6 male and 11 female patients; while in group B there were 76 male and 74 female patients which is significant (P=0.017).

Pelvic appendix: in group A there were 4 male and 4 female patients; while in group B there were 21 male and 23 female patients which is not significant (P=0.88).

Paracecal appendix: in group A there were no patient; while in group B there were 2 male and 1 female patient which is not significant (P=0.083).

Preileal appendix: in group A there were no patient; while in group B there were 1 male and 1 female patient which is not significant (P=0.95).

Unfortunately 3 patients were died in the group A in the first postoperative day due to uncontrolled septic shock which is significant (P = < 0.01). Table 2 shows the site of the appendix at the time of appendectomy in group A and B.

The patients in group A had longer hospital stay (more than two days postoperatively) due to complications (generalized peritonitis and risk of septic shock) as compared with the patients in group B whom had no serious complications and were discharged from the hospital in the 2nd postoperative day, which is significant (P = < 0.01).

Late complications after discharge from the hospital in group A was 5 (8.57%) patients, three male and two female patients had recurrent admission to the hospital due to complications (adhesions and subacute intestinal obstruction); and they were improved on conservative management which is significant (P = < 0.01). Table (3) shows the incidence of complications and mortality in both groups.

Table 2. Site of the appendix at appendectomy in group A & B patients

Site of the appendix	Group A (total 35)		Group B (total=201)		P value
	male	female	male	female	
Retrocecal	6	11	76	74	0.017
Pelvic	4	4	21	23	0.88
Postileal	2	4	1	0	0.0001
Subhepatic	2	2	1	0	0.0004
Paracecal	0	0	2	1	0.83
Preileal	0	0	1	1	0.95
Total	14	21	102	99	

Table 3. Postoperative major complications and mortality in group A &B patients

Complications	Group A=35		Group B =201	
	Male	female	Male	female
Long hospital stay (> 2 days) due to complications	14 (100%)	18(85.7%)	0	0
Generalized peritonitis	14(100%)	21(100%)	0	0
Recurrent admissions for SIO due to adhesions	3(8.57%)	2(5.71%)	0	0
Mortality (in the 1st postoperative day)	0	3(8.57%)	0	0

SIO = sub acute intestinal obstruction

All the patients 35 (100%) in group A had increased white blood cell count (more than 10,000⁄mm^3); and in many patients especially those with subhepatic appendicitis the diagnosis of appendicitis and the decision for exploration and appendectomy were based mainly on the increased white blood cells (WBC) count, because those patients were presented with acute poorly localized abdominal pain for few days without tenderness in the right iliac fossa and with absence of classical visceral-somatic sequence of pain but there was high index of suspicion of acute appendicitis (nausea, anorexia, vomiting); and their history was long enough to increase their white blood cells as in any acute inflammatory disease, so the decision of exploration and appendectomy was taken. While in group B, only 46 (23%) of the patients had increased WBC count (more than 10,000⁄mm^3); and the rest of the patients was normal WBC count because the diagnosis of acute appendicitis was early and straight forward due to the presence of the classical visceral-somatic sequence of pain and tenderness in the right iliac fossa so their WBC count range between 4000-10,000⁄mm^3 and there is no enough time for the inflammatory process to increase WBC.

Discussion

Appendectomy for acute appendicitis is the most common non elective procedure performed by general surgeons [6]. It has generally been accepted that an appendectomy should be performed within a few hours of diagnosis and that a delay in the operation may lead to an increase in incidence of the morbidity and mortality [7].

A number of clinical and laboratory-based scoring systems have been devised to assist diagnosis of acute appendicitis. The most widely used is Alvarado scale. This scoring system was designed to improve the diagnosis of appendicitis and was devised by giving relative weight to specific clinical manifestation. Patients with scores of 9 to 10 are almost certain to have appendicitis; there is

little advantage in further workup, and they should go to the operating room. Patients with scores of 7 to 8 have a high likelihood of appendicitis, while scores of 5 to 6 are compatible with but not diagnostic of appendicitis [8]. Contrast-enhanced CT scanning is most useful but it is cost effective [2]. On the other hand, it is difficult to justify the expense, radiation exposure time, and possible complications of CT scanning in those patients whose scores of 0 to 4 make it extremely unlikely "but not impossible" that they have appendicitis.[1]and it is important to remember that if the patient has Alvarado score 0 and no signs and symptoms of the classical appendicitis it is not impossible that the patient may complained of acute appendicitis due to hidden appendix and variable anatomical site of the appendix which produce non typical signs and symptoms.

Acute appendicitis is relatively rare in infants, and becomes increasingly common in childhood and early adult life, reaching a peak incidence in the teens and early 20s. After middle age; the risk of developing appendicitis is quite small. The incidence of appendicitis is equal among males and females before puberty. In teenager and young adults, the male-female ratio increases to 3:2 at age 25years; thereafter, the greater incidence in males declines [2].

In this study, the age incidence in both groups is comparable and relatively equal and no significant difference between both groups (P> 0.05). Delayed decision of appendectomy in group A is more common in female 21(60%) patients than male 14(40%) patients. In group B, the sex incidence is nearly equal (102 male and 99 female). Table 1 shows the age incidence in both groups and its significance.

In group A, the patients had acute appendicitis for more than 72 hours because they were miss diagnosed (due to absence of tenderness in the right iliac fossa and according to the anatomical variation of the appendix); and then after the 72 hours, laparotomy for acute abdomen was performed. In group A, 35(100%) had generalized peritonitis, and 3 (8.57%) patients were died in the first postoperative day due to septic shock which is significant (P=<o.o1), and 5(14.29%) patients had recurrent admission to the hospital due to complications and intestinal obstruction. While in group B, were all the patients underwent appendectomy before 72 hours of the onset of first symptom of appendicitis, all the patients 201(100%) had no serious complications and no mortality and they were discharged well in the 2nd postoperative day. Table 3 shows the incidence of major complication and mortality in both groups.

The differential diagnosis of acute appendicitis depends upon four major factors: the anatomic location of the inflamed appendix; the stage of the process (i.e., simple or ruptured), the patient's age; and the patient's sex [5].

In group A the delay in the time of appendectomy were attributed to miss diagnosis of the patient's condition due to unusual presentation and non typical signs and symptoms of appendicitis (due to absence of tenderness in the right iliac fossa according to the anatomical variation of the appendix); until the patient's condition were deteriorated and (laparotomy for acute abdomen was performed which was proved to be generalized peritonitis due to perforated appendix). Table 2 shows the anatomical sites of the appendix in this group.

The signs and symptoms of patients in group A were attributed to other diseases like gastroenteritis (in postileal appendix), ureteric colic and urinary tract infection and other causes of the wide differential diagnosis of acute appendicitis. Table 2 shows the site of the appendix at the time of appendectomy in both groups and its significance in the delayed diagnosis of acute appendicitis.

In postileal appendix; the inflamed appendix lies behind the terminal ileum away of the right iliac fossa and not in contact with the parietal peritoneum of the anterior abdominal wall of the right lower abdomen [in classical appendicitis, once the inflamed appendix been

in contact with the parietal peritoneum of the anterior wall of the abdomen in the right iliac fossa, the visceral abdominal pain will shift to the right iliac fossa because the parietal pain is more sever and more precise] [(2)]. So postileal appendicitis presents the greatest difficulty in diagnosis because the pain may not shift, diarrhea is a feature and marked retching may occur. Tenderness, if any, is ill defined, although it may be present immediately to the right of the umbilicus (ill defined deep tenderness due to pressure on the inflamed appendix behind the ileum [(2)]. So it is very significant cause of delayed diagnosis of acute appendicitis in the study group and delayed decision of appendectomy (P=0.0001).

In group A; four patients had subhepatic appendicitis presented with abdominal pain and malaise without any tenderness in the right iliac fossa but their WBC count was highly increased, and in one patient there was air under the diaphragm shown by plain X-ray of the abdomen due to perforated appendix.

An inflamed appendix in the pelvis may never produce somatic pain involving the anterior abdominal wall (because the inflamed appendix not in contact with the parietal peritoneum of the anterior abdominal wall of the right lower abdomen), but may instead cause suprapubic discomfort and tenesmus. In this circumstances, tenderness may be elicited only on rectal examination and is the basis for the recommendation that a rectal examination should be performed on every patient who presents with acute lower abdominal pain [(2)].

Occasionally early diarrhea results from an inflamed appendix being in contact with the rectum. When the appendix lies entirely within the pelvis, there is usually complete absence of abdominal rigidity. And often tenderness over McBurney's point is also lacing [(2)].

An inflamed appendix in contact with the urinary bladder may cause frequency of micturition. This is more common in children (because children have shallow pelvis and the inflamed appendix been in contact with the urinary bladder causing irritation of it and frequency of micturition [(2)]. Presence of pus cells in the general urine examination, urinary tract infection; and diarrhea not exclude diagnosis of appendicitis because of irritation to the urinary bladder and to the rectum by the inflamed appendix according to its anatomical site can produce these misleading symptoms; so acute appendicitis should always be kept in mind.

The principal factors in mortality are whether rupture occurs before surgical treatment and the age of patient. Death is usually attributed to uncontrolled sepsis-peritonitis, intra-abdominal abscesses, or gram-negative septicemia [(1)].

Unfortunately 3 patients were died in group A in the first postoperative day due to uncontrolled septic shock which is significant (P = < 0.01). The first patient was 28 year old female patient presented to the emergency department with cyanosis, cold stage of septic shock, abdominal pain in the left iliac fossa and dark color urine for more than 3 days which was miss diagnosed as urinary tract infection and ureteric colic, but proved to be generalized peritonitis due to perforated postileal appendix.

The 2nd patient was nine years old female child presented to the emergency department with acute abdomen and she was treated as (typhoid fever and abdominal pain) for more than three days but proved to be generalized peritonitis due to perforated retrocecal appendix.

The 3rd patient was seven years old female child presented with abdominal pain for more than three days and was treated with antibiotics but proved to be generalized peritonitis due to perforated postileal appendix.

In group B, tenderness in the right iliac fossa and increased white blood cell (WBC) count save life of many patients; two adult females patients were underwent cesarean section one week before they develop acute appendicitis and they improve and survive after appendectomy because of high suspicion of

appendicitis and increased white blood cells (WBC) count with tenderness in the right iliac fossa (retrocecal appendix). Other two children had viral hepatitis A and jaundice presented with acutely inflamed appendix, but they improve after appendectomy because of high suspicion of appendicitis due to tenderness in the right iliac fossa (retrocecal appendix). While in patients with postileal and subhepatic appendix there was no tenderness in the right iliac fossa (because the inflamed appendix not in contact with the parietal peritoneum of the anterior abdominal wall of the right lower abdomen).

Tenderness in the right iliac fossa is helpful in decision for appendectomy in patients with viral hepatitis in spite of jaundice and saves the patient's life; but in patients with postileal and subhepatic appendix there were no tenderness in the right iliac fossa and the patient's condition progress to perforation of the appendix and generalized peritonitis.

All the patients in group A and 46 (23%) of patients in group B had increased WBC count. In some cases the decision for appendectomy depends totally on increased WBC count like in subhepatic appendicitis of 33 years old male patient in group B (he was a surgeon).

Supine abdominal radiograph may be of benefit. Plain X-ray of the abdomen in erect position and X-ray of the diaphragm can be helpful in decision for appendectomy when there were air under the diaphragm which indicates perforated viscous.

In comparison with other studies in this field; Horwitz et al find that diarrhea is important confusing symptom in making the diagnosis of acute appendicitis in very young children [9]. Gamal *et al* showing that diarrhea is very often a concomitant symptom in appendicitis [10].

The squeal of delayed diagnosis may result from late presentation by the patient but are sometimes due to the initial failure of the clinician to make the correct diagnosis [11].

Diagnostic uncertainty due to non-classical evolution of acute appendicitis may occur when the appendix is anatomically mal-located. At any age, variation in location of the appendix due to adhesions or developmental anomalies such as fetal intestinal mal-rotation leads to non-typical presentation, delays in diagnosis and increased adverse outcomes [12]. Subhepatic appendicitis was first described in 1955 by King [13], but has rarely been reported since, and includes a case of delayed diagnosis leading to perforation [14].

Despite an increased use of ultrasonography, computed tomography (CT) scanning, and laparoscopy, the rate of misdiagnosis of appendicitis has remained constant (15.3%), as has the rate of appendiceal rupture [1].

The use of a diagnostic protocol incorporating both the Alvarado score and graded compression ultrasonography failed to produce better outcomes than unaided clinical diagnosis [15].

On the other hand; Surana et al [16] studied the effects of delaying an appendectomy for acute appendicitis. They found no statistical difference in the rate of complications between children who underwent appendectomies within 6 hours of diagnosis and those who underwent appendectomies between 6 and 18 hours of diagnosis (2.3% to 4.2%, respectively; P = 0.28). A similar study by Yardeni et al [7] on the effects of delaying appendectomies by 6 to 24 hours in children showed no significant increase in the rate of perforation, operative time, or complications when compared with children who underwent the appendectomies within 6 hours. Furthermore, some studies suggest that the rate of perforation is due to a delay in patient presentation rather than to a delay in treatment [17-18].

Conclusion

The signs and symptoms of acute appendicitis vary according to the site of the appendix; and absence of tenderness in the right iliac fossa dose not exclude appendicitis like in postileal, subhepatic and pelvic appendix. The postileal and subhepatic site of the inflamed appendix are very significant causes of the delayed decision of appendectomy. In the study group,

the incidence of major complications (generalized peritonitis and septicemia) was 100% and the incidence of mortality was 8.57% due to uncontrolled septic shock.

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Hirschsprung's Disease: a Comparison of Swenson's and Soave's Pull-through Methods

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Abstract

Background Hirschsprung's disease or the congenital intestinal aganglionosis is the result of arrested fetal development of the myentric nervous system, but the precise pathogenic mechanisms involved are unknown. The successful treatment of infants and children with Hirschsprung's disease depends on prompt diagnosis and early treatment.

Objective To compare the complications of Swenson's and Soave's pull-through procedures for the management of Hirschsprung's disease.

Methods This study was conducted by patient's relative interview in Central Teaching Hospital of Pediatrics in Baghdad for the period from June 2006 to June 2010 .All the patients were under (10) years old who were admitted and underwent surgical interference and followed up under the study group. This study included 40 infants and children with Hirschsprung's disease underwent either Swenson's or Soave's pull-through procedures. On average, the post operative follow-up period was six months after the last stage of operation (closure of colostomy).

Results The patients consisted of 32 males (80%) and eight females (20%), a ratio of 4:1. Their age ranged from 1 day to 10 years. History of failure to pass meconium was the commonest presentation and was found in (87.5%). Twenty-five patients (62.5%) underwent a Swenson's pull-through and fifteen patients (37.5%) underwent Soave's pull-through. The incidence of the complications following Swenson's procedure was 24% while after Soave's procedure was 20%. Wound infection and adhesive intestinal obstruction were the commonest complications after Swenson's pull-through and occurred in (12%) while anastomatic stricture was the commonest complication after Soave's procedure and occurred in (20%).

Conclusion The rate of complications was higher following Swenson's pull-through in comparison to Soave's pull-through procedure.

Keywords Hirschsprung's disease, Swenson's pull-through, Soave's pull through, complications.

Introduction

Harold Hirschsprung's, a Danish pediatrician is credited with the first definitive description of the disease in 1888 that bears his name [(1)]. Hirschsprung's disease or congenital megacolon is a developmental anomaly caused by migratory failure of neural crest cells. When these primitive neurogenic cells fail to take up positions in the Submucosal and intermyenteric plexus of the bowel from lips to anus, motility disturbances result; that most routinely present as chronic constipation in a newborn child [(1-4)].

Ninety-eight percent of babies pass meconium within 24-48 hours of birth. In babies with Hirschsprung's disease 90% fail to achieve this

passage. The disease progresses to abdominal distension, bilious vomiting, and possibly obstructive enterocolitis. If a neonate leaves the hospital without diagnosis, he will generally reappear with chronic constipation within two years. This constipation often accompanies a dietary change such as the change from breast milk to formula or formula to solid foods. Rarely, children escape diagnosis until more advanced ages when chronic constipation and failure to thrive are seen. The most common physical findings are abdominal distension, visible bowel loops with peristalsis, and poor muscle development secondary to poor nutrition. Rectal examination reveals a spastic rectum with no or little stool because stool is high above the spastic colonic segment.

When the clinical suspicion of Hirschsprung's disease has been raised, plain abdominal radiographs in the anteroposterior projection or in left lateral decubitus position are obtained to look for evidence of intestinal obstruction or free intraperitoneal air or both. This study can be followed by unprepared barium enema or anorectal manometry and full thickness or suction rectal biopsy. The specimen must be obtained at least 1.5 cm above dentate line [2-5].

Surgery is the only mode of treatment for Hirschsprung's disease. The child usually has a colostomy, which was placed several months previously. This colostomy decompresses the bowel and returns it to normal caliber. The definitive operation generally is performed when the child is 6 to 12 months old. Over the past 50 years, different methods of varying efficacy have been adopted to correct the underlying abnormality. The first definitive operation was described by Swenson's and Bill in 1948. This procedure involves resection of aganglionic colon bowel and anastomosis of the distal rectum to ganglionated colon by combined abdominoperineal approach. Essential to this operation is maintenance of dissection immediately adjacent to the rectal wall to avoid injury to the pelvic nerves responsible for rectal and bladder innervations and sexual function. Soave's method involves removing diseased mucosa and submucosa, followed by pulling the ganglionated intestine through the muscular cuff and end to end coloanal anastomosis [5-8].

Enterocolitis of Hirschsprung's disease remains the major cause of significant morbidity and mortality today. The entity is manifested clinically by explosive diarrhea, abdominal distension, and fever. Pathologically, enterocolitis is defined as an acute inflammatory infiltrate into crypts and mucosa of either the colonic or the small intestinal epithelium [8,9].

Methods

Forty patients with a diagnosis of Hirschsprung's disease were admitted to Central Teaching hospital of pediatrics between 2006 and 2010. A standardized data sheets were prepared for collection of information including age, sex, body weight, and natal history, family history, age at presentation, associated anomalies, methods of diagnosis, type of the surgical procedure performed for treatment and post-operative complications. All our patients underwent three staged surgical procedures including colostomy which was placed in the ganglionic segment of colon as the first stage in order to decompress the colon and return it to normal caliber while the definitive surgical procedure done in the second stage after six months from the time of colostomy. The last stage is closure of colostomy which was done after two months from definitive surgery. On average, the post-operative follow-up period was six months after the closure of colostomy (third stage).

Results

During four years period from June 2006 to June 2010; forty cases of Hirschsprung's disease (congenital megacolon) were admitted to the hospital. There were 32 male (80%) and eight female (20%) patients, with male: female ratio being 4:1. Their ages ranged from 1 day to 10 years. Table 1 shows age distribution of the studied sample.

Table 1. Age distribution of the studied sample

Age	Number of patient	%
< 28 days	7	17.5%
28 days -1 year	23	57.5%
1 year – 6 years	7	17.5%
> 6 years	3	7.5%

History of delayed passage of meconium in the first 48 hours of life was the most common mode of presentation, occurring in 35 patients (87.5%), followed by bile stained vomiting in 25 patients (62.5%), while 28 patients(70%) had abdominal distension and 30 patients (75%) presented with constipation. Other modes of presentation include fecal impaction (10%), Encopresis (5%) ad Enterocolitis (5%) as noticed in table 2.

Table 2. The mode of presentation of patient with Hirschsprung's disease

Signs and symptoms	Number of patients	%
History of Failure to pass meconeum	35	87.5%
Bile-stained vomiting	25	62.5%
Abdominal distension	28	70%
Constipation	30	75%
Fecal impaction	4	10%
Encopresis	2	5%
Enterocolitis	2	5%

Rectal biopsy was performed in 37 patients, and three patients had been referred from other hospitals with rectal biopsy already done. All biopsies were performed 2-3 cm above the dentate line, four samples were initially reported as inconclusive, but the patients continued to have symptoms and biopsy was repeated, all these samples showed the absence of ganglion cells. No morbidity or mortality was associated with these biopsies during the follow-up period. Barium enema was performed in all our patients (even in patients with acute intestinal obstruction where Ba-enema done two weeks after creation of stoma) and was diagnostic only in thirty patients (75%) showing spastic distal segment. Down syndrome was the most common associated anomaly and was present in six patients (15%).

Congenital heart disease was present in four patients (10%), and congenital hip dislocation was observed in three patients (7.5%).Other associated anomalies malrotation (7.5%), inguinal hernia (5%), umbilical hernia in(5%), annular pancreas (2.5%), and ileal atresia in (2.5%). Long segment Hirschsprung's disease (extending to colon) was the most common type as shown in table 3.

Table 3. Types of Hirschsprung's disease according to extension of aganglionic cells (independent on Ba-enema and colonic biopsies).

Surgical procedure	No. of patients	%
Swenson's pull-through	25	62.5%
Soave's pull-through	15	37.5%
Total	40	100%

Twenty-five patients (62.5%) underwent Swenson's pull-through and fifteen patients (37.5%) underwent Soave's pull-through, based on surgeon preference and operative finding if there is adhesion between mucosal and muscular layers we prefer to do Swenson's pull-through and this is usually happened in those patients who had long history of presentation with recurrent enterocolitis, while if there is no adhesion and Submucosal dissection coming easy we prefer to do Soave's pull- through which usually occur in younger age and with those who had short history of presentation.

Table 4. The surgical methods used in treating Hirschsprung's disease

Type	No. of patients	%
Short segment(localized to rectosigmoid)	11	27.5%
Long segment (extend to colon)	28	70%
Total colonic aganglionosis	1	2.5%
Total	40	100%

The incidence of complications following Swenson's pull-through was 24% and that after Soave's pull-through was 20%. The most common complications after Swenson's pull-through were wound infection and adhesive intestinal obstruction (12%), one of those patient with adhesive intestinal obstruction not responding to conservative treatment and underwent surgical treatment.

Anastomatic stricture was most common complication after Soave's pull-through and occurred in three patients (20%) all of them responding to the frequent dilatation under general anesthesia. Enterocolitis was most common after Soave's pull-through (13.4%) in compare to Swenson's pull-through (8%) all of them were admitted to hospital and treated with bowel rest and parental antibiotics. Two patients (8%) continued to be constipated after Swenson's pull-through while no patient remained constipated after Soave's pull-through (Table 5).

Table 5: Complications after pull-through method

Complication	Surgical procedure	
	Swenson's pull-through	Soave's pull-through
Wound infection	2(4%)	1(6.7%)
Anastomatic leak	1(4%)	0
Anastomatic stricture	2(8%)	3(20.1%)
Adhesive intestinal obstruction	3(12%)	1(6.7%)
Constipation	2(8%)	0
Fecal incontinence	1(4%)	0
Enterocolitis	2(8%)	2(13.4%)
Voiding dysfunction	2(8%)	0

Voiding dysfunction occurred in two patients (8%) after Swenson's pull-through while no patients developed such problem after Soave's pull-through. All those patients with voiding dysfunction treated with urinary bladder catheterization for one month; then intermittent catheterization done for another one month until they become with normal urination.

Discussion

Since 1888, when Harold Hirschsprung's presented his classical description of congenital megacolon for the first time, numerous approaches have adopted for the diagnosis and surgical treatment of Hirschsprung's disease. In this study, delayed passage of meconium was the most common presentation (87.5%) followed by bile-stained vomiting (62.5%) which similar to O'Donovan et al [5], but differ from Taxman et al (70%) [6], Stockman and Philippart (80%) [7]. Barium enema and rectal biopsy used as diagnostic procedures, barium enema was diagnostic in 75% showing spastic distal colonic segment and this result was similar to O'Donovan et al (77%) [5], but differ from Taxman et al [6] (80%) and Stockman and Philippart (85%).

We performed rectal biopsy in 37 patients using open method under general anesthesia. The failure in first attempted biopsy happened in 4 biopsies (8%), which is higher than range reported by Andrassy et al [8] (2.3%), but low in comparison to Ghos and Griffen study [9] (14%). This failure rate attributed to technical error resulting in biopsy being taken close to dentate line or too superficially.

In our center, both the multistage Swenson's and Soave's approach were used, with colostomy being performed within one month from the time of rectal biopsy .There were no intraoperative or early post-operative deaths during the period of this study. These results similar to Fortuna et al[10] and Taxman et al (70%) [6] who reported no mortality, but it was differ from those shown by Ikeda and Goto [11] as well as Kleinhaus et al [12] ,where the mortality was 0.3%.

The incidence of complications following Swenson's procedure was 24% whereas after Soave's procedure was 20%, this results are comparable with the series reported by Stockman and Philippart, who observed similar complication rates following Swenson's (22%) and Soave's procedures (18%), whereas Fortuna et al reported higher complication rate after Soave's (30%) and Swenson's (40%) procedures. In our study adhesive intestinal obstruction was commonest complication after Swenson's procedure (12%) while (6.7%) after Soave's procedure, a rate higher than Ikeda and Goto study (8% after Swenson's 4.2% after Soave's procedures).

Anastomatic stricture was more common after Soave's (20.1%) than after Swenson's procedure (8%), which is higher than Kleinhaus et al study [12] (11.3%) after soave's while 5.5% after Soave's procedures). Voiding dysfunction occurred in two patients (8%) who underwent Swenson's pull-through while no patient developed this problem after Soave's procedures and this may be attributed to massive perirectal dissection leading to injury to the pelvic nerves that supply of the urinary bladder in Swenson's procedure, but this result differ from other reported studies.

There was no mortality in our study whereas similar comparative studies (Kleinhaus et al and Ikeda and Goto studies) have shown low mortality after Swenson's (1.8%) and Soave's (2.2%). Although in our study we did three stage pull-through to reduce the incidence of postoperative complications especially Anastomotic leak, recent literatures favor the use of single stage pull-through which eliminates the complications and cost secondary to enterostomy [13-16].

Conclusion

The rate of complications is higher with Swenson's pull-through procedure more than Soave's procedure especially voiding dysfunction, postoperative adhesive intestinal obstruction and fecal incontinence.

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Enzymatic Liver Changes among Workers Exposed to Vinylchloride

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Abstract

Background Polyvinyl chloride (PVC) is used in production and manufacturing of many essential tools for example plastic pipes, fabric, cables, decorative products etc.). Its production is impossible without the use of vinyl chloride monomer (VCM), which can cause liver damage in long-term.

Objective To assess the effects of mild to moderate long term exposure to VCM on liver and to assess the importance of liver enzyme measurements as screening tools.

Methods In this study, measurement of serum levels of liver enzymes of 64 exposed workers and 61control workers was carried out starting from the first of October 2010 till the end of January 2011. All of the studied cases were worked in a poly vinyl chloride (PVC) production unit in three polyvinyl chloride factories and considered as target population for detection of any possible industrial vinyl chloride associated liver enzymes changes. The controls were randomly selected from office personnel of the same factories. Biochemical paramedics and a questionnaire method were used for analysis and in both groups.

Results Both groups have a similar age structure. Statistical difference was noted between the alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) mean values for both the exposed and non-exposed groups. The mean values for alpha-2-globulin and gamma-globulin in both exposed and non exposed groups of serum electrophoresis were statistically significant. The relative risk for the exposed workers was higher than that one for all other variables. It was the highest and most significant for gamma-globulin abnormal values associated among the exposed group followed by the relative risk of alpha-2- globulin.

Conclusion Liver function tests with serum protein electrophoresis are useful to detect hepatic damage among workers exposed to polyvinylchloride.

Key words Liver Enzymes, Workers, Protein Electrophoresis, Vinyl Chloride

Introduction

Vinyl chloride (VC) is a colorless organic gas with a sweet odor, and is used to make polyvinyl chloride (PVC) plastic and vinyl products [(1,2)]. It is used in the manufacture of numerous products in building, construction, the automotive industry, electrical wire insulation, cables, piping, industrial and household equipment, medical supplies, rubber, paper, and glass industries [(1,2)].

VC is a known human carcinogen (cancer-causing agent) [(3)]. VC is also a known genotoxicant, causing chemical alterations of DNA in tissues that may lead to cancer

following exposure of humans and experimental animals [3]. The primary target organ for VC exposure is the liver [4]. The association between angiosarcoma of the liver and vinyl chloride exposure is well documented for occupational exposures [4]. Noncancer liver pathologies have also been associated with VC exposure, including liver necrosis and cysts [4]. Several studies in experimental animal models have demonstrated that early life exposure to VC can increase susceptibility to cancer later in life [5]. VC is a synthetic chemical used as a chemical intermediate in the polymerization of PVC [6]. At room temperature and pressure VC is poorly soluble in water. Structurally, VC is a haloalkene and is related to vinylidene chloride and trichloroethylene. Human and animal data indicate that VC is rapidly and efficiently absorbed via the inhalation and oral routes, is rapidly converted to water-soluble metabolites, and is rapidly excreted. At low concentrations, VC metabolites are excreted primarily in urine, while at high exposure concentrations; unchanged VC is also eliminated in exhaled air. Overall, the data indicate that neither VC nor its metabolites are likely to accumulate in the body.

Absorption of VC in humans after inhalation exposure is rapid. A study conducted in five young adult male volunteers inhaling VC at concentrations of 7.5 to 60 mg/m^3 showed that 42% was retained, maximum retention was reached within 15 minutes, and the percent retention was independent of inspired VC concentration [7].

VC is produced on a substantial scale - approximately 31.1 million tons were produced in 2000[7]. An important subject in health preservation of workers exposed to VCM is the early detection of their effects. Unfortunately minor liver damages can be detected through routine screening tests such as aminotransferase measurement and needs more specific tests such as the measurement of others liver enzymes level [8]. It is used in the manufacture of personal protective equipment such as in the gloves material for hand protection used by the forensic scientists during crime scene investigation as it is resistant to alkalies, oil and limited concentration of nitric and chromic acids [9].

Several studies have been conducted on the detection of early effects of VCM on workers with contradictory results in many factories where the PVC workers are exposed to below the threshold levels of VCM [10]. Regarding the increase in the number of such workers in our country and no study was conducted about the problem, we designed this study.

Methods

A cross sectional study was carried out to determine the prevalence of enzymatic liver changes among exposed and unexposed workers, 64 exposed workers were compared to 61 control_workers during September 2010 through February 2011. All cases were working in a PVC production unit in three PVC factories of the national chemical and plastic industries which was established in 1983 and situated in Zafarania\Baghdad and considered as target population for detection of any possible industrial VC associated liver enzymes changes. The controls were randomly selected from office personnel of the same factories. This factory is specialized in the production of PVC granules (total product 85tons/day) which are used in decorative products, cables, houses and fabric industry.

Data collection

After explaining the objectives of the study to the workers and taking their verbal consent, the data were collected from the workers by using specially constructed questionnaire.

Demographic data gathered in the questionnaire include age, sex, marital status, weight, height, work experience, alcohol consumption, tobacco smoking, past medical history, drug history, performing heavy exercises, work history including any changes

of the job and second job, history of surgery and history of blood transfusion .

A thorough clinical examination, signs and symptoms , with special attention to the signs that may be related or associated with liver disease such as jaundice, clubbing of fingers, palmer erythema, spider naevi, ascites, hepatomegally and splenomegally. The blood samples were drawn from the workers through a venepuncture 1.0 ml of the blood was added to 0.2 ml of 0.11 molar sodium citrate in a test tube to be used for the determination of Prothrombin time (PT). The remaining part of the blood sample was allowed to coagulate, centrifuged and the serum separated was divided and stored into three labeled test tube at 20^{o}C for other measurement , the first one for the determination of total serum bilirubin (using the method of Malloy with the normal range being 0.2-1.0 mg /100ml), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP); the second one for gamma glutamyl transferase (GGT); and the third one for total serum protein and protein electrophoresis. The serum was deep freeze until used for these parameters ,usually within less than 48 hours.

Methods used for Biochemical investigations in the study:

a) Total serum bilirubin ;
b) ALT: The spectrophotometric methods of Reitman and Frankel (normal range of 2-20 I.U/L).
c) AST: The spectophtometric methods of Reitman and Frankel (normal range of 2-15 I.U/L).
d) GGT: GGT reagent cartridge kit (normal range 7-64 I.U/L) [11].
e) ALP: Spectophtometric assay was used (normal range being 3-14 K.A.U/100L) [12].
f) PT: This was done by the Quick one stage method (normal range of 10-13 second) [13].
g) Total serum protein Electrophoresis: The Biuret method for the determination of total protein in serum (normal range of 62-77 g /L) [14].

The serum electrophoresis was carried out according to normal values: albumin = 35-50 g/L, α_1-gloubulin = 1-4g/L, α_2-gloubulin = 4-8g/L, β-globulin =5-10 g/L and γ-globulin =60 - 13 g/L. All these biochemical investigations were performed in the factories of National Center of Occupational Health and Safety in Baghdad.

Data analysis*:* was done by using:

a. Descriptive statistic: tables (frequency and percentage)
b. The relative risks (RR) with their 95% confidence intervals (CI) were estimated [15].
c. Inferential statistic: t-test was used to test the statistical differences between group means (Minitab version 13)

Result

Sixty four workers occupationally exposed to VC were studied and compared with 61 non-exposed workers. Both groups have a similar age structure (Table 1) with mean of 36.79 ±8.60 years for the exposed and a mean of 37.52± 8.85 years for the non exposed workers. No statistical difference could be detected between the two age means (p>0.05).

The majority 31 (48.88%) of the exposed workers and 29 (45.31%) of the non exposed workers fall in the age group 30-39 years. All workers (exposed and unexposed were males), and all of them were Iraqis. The mean duration of employment for the exposed workers was 5.53±3.51years. Twenty nine (45.31%) have a duration of employment of 1-5 years and quite a large number 23 (35.94%) have a duration of employment 6- 10 years (Table 2). Table 3 shows positive clinical findings relevant to the liver disease in VC exposed and non exposed workers .Hepatomegally was detected in 7. 81% of the exposed and in 3.28% of the non exposed workers .Exposure to VC carries more than twice the risk for developing hepatomegaly (RR= 2.38, 95% CI=0.48 – 11.8).

Splenomegaly was found in 1.56% of the exposed while none of the non exposed

workers had such a finding. Clubbing of the fingers was detected in 4.69% of the exposed and in 1.64% of the non exposed which carries a relative risk of 2.86 and a 95% CI of 0.31-26.58. None of exposed and non exposed workers was jaundiced at the time of examination. Spider naevi, palmer erythema, ascites and other signs of liver disease were not detected in any of the studied groups (Table 4).

Table 1. Age distribution of exposed and non exposed workers to vinyl chloride

Age groups (years)	Exposed workers (64)		Non-Exposed workers (61)		mean±Sd	T test	Level of significance
	No.	%	No.	%			
20-29	12	18.75	11	18.03	26.27±1	0.3	p>0.5
30-39	31	48.44	29	45.31	34.09±2.45	0.16	p>0.5
40-49	13	20.31	13	21.31	43.18±3.18	1.23	p>0.5
50-59	8	21.5	8	13.11	53.50±1.77	0.66	p>0.5

Table 2. Duration of employment for workers exposed to vinyl chloride

Duration of employment	Number of workers	Percent of total (n=64)
≤1 years	4	6.25
1-5 years	29	45.31
6-10 years	23	35.94
≥10	8	12.5

Table 3. Clinical findings relevant to liver disease in vinyl chloride exposed and none exposed workers.

Clinical findings	exposed workers (64)		Non-exposed workers (61)		Relative risk	95% CI for Relative risk
	No.	%	No.	%		
Hepatomegally	5	7.81	2	3.28	2.38	0.84-11.83
Splenomegally	1	1.56	0	0	-	-
clubbing fingers	3	4.69	1	1.64	2.86	0.31-26.58

Table 4. Past medical history and symptoms relevant to liver disease in vinyl chloride exposed and non-exposed workers

Medical history and symptoms	exposed workers (64)		Non-exposed workers (61)		Relative risk	95% CI for Relative risk
	No.	%	No.	%		
Jaundice	2	3.13	1	1.64	1.91	0.18-20.49
Upper abdominal discomfort	17	28.56	9	14.75	1.80	0.87-3.74
Loss of appetite	8	12.50	5	8.20	1.53	0.53-4.44
Nausea	7	10.94	5	8.20	1.33	0.45-3.92
Loss of weight	3	4.69	1	1.64	2.86	0.31-26.58
Hepatitis	3	4.96	2	3.28	1.43	0.25-8.33

Table 5 shows a statistically significant difference in the ALT and GGT mean values between exposed and non exposed groups (p< 0.05). Other test i.e. total serum bilirubin, AST, ALP, and PT showed no statistically significant difference in both study groups(p> 0.05), although such values were all higher in the exposed than the non-exposed groups.

Table 5. Liver function tests in vinyl chloride exposed and none exposed workers

Liver Function Tests	Exposed workers n=(64)	Non-exposed workers N=61	T test	P value
Total serum bilirubin	0.44±0.17	0.43±0.11	0.39	P>0.05
ALT	10.06±7.39	7.10±4.89	2.65	P<0.005
AST	10.24±4.69	9.70±4.00	0.69	P>0.05
GGT	34.62±35.96	23.23±15.55	2.32	0.05>P>0.01
ALP	9.74±5.27	8.73±3.74	1.23	P>0.05
****PT**	13.03±0.26	13±0	0.88	P>0.05

* This result applies to 58 workers only

Table 6 shows the mean values for different components of serum protein electrophoresis. There was statistically significant difference between α_2-globulin mean values for both the exposed and non exposed groups (p< 0.05 and p<0.005 respectively). The mean concentration of the total protein, albumin, α_1-globulin, and β-globulins, where not statistically significantly different in both study groups (p> 0.05).

Table 6. Serum protein electrophoresis and total protein in vinyl chloride exposed and none exposed workers

Serum protein electrophoresis and total protein	Exposed workers n=(64)	Non-exposed workers N=61	T test	p- value
Albumin	3.99±0.38	4.02±0.34	-0.47	P>0.05
α_1-globulin	0.31±0.14	0.29±0.05	1.07	P>0.05
α_2-globulin	0.71±0.14	0.67±0.08	1.97	0.05>P>0.025
β -globulin	0.91±0.23	0.89±0.14	0.59	P>0.05
γ-globulin	1.57±0.77	1.13±0.27	2.54	P<0.05
Total protein	7.39±0.50	7.25±0.45	1.65	P>0.05

Table 7 illustrate the relative risk of exposed group ranged between 1.27 for AST and 2.38 for GGT with other values for total serum bilirubin, ALT and ALP falling in between. All 95% confidence intervals built around such relative risks had lower limits of less than one.

Table 8 shows that the relative risk for the exposure was higher than one for all variables being the highest and most significant (RR=3.81, 95% CI=1.12- 13.07) for γ-globulin abnormal values associated with exposure followed by that of α_2-globulin (RR=3.34, 95% CI=0.72-15.33).

Table 7. Rates for abnormal components of serum protein electrophoresis in vinyl chloride exposed and none exposed workers

Serum protein electrophoresis	exposed workers (64)		Non-exposed workers (61)		Relative risk	95% CI for Relative risk
	No.	%	No.	%		
Albumin	7	10.94	3	4.92	2.22	0.60-8.25
α_1-globulin	2	3.31	1	1.64	1.91	0.18-20.49
α_2-globulin	7	10.94	2	3.28	3.34	0.72-15-33
β -globulin	3	4.69	2	3.28	1.43	0.25-8.25
γ-globulin	12	18.75	3	4.92	3.81	1.21-13.07

Table 8. Liver function tests abnormality (rate percent in vinyl chloride exposed and none exposed workers)

Liver Function Test	exposed workers (64)		Non-exposed workers (61)		Relative risk	95% CI for Relative risk
	No.	%	No.	%		
Total serum bilirubin	2	3.13	1	1.64	1.97	0.18-21.12
ALT	9	14.06	4	6.56	2.14	0.70-6.55
AST	4	6.25	3	4.92	1.27	0.30-5.47
GGT	5	7.81	2	3.28	2.38	0.48-11.8
ALP	7	10.94	3	4.92	2.22	0.60-8.25
PT	1	1.75	0	0.00	0.00	0.00

Discussion

Clinical symptoms associated with vinyl chloride exposure were mainly that of digestive manifestations including anorexia, nausea, abdominal distention, epigasteric pain, pain in the right and left hypochodrium, and loss of weight [16]. In our study low values of digestive manifestations may be explained on the basis of low exposure levels, and that digestive manifestations associated with high exposure levels are sometimes accompanied by some neurological manifestations [17]. Such neurological manifestations were totally absent in our study. Clinical features related to liver disease that could mainly be associated with VC-exposure include hepatomegally, splenomegally, hematemesis and melena, jaundice, spider naevi, palmer erythema and ascites [18]. The low rate of hepatomegally in this study may also due to effect of transfer from one to another section in the studied factories because workers in these factories move around among different places at different intervals during the year depending on factory needs and priorities, hence they might be exposed to different (usually lower) concentration of VC than actually suggested by their working shift time. In our study only 1.56 % of exposed workers had splenomegally and none had the history of haematemesis or melena as well as spider navei, palmer erythema, jaundice and ascities were not found on clinical examination. So our result showed lower rates than those shown by other studies and such high rates in other studies may be related to more advanced stage of the disease that might have not existed in our result [19].

In the present study only 3.13 of the exposed workers had elevated total serum bilirubin also our study has demonstrated that 14.1% of the exposed workers have elevated ALT. Elevated AST were found in 6.26% of the studied cases.

Elevated ALP values were recorded in 10.9% of the exposed study workers.

The abnormalities of total serum bilirubin, ALT, AST and ALP of this study are generally lower as compared to the other studies [20]. Such discrepancy could be explained by shorter period of exposure, discontinuity of exposure and lower level of VC. In addition, the surveyed workers in other studies were usually selected on the basis of clinical manifestations beside the effect of social factors such as alcohol intake where 21.88% of our exposed workers had history of alcohol intake while others studies had high rate of alcohol intake [21]. Regarding PT and GGT, no comparable studies could be found to compare our result with. In this study there is a significant difference between the mean values of ALT and GGT for the VC in exposed and non exposed workers while no significant difference was found concerning values of total serum bilirubin, AST, ALP and PT. The significant difference of ALT may be explained on the basis that VC or it is metabolites. Also significant elevation of ALT and GGT may indicate that the effect of vinyl chloride or it is metabolites are mainly on the liver cells because mechanism of toxicity and carcinogenity of VC is hypertrophy and hyperplasia of hepatocytes and sinusoidal dilatation and destruction, hepatocyte destruction, portal tract fibrosis and binding of coloroethylene oxide (VC metabolite) to DNA and RNA [22].

For PT significant difference was not found between exposed and non exposed workers and this may be attributed to the fact that the liver changes are not so advanced to disturb the synthetic function of the liver to the extent to cause decreased clotting factors synthesis .In our study values for components of serum protein electrophoresis were done for both of studied groups. It is obvious clear to note that all of our results are lower that obtained by another studies. [22] Such different between our study and another studies may explained by more advanced liver changes which is produced by longer and higher level of exposure to VC, also such higher result in another studies could be explained by the selection of those workers [23].

In our study significant difference was found between VC exposed and non exposed workers for the means values of α_2-globulin and γ-globulin, such findings could be explained by the presence of liver injury by VC or it is metabolites [23]. Elevated γ-globulin level could be noticed whenever there is prolonged and marked immune response [24] considered VC disease an immune complex disease.

Conclusions

Our results indicate that although the laboratory results were all within normal range but liver involvement in PCV processing workers is still possible and should be given full attention in the medical surveillance of the workers. Our results showed that laboratory tests were of limited values in the identification of VC associated liver disease, but it is wise to run the usual battery of tests annually for the sake of early detection of changes that could accompany other findings detected by other methods of investigation Such as ultrasonagraphy, computerized tomography, serum bile acid levels and Indocyanin clearance test [25].

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Quantification of Pain Threshold in Parkinson's Disease

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Abstract

Background Parkinson's disease (PD) is the second most common degenerative neurologic disorder after Alzheimer's disease. Pain is one of the major clinical symptoms of Parkinson's disease, occurring in 50-83% of patients. Pathways mediating pain are complex and include basal ganglia and thalamocortical-basalganglia circuits.

Objective To quantitatively assess pain perception in Parkinson disease patients, by determining pain threshold in patients with and without pain through using electrical stimulation.

Methods A cross sectional observational study recruiting 18 patients with a clinical diagnosis of Parkinson disease and healthy controls from the neurologic unit in Al-Kadhimiya teaching hospital in Baghdad; between May 2010 to Jan 2011. There were 13 men and 5 women with a mean age of (66.5 ± 10.2 years). The control group includes 18 healthy subjects, [12 males/ 8 females] with a mean age of 56.6±6.74 years. Quantitative sensory testing was carried at the neurophysiology laboratory in Al-Kadhimiya hospital; using bipolar stimulating electrodes on the forearm, index finger, mid leg, and big toe.

Results Fourteen Out of 18 patients (77.7%) reported pain, while 4 (22.3%) had no pain. There was a highly significant statistical difference in electrical perception between the affected and unaffected side, and between Parkinson disease patients and the controls. There was no statistically significant difference between males and females [p =0.8248], and between patients with and those without pain [p =0.3279]. And between upper and lower limbs on the affected side [p =0.1412], and body side involvement whether right or left in both the patients and controls.

Conclusion Chronic pain is present in 77.7% of Parkinson disease. Patients with Parkinson disease had lower pain threshold compared to controls. The affected side had lower pain threshold. The left or right body side and gender had no effect on pain threshold.

Key worlds Parkinson disease, Pain

Introduction

Parkinson's disease (PD) is the second most common neurologic degenerative disorder after Alzheimer's disease. Pathophysiologically there is neuronal loss within the substantia nigra of the midbrain and the neocortex. Pain was reported in (50-83%) of Parkinson's disease [1-3]. Pain is a complex symptom involves sensory pathways within the basal ganglia and the thalamocortical-basal ganglia circuits. Although standard sensory assessments have proved that conduction along peripheral and central pain

pathways is normal in patients with Parkinson disease. Studies assessing more delicate sensory functions, such as spatial, proprioceptive, and tactile-discrimination sensations, showed abnormalities. There is also evidence of a dopaminergic modulation of the objective pain threshold in PD patients [3-5].

Patients with PD often have joint and muscles pains secondary to the rigidity and abnormal postures associated with the disease. Levodopa therapy as well as physiotherapy may alleviate these pains to some extent. Other causes of pain in PD include compression of nerve roots or dystonia-related muscle spasms. In rare cases, people with PD may develop unexplained burning or stabbing sensations, this type of pain, called "central pain," originates in the brain. Dopaminergic drugs, opiates, antidepressants, and other types of drugs may all be used to treat this type of pain [4-6].

The objectives of this study were to quantitatively assess pain perception in patients with PD, with or without pain using electrical stimulation to assess pain threshold.

Methods

A cross sectional observational study recruiting eighteen patients with a clinical diagnosis of PD and controls from the neurologic unit in Al-Kadhimiya teaching hospital in Baghdad; between May 2010 to Jan 2011. There were 13 men and 5 women with a mean age of 66.5 years (± 10.2, range: 54 to 79). Eighteen healthy subjects were studied as a control group [12 males/ 8 females] with a mean age of 56.6 ± 6.74 years. Patients were included if they fulfilled the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria [1]. Patients and controls were excluded from the study if they had peripheral neuropathy, joint problems and diabetes mellitus, or patients on antidepressants or antiepileptic medications. Patients consent to participate in the study was taken verbally from the patient and his or her companion; and the study was approved by ethical committee of Al-Nahrain College of Medicine. A full detailed history of the neurologic symptoms was obtained from the patient by filling a structured questionnaire form. Patients underwent a full general and neurological examination in the morning before intake of medication in the neurology unit Al-Kadhimiya teaching hospital. All patients received their last anti parkinsonian medication on the evening before examination.

Quantitative sensory testing of the patient and controls were performed at the neurophysiology laboratory in Al-Kadhimiya hospital; using bipolar stimulating electrodes on the forearm and index finger in the upper limbs and the mid leg and big toe in the lower limbs bilaterally. The electrical sensory threshold was determined by progressive increasing of intensity of stimulation starting at an intensity of zero mill amperes at a frequency of 1 hertz [HZ].

The patients indicated verbally the point at which a first sensation of pain was perceived, considered to be minimum pain thresholds and when the pain became severe, considered to be the maximum pain thresholds.

Statistical analysis: Statistical analysis was done using graph pad software. Quick calculation for scientist to analyze the difference between continuous variables. Level of significance was set at P value equal to or less than 0.05 (7).

Results

Out of 18 patients 14 (77.7%) reported pain while 4 (22.3%) had no pain. There was no statistically significant difference in pain perception from the affected side between males and females [p =0.8248] (Table 1). There was a statistically significant difference in pain thresholds between affected and unaffected sides in patients with PD [p =0.0427], and between patients with PD and controls [P< 0.0001] (Table 1). There was no statistically significant difference in pain thresholds between PD patients with and without pain [p =0.3279],

(Table 1) or upper and lower limbs on the affected side [p =0.1412] (Table 1).

Table 1. Pain threshold testing in Parkinson disease patients

Character		Minimum (mAamp)	Maximum (mAamp)	Mean (mAamp)	The two-tailed P value
Gender	Male[13] Female[5]	7 8	14 11	10±1.8 9.8 ± 1.3	0.8248
Body side	Affected side Normal side	14 10	7 19	10.5 ± 4.9497 14.5 ±6.36	0.0427
Presence of Pain	Yes No	9 8	14 18	10.5± 4.9497 13.5±6.36	0.3279
Limb affected	Upper Limb Lower Limb	8 6	18 13	13±7.07 9.5± 4.9497	0.1412

Comparison in pain threshold measured showed highly significant difference between Parkinson disease patient and the control subjects but no significant difference of body side involvement whether right or left body side in both the patients and the control (Table2).

Table 2. Pain threshold testing for different sides for the study sample

Tested Side		Pain Threshold In mAmp	t test	P value
Within Cases	Right side Left side	10.7 ± 2.3 11.9 ± 3.8	1.15	0.26
Within Control	Right side Left side	14 ± 1.8 14.4 ± 2.2	0.46	0.56
Between Cases and Control	Right side (cases) Right side (control) Left side (cases) Left side (control)	10 ± 1.6 14 ± 1.8 10 ± 1.6 14.4 ± 2.2	6.3	< 0.0001

Discussion

Pain was reported in 77.7% of parkinsonian patients in the present study. This rate is similar to the one reported in the study by Nègre-Pagès *et al* [3], but higher than the rate seen in the Tinazzi *et al* study [5].

The present study showed a significantly lower electrical pain threshold in Parkinson disease patients compared to control subjects, in agreement with the studies by Tinazzi *et al*, Schestatsky *et al*, Mylius *et al*, and Lee *et al* [5,8-10]. The present study as well as the study of Tinazzi *et al* were used electrical stimulators to assess pain threshold while Mylius *et al* determined electrical pain thresholds during painful heat stimulation (conditioning stimulation) and during innocuous stimulation (control stimulation) [5,9].

The accuracy of psychophysical results that depend on subjective reaction may be hampered in patients with disorders like PD because of the slowness of reaction of Parkinson disease, which

can cause art factual threshold elevations. The change in pain perception in patients with PD is based on anatomophysiologic studies showing that the basal ganglia contain neurons with somatosensory function [11].

The present study showed lower pain thresholds for electrical stimulation on the affected side compared to the normal side reflecting the role of central dopaminergic pathways in the generation of pain. This is in agreement with the study of Schestatsky *et al* and the known fact that levodopa therapy alleviates pain in patients with Parkinson disease [8, 12,13].

There were no significant differences in electrical pain thresholds between male and female patients with Parkinson disease in the present study as well as in other studies [8-10].

We did not find prove any significant correlation between pain thresholds and body side involvements or upper versus lower limbs. The role of the basal ganglia in pain modulation was provided by studies showing that stimulation of the substantia nigra activates neurons in lamina V of the spinal cord, resulting in inhibition of the response to nociceptive stimuli. Furthermore, the descending pain-inhibition pathway originating in the midbrain is partially dopaminergic. Nigral neurons respond to low-intensity mechanical stimulation, and striatal neurons respond to noxious stimulation. In both cases, the cutaneous receptive fields are large and bilateral and may include the whole body and this may explain the approximately similar pain thresholds of upper and lower limbs [11].

Conclusion

Chronic pain is present in 77.7% of Parkinson disease. Patients with Parkinson disease had lower pain threshold compared to controls. The body side involved and gender had no effect on pain threshold

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Immunocytochemical Study of Smooth Muscle Actin (SMA) in Fine Needle Aspiration Cytology (FNAC) of Benign and Malignant Breast Tumors

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Abstract

Background Myoepithelial cells play an important role in the interpretation of breast fine needle aspiration cytology, since these cells are believed to be a component of the benign process in the breast lesion. Myoepithelial cells distinction may be difficult occasionally, so their demonstration by immunocytochemistry method through myoepithelial cell marker like smooth muscle actin is a useful diagnostic tool to differentiate between benign and invasive malignant lesions.

Objective To study the role of smooth muscle actin as immunocytochemical marker in the demonstration of myoepithelial cell as an aid in the diagnosis of benign breast lesions in fine needle aspiration cytolog of breast.

Methods Forty five cases of fine needle aspiration cytology of palpable female breast lesions were selected. 25 out of these 45 cases comprised 20 invasive breast carcinoma and 5 fibrocystic diseases with atypia stained with smooth muscle actin by immunocytochemistry method directly and 20 cases comprised of 10 fibroadenoma and 10 fibrocystic disease stained with smooth muscle actin after destaining from H&E stain. All of these cases had a confirmatory histological diagnosis.

Results Smooth muscle actin staining consistently highlighted the myoepithelial cells in smears of all histologically proven benign lesions such as fibroadenomas and fibrocystic changes. In contrast, invasive breast cancers demonstrated absence of staining with smooth muscle actin that seen in benign breast lesions**.**

Conclusion Application of smooth muscle actin immunostaining in breast fine-needle aspirates is feasible and practical. The use of destained H&E smears provides an effective means to directly examine any atypical cluster of cells for the presence of MEC differentiation. The demonstration of presence or absence of MEC differentiation in atypical cases can provide sufficient evidence to decrease error in diagnosis (false positive) of breast fine-needle aspirates.

Keywords Smooth muscle actin, fine needle aspiration cytology, breast lesions

Introduction

The identification of myoepithelial cells (MECs) located between ductal epithelial cells and the basal lamina is useful in breast pathology for differentiating benign breast lesions from invasive breast carcinoma [(1)].

MECs also play an important role in the interpretation of breast fine-needle aspiration cytology (FNAC) [(2)].

These cells are believed to be a component of a benign process in breast lesions. Since MECs are not always easily identified in routinely stained

cytologic slides, their immunocytochemical demonstration would be a reliable ancillary study [3].

The various antibodies that have been studied for this purpose [4] include S100 protein [5], smooth muscle myoscin heavy chain (SMM-HC) [6,7] and p63 [8,9],CD10 [10] smooth muscle actin (SMA) [1,11]. Previous studies have reported that SMA appeared to be the most reliable marker for the recognition of MECs [2,3,11-16].

In the present study we apply SMA immunocytochemistry for staining breast FNAC for different benign and malignant tumors. Our purpose was to study the role of SMA as immunocytochemical marker in the demonstration of myoepithelial cell as an aid in the diagnosis of benign breast lesions in FNAC of breast.

Methods

Forty five histologically proven cases of FNAC of palpable breast lesions were selected, 20 infiltrative ductal carcinoma and 25 cases of benign lesions which were from female breast (10 fibroadenoma and 10 fibrocystic changes and 5fibrocystic changes with atypia).

Twenty five out of these 45 cases were prospective collected from breast center in Al-Kadhimiya teaching hospital and 20 were retrospective and they were retrieved from cytology files from Al-Yarmouk hospital laboratories for the period between Jan. 2010 - August 2010 (10 fibroadenoma, 5 fibrocystic changes and 5 invasive ductal carcinoma) all of these cases had a confirmatory histological diagnosis with excisional biopsy results.

Immunocytochemical staining protocol:

Prospective cases

Preparation of cytological specimen to be examined immunocytochemically was the same procedure of conventional cytology, after smearing the slides; they are quickly placed in the fixative (95% ethanol) to decrease the air drying artifact for about 20-30 min. Then we proceed in the immunocytochemistry staining protocol which is similar to immunohistochemistry (IHC) protocol except in:

1. Cytology smears are not contain wax so there was no need for overnight backing in oven and no need for putting the slides in xylol**.**
2. Cytology smears are not subjected to formalin so the epitope retrival technique is unnecessary for immunocytochemical staining of these specimens.

We begun with *Rehydration*: through descending alcohol series (Fresh absolute ethanol, 95% ethanol, 70% ethanol, 30% ethanol and distilled water) for 5 minutes for each step then ***Peroxidases block****:* 50μL of peroxidase blocking reagent was placed onto the sections and incubated for 20 minutes in the humid chamber; then washing in phosphate buffer saline. Slides are drained and blotted.

Primary antibody 50μL of pre diluted primary antibody smooth muscle actin (Dako Clone HHF35 code M0635**,** Mouse- antihuman, monoclonal) was placed onto the sections (the dilution was 1:50) and incubated in the humid chamber at 37C° for 15 minutes then we leave the humid chamber for one hour. Then we place the slides in fresh buffer bath for 5 minutes.Drain and blot gently.

Enough drops of ***secondary (biotinylated link) antibody*** (Dako, Denmark) were applied to cover the specimen and slides were incubated for 1 hour at 37C° in humid chamber. The slides were rinsed with tris phosphate buffer solution and then drained and blotted gently.

Enough red drops of ***streptavidin HRP*** **complex** reagent were applied onto the section and then were incubated for 30 minutes at 37°C in humid chamber. Slides are rinsed with PBS then drawn and blotted gently.

Substrate-chromogen solution*:* we apply enough drops of diamino benzedine (DAB) substrate chromogen solution in dark field. Substrate chromogen solution was prepared freshly in each run by adding the substrate drops in graduated

tube until 1mL then we add one drop of chromogen. The slides then are put in the humid chamber for 10 minutes at 37°C. Rinse gently with distilled water. Slides are immersed in the Mayer's ***haematoxylin*** for about 10 seconds then slides are rinsed with slowly running tap water. Slides are then immersed in distilled water for 3 minutes. The slides are drained and blotted and left to dry.

One to two drops of aquous mounting media (Dako, Denmark) are applied onto the sections and the sections are quickly covered with cover slips and left to dry overnight. Slides are examined under light microscope for the assessment of immunostaining .

Retrospective cases

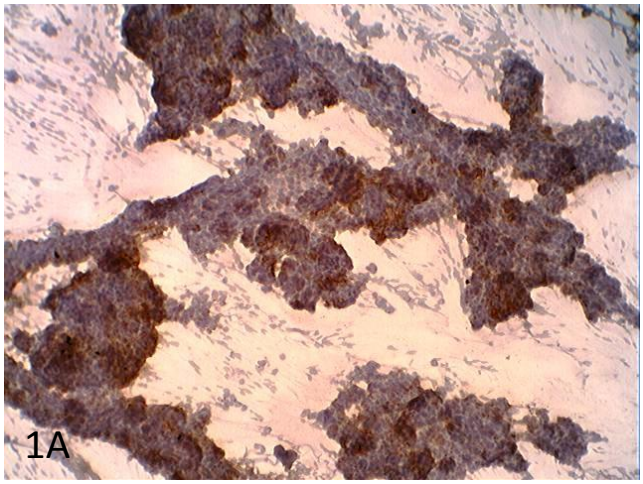
FNAC cases were selected and Immunocytochemical studies were performed following destaining of hematoxylin and eosin stained slides by immersion in 1% acid alcohol (prepared by adding 1 ml of HCL to every 100 ml of 70%of alcohol) for 10-30 min. then we proceed in the same above protocol (rehydration, peroxidase block. Slides were then incubated for 30 min in a humid chamber using a monoclonal mouse anti-human SMA. Slides were then incubated consecutively with a biotinylated anti-mouse secondary antibody, peroxidase labeled streptavidin detection, and 3-3 diaminobenzedine **(LSAB** Kit **code K0675,** Dako) and **DAB** substrate. Following hematoxylin counter staining, dehydration, and cover slipping were performed, slides were examined by light microscopy.

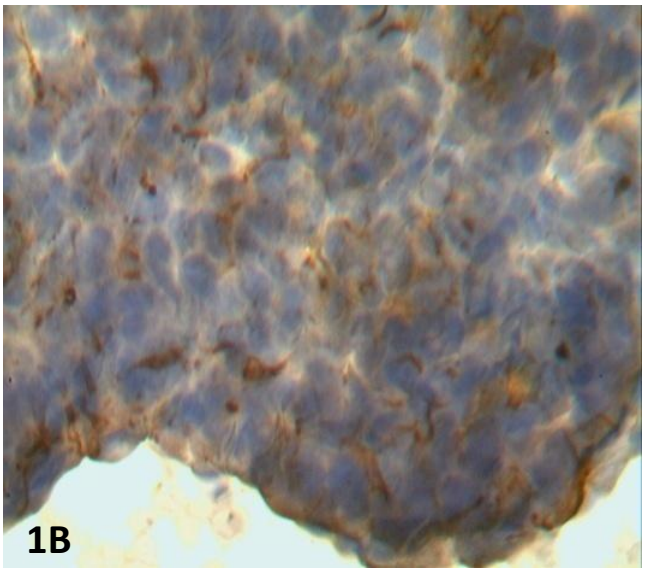
Positive control (smooth muscle of uterine leiomyoma) and technical negative control by omitting primary antibody and adding diluents only were included in each run.

All the slides were examined under light microscope. A positive immunocytochemical reaction will appear as a brownish discoloration of the cytoplasm of myoepithelial cells**.**

Results

SMA staining consistently highlighted the myoepithelial cells in both smears as well as all histologically diagnosed benign lesions such as fibroadenomas and fibrocystic disease. The SMA positivity was demonstrated predominantly in the cytoplasm of individual spindle-shaped myoepithelial cells intertwined within the clusters of epithelial cells which characteristically showed no immunostaining (Figure 1 A,B).





Some myoepithelial cells were contained along the perimeter of these fragments, and these also were decorated (Figure 1D).

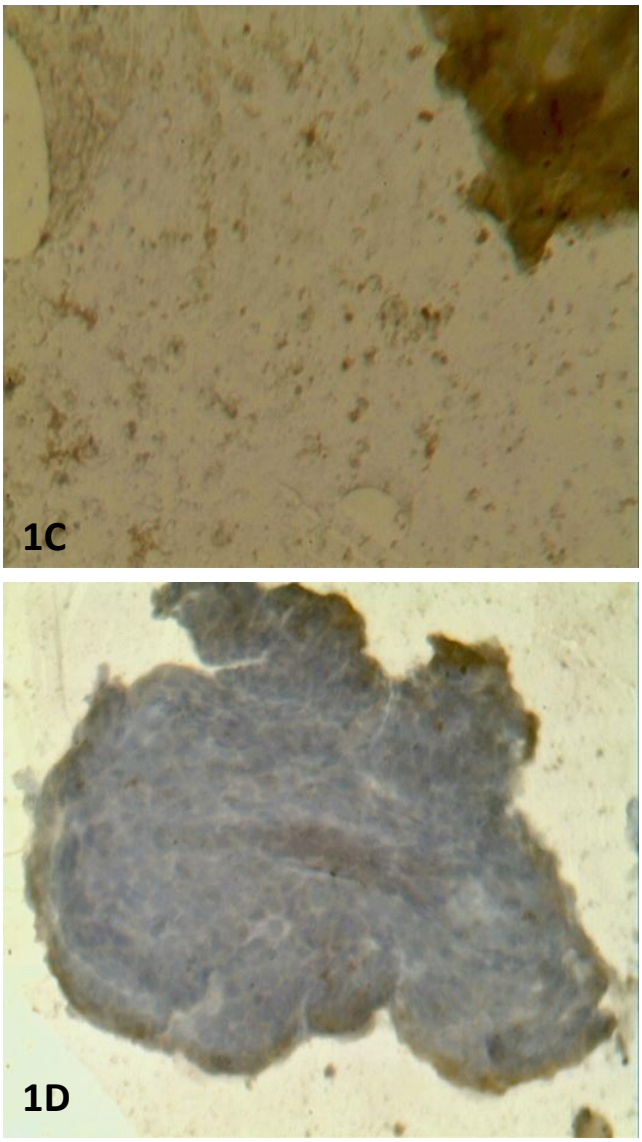
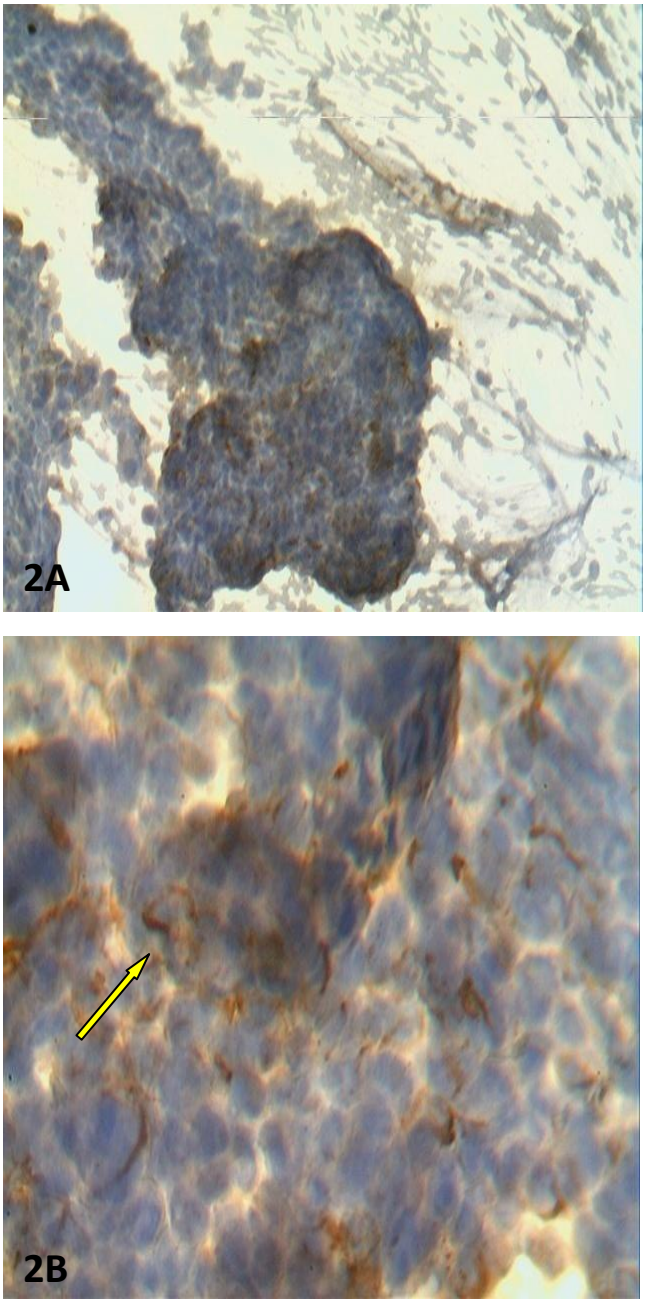


Figure 1. Expression of SMA by immunocytochemistry [A&B]: fibroadenoma showing positive staining for SMA as a brownish color [C]: fibroadenoma with positive MEC s in the background of the specimen (400X)[D]: a case of fibrocystic changes showing positive staining of the peripheral cells surround the epithelial cells (400X)

Expression of SMA was also clearly demonstrated in spindle cells scattered in the background. However, some spindle bipolar stromal cells, in the aspirates of proliferative fibrocystic breast disease or fibroadenomas, showed no immunostaining while SMA immunostaining could be seen in the adjacent cells.



In 3 out of 15 fibrocystic disease cases showed cluster of atypical cells but with abundance of MECs in the background and within the clusters these cases proved to be on histopathological examination as prolifertive fibrocystic changes with mild –moderate atypia.

In contrast, invasive breast cancers demonstrated absence of staining seen in benign breast lesions in 18 cases (Figure 2 C,D), while 2 of malignant cases showed positive staining of some scattered spindle cells in the background.

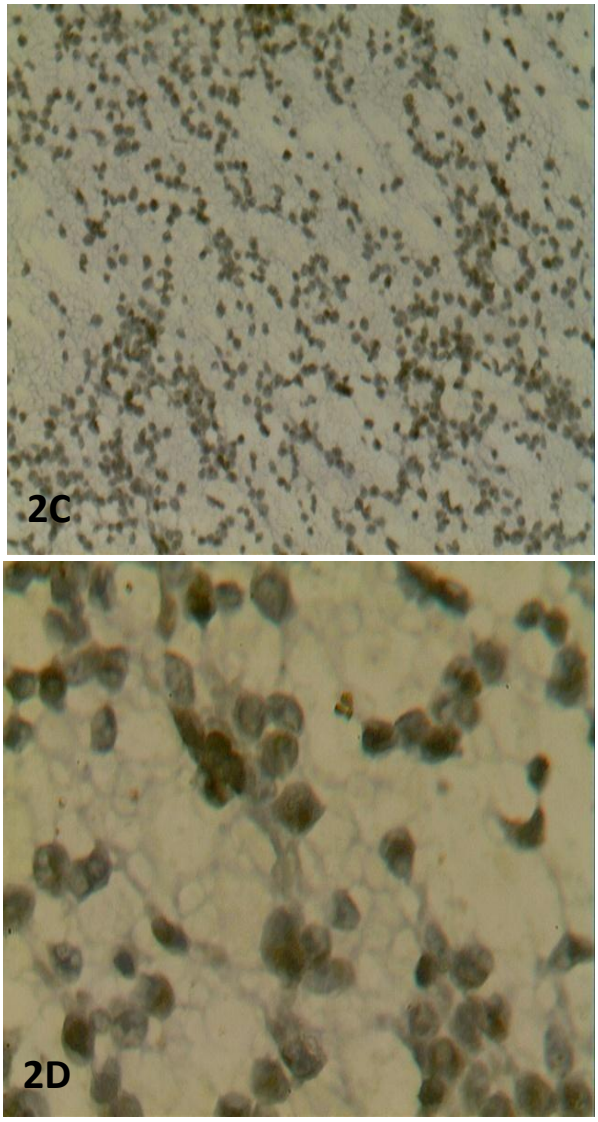


Figure 2. Expression of SMA by immunocytochemistry: [A,B]: case of fibrocystic disease with atypia showing positive staining for SMA as a brownish color in different magnification (200x, 400x respectively) [C]: invasive ductal carcinoma showing negative staining for SMA(200X).[D]. Same previous case in (Figure C) in high magnification (400X)

Discussion

As the identification of MECs is useful in breast pathology for the differentiation between benign breast lesions and invasive breast carcinoma it gains the same importance in breast cytology [(1)].

The presence of myoepithelial cell (ME) has long been recognized as a prominent feature of benign breast diseases [(11)].

In histology MECs identification based on both cytomorphologic features and their structural location on the other hand in cytology since the exfoliative cells do not demonstrate a lot of structural informations. MECs are almost always identified only by cytomorphological features. Therefore immunocytochemical staining is thus considered to be a reliable ancillary study to identify MECs [(1)].

The MEC markers were not unique to MECs. In contrast, immunostaining for SMA has been found to be the most reliable marker for the recognition of MECs [(1-3,11-15)].

The observed staining of MECs in benign and proliferative fibrocystic breast disease can be used as a strong differentiating feature in interpretation of atypical breast fine-needle aspirates.

This will potentially maximize the diagnostic accuracy of fine-needle aspiration biopsies and help to reduce the number of inconclusive cytologic diagnoses.

In the present study, spindle cells in benign cases occasionally showed no expression of SMA, while characteristic cytaplasmic staining as evidenced by SMA expression was clearly seen in the adjacent cells. This may be explained that these cells are connective tissue cells and not MECs [(6)], or these cells may be the indeterminate cells may elongate and be mistaken for the MECs by light microscopy [(2)].

The explanation to the presence of scattered positively staining cells with SMA in malignant cases is that these cells is possibly the SMA positive myofibroblast that exfoliate on cytologic smears and not MECs [1]and the tissue sections of

these cases proved to be positive for these cells, another possible explanation is that the cells had been entrapped during passage of the needle through the benign component of a malignant breast lesion [(2)].

In the present study, it was found that SMA positive myoepithelial cells are commonly seen in the background of non-neoplastic conditions.

This study suggests that the application of SMA immunostaining in breast fine-needle aspirates is feasible and practical. The use of destained H&E smears provides an effective means to directly examine any atypical cluster of cells for the presence of myoepithelial cell differentiation. The demonstration of presence or absence of myoepithelial cell differentiation in atypical cases can provide sufficient evidence to decrease the error in diagnosis (false positive) in breast fine-needle aspirates.

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Sensorineural Hearing Loss in Chronic Suppurative Otitis Media

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Abstract

Background Chronic suppurative otitis media is a common disease affecting young age group, it is typically a persistent disease, often capable of causing severe destruction and irreversible sequelae and clinically manifest with deafness and discharge.

Objectives To evaluate the degree of sensorineural hearing loss component in patients with chronic suppurative otitis media with and without pathological sequelae, which necessitate early detection as it increase in severity with time.

Methods A prospective study of 140 patients with unilateral chronic suppurative otitis media was evaluated otologically and audiologically for bone conduction hearing loss in the diseased ear throughout one year. The patients mean age was 26.5±0.5 years ranging from 10-45 years and the mean duration of the illness were 6.9±2.5 years ranging from 1-30 years.

Results Eighty-eight and a half percent of the patients with unilateral chronic suppurative otitis media had some loss of cochlear function.

Conclusion Most patients had a difference of less than 10 db in bone conduction between diseased and uninvolved ears. The range difference between diseased and uninvolved ears was wider at the higher frequencies, related mainly to the duration of the disease and its pathological sequelae such as cholesteatoma and or granulation tissue.

Key word Sensorineural, otitis media, round window, cholesteatoma

Introduction

Chronic suppurative otitis media is an inflammation of part or all of the mucoperiosteal lining of middle ear cleft. The disease has 2 types; mucosal chronic suppurative otitis media and sequamous epithelial disease (cholesteatoma).

All perforations of pars tensa are central indicative of tubo-tympanic disease. All attic disease is attico- antral and marginal.

The typical feature of atticoantral disease is the presence of a cholesteatoma (Keratinizing sequamous epithelium in the middle ear cleft [1]. The bony involvement by the disease may give rise to granulation or polyp.

Round window membrane is the only soft tissue barrier separating the middle ear from the inner ear this semipermeable membrane appears to be the main portal for the passage of noxious substance from the middle ear cavity to the labyrinth. The round window membrane in chronic suppurative otitis media commonly

changes its thickness 3-5 times than that of the control [2].

Round window membrane is permeable to certain biological substance which has the potential to cause inner ear damage leading to functional disturbance [3], it is known that endotoxin alter the permeability and penetrate the round window membrane to cause transient dysfunction of inner ear.

Paparella et al (1972) reported the presence of serofibrinous and inflammatory cells in the cochlea of mostly adult patients with chronic suppurative otitis media [4], they thought that clinically observed sensorineural hearing loss result from inflammatory and immunological effect [4].

The chronic inflammatory process can produce some circulatory disturbance such as vasodilatation and vasoconstriction of the mucosal vessels of the round window membrane which could influence the inner ear [5].

Methods

This study was carried on 140 patients complaining of long standing unilateral chronic suppurative otitis media prospectively studied and to evaluate the effect of chronic suppurative otitis media on sensorineural hearing loss.

The patients were selected in accordance with clinical criteria of at least one year prior to inclusion in this study as prerequisite for selection the normal ear was taken as control. The age ranged from 10-45 years.We considered the age, sex, duration, size, and site of the perforation and whether the ear is dry or wet.

After the patients were being examined clinically they were evaluated by pure tone audiometry examination. Bone conduction thresholds were measured at frequencies (500, 1000, 2000, 4000 H2) utilizing diagnostic audiometer model TA 155. The opposite ear was always masked while bone conduction results were obtained, statistical analyses used normal distribution statistics and unpaired t-test distribution to determine significant differences between variables.

Results

The male percentage was (47.2%) while female percentage was (52.8%), (58.5%) were right diseased ears and (41.5%) were left diseased ears. The mean age were 26.5±0.5 years ranging from 10-45 years. Discharging ears formed 77.2% while only 22.8% were dry at the examination. Sixty-Two and eight percent of patients complained of tinnitus at the time of examination.

Eighty-eight and half percent of the patients with unilateral chronic suppurative otitis media had some loss of cochlear function as showed by pure tone audiometry.

Table 1 shows the distribution of patients according to duration of pathology the mean duration of illness was 6.9 ± 2.5 years ranging from 1-30 years. Table 2 shows the distribution of patients according to the type of pathology.

Table 1. Distribution of the patients according the type of pathology

Duration (years)	No. of patients	%
1-5	84	60%
6-10	26	18.57%
11-15	12	8.57%
16-20	12	8.57%
> 20	6	4.28%

Table 2. Distribution of patients according to to the duration of pathology

Pathology	No. of patients	%
Perforated TM	104	74.28%
Granulation or polyp	24	17.14%
Cholsteatoma	12	8.57%

TM: Tympanic membrane

Table 3 shows the mean and median bone conduction in dB of each frequency in the normal ears and diseased ears. Table 4 shows mean bone conduction hearing threshold at each frequency in the diseased ear in relation to the duration of pathology.

It shows increase hearing loss with increasing duration of pathology this relation was statistically significant ($p < 0.05$). There is 15dB or greater bone conduction hearing loss at one frequency or more in 42.8% of the patients involved in the study and there is 15dB or greater hearing loss at two frequencies or more in 21.4% of the patients

Table 5 shows mean bone conduction hearing thresholds at each frequency in relation to the type of pathology. There is increase in bone conduction hearing threshold with granulation tissue, polyp, and cholesteatoma. This relation was statistically significant ($p < 0.01$). The site and size of the perforation have no significant effect on bone conduction of diseased ear.

Table 3. Bone conduction in dB at each frequency in normal and diseased ear

Frequency (Hz)		Mean (dB)	Median (dB)	Range (dB)
Normal ear	**500**	5.85± 0.14	5	0-30
	1000	7.42 ± 0.12	5	0-35
	2000	9.50 ± 0.1	10	0-35
	4000	10.71 ± 0.09	10	0-50
Diseased ear	**500**	9.00 ± 0.19	10	0-40
	1000	13.00± 0.16	10	0-45
	2000	16.57 ± 0.13	15	0-50
	4000	21.00 ± 0.12	20	0-60

Table 4. Bone conduction in dB in the diseased ear at each frequency in relation to duration of pathology

Duration (years)	500Hz	1000Hz	2000Hz	4000Hz
1-5	6.07±0.7	8.45±0.4	13.09±0.2	17.50±0.09
6-10	10.38±0.4	15.38±0.35	16.15±0.25	20.76±0.15
11-15	13.30±0.3	21.60±0.2	23.30±0.09	27.50±0.85
16-20	17.50±0.2	25.00±0.09	28.30±0.07	33.33±0.06
>20	20.00±0.1	26.60±0.08	30.00±0.05	36.60±0.04

Table 5. Bone conduction threshold according to the pathology at each frequency

Pathology	500Hz	1000Hz	2000Hz	4000Hz
Perforated tympanic membrane	8.60±0.45	10.48±0.4	15.60±0.2	20.38±0.15
Polyp or granulation tissue	13.75±0.35	15.41±0.25	19.58±0.2	22.50±0.1
Cholesteatoma	13.33±0.3	15.80±0.2	20.80±0.15	23.30±0.09

Table 6. Bone conduction difference in the diseased and normal ear calculated by T- test

Frequency	Bone conduction		Difference square	Difference (dB)
	diseased ear (dB)	normal ear (dB)		
500 (dB)	9.0	5.85	3.15	9
1000(dB)	13.0	7.42	5.58	31
2000 (dB)	16.57	9.50	9.07	82
4000 (dB)	21.00	10.71	10.29	105
Total	59.57	33.48	28.09	227

Discussion

Our results shows there is a difference in bone conduction thresholds between diseased and control ears at all frequencies particularly at higher frequencies, this difference increase with duration and the presence of pathological sequelae such as a cholsteatoma, granulation tissue and polyps these results agrees with the majority of studies done on this subject .

The mean bone conduction difference for pure tone average was 6.52±2.5dB, median of 6.3dB this finding is in agreement with that result obtained by Dumich et al (1983) who noted median bone conduction threshold difference between diseased and uninvolved ear with in 5dB [(6)].

However it has bean reported by Levine et al (1989) that the majority of patients had little difference between diseased and control ears the mean bone conduction difference for the pure tone average was 9.1dB across the frequencies [(7)].

Rice found sensorineural hearing loss of 20 dB or more in 34% of 225 consecutive ears undergoing tympanoplasty for various reasons [(7)]. Cusimano et al (1989) reported an increased mean bone conduction difference of 5.5dB for every ten years duration of chronic otitis media [(5)].

A 24 percent of sensoineural hearing loss was found, garticularly involving the higher frequencies. Moreover, the incidence of sensorineural hearing loss progressively increased with the increase in duration of chronic suppurative Otitis media. [8] SNHL occurred in 13% of the patients with CSOM, and was correlated with older age, but not with the presence of cholesteatoma or longer duration of ear disease [(9)].

The bone-conduction threshold averages for the normal side were lower than those for the ear with chronic otitis media. The threshold shift was statistically significant for each frequency ($P < 0.0001$, Student's T test).

There were differences between the groups when analyzed for age (500 and 1,000 Hz) or the presence of cholesteatoma (1,000 Hz). This study shows that chronic otitis media is associated with a decrease in cochlear function [(10)]. In our study some patients showed better bone conduction in the diseased ear, this agrees with finding of Dumich et al (1983) [(6)].

Conclusion

The majority of patients with chronic supprutive otitis media had little difference in bone conduction between diseased and control ear, clinically significant sensorineural hearing loss is uncommon.

In spite of extensive middle ear and mastoid disease were found causing significant loss of cochlear function, high frequencies were more affected.

Statistically significant sensorineural hearing loss was related to the duration of the disease and its pathological sequelae such as cholesteatoma and granulation tissue.

Sensorineural hearing loss in chronic suppurative otitis media is not as prevalent as expected to the relatively prevalent otitis media; it seems that the round window membrane provides a protective barrier to the passage of toxic materials through it to the cochlea.

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Spleen Rupture as the First Presentation of Chronic Phase Chronic Myeloid Leukemia. Case Report

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Abstract Spleen rupture is a rare life threatening emergency that mostly occurs in pathologically enlarged spleens. Causes may include infections, congenital cyst, metabolic, degenerative and malignancy (leukemia). Several possible mechanisms have been proposed for spleen rupture in patients with hematological disorders. Most commonly, malignant cells of lymphoproliferative or myeloproliferative origin infiltrate the spleen directly. Their sheer volume exceeds the capacity of the relatively non-distensible splenic capsule, causing splenic rupture and splenic hemorrhage.

In this report we present a 34 years old male patient presented with an acute abdomen after 3 days from sustaining blunt hit over the abdomen. After immediate resuscitation, exploratory laporotomy had done that revealed old altered blood in the peritoneal cavity and large spleen with non-bleeding laceration in addition to hepatomegaly and dilated mesenteric veins. Evacuation of the clotted blood was done with splenectomy.

Key word Massive splenomegaly, chronic myeloid leukemia, spleen rupture

Background

Chronic myeloid leukaemia (CML) is a BCR-ABL1+ve myeloproliferative neoplasm results from an acquired genetic change in a pluripotential haemopoietic stem cell [(1,2)].

It results from translocation of ABL1 on chromosome 9 to the region of the BCR gene on chromosome 22 (Philadelphia chromosom) [(1,2)].

It occurs in about 1.0 – 1.5 per 100 000 of the population per annum, with a median age of onset is 50 – 60 years [(1)].

Most cases of CML are diagnosed in the chronic phase [(1,2)] as the majority of patients presented with symptoms, usually attributable to splenomegaly, haemorrhage or anaemia, however asymptomatic patients are reported .The patient may have severe pain or discomfort in the splenic area, often associated with splenic infarction [(1)].

The peripheral blood smear shows leukocytosis; owing to neutrophils in different stages of maturation [(1-3)]. Blasts are usually less than 2% of the leucocytes, but absolute basophilia is invariably present [(1,2)]. Bone marrow specimens show increased cellularity owing to granulocytic proliferation with a maturation pattern similar to that in the blood, and blasts account for fewer than 5% of the nucleated bone marrow cells [(1-3)].

We report a case of a Philadelphia positive chronic myeloid leukemia which presented

with spontaneous splenic rupture as the initial manifestation of the disease.

Case presentation

A 34 years old male patient presented to emergency ward complaining of an upper abdominal pain following simple hit by blunt object 3 days before.

It starts as suddenly as sharp pain at the upper abdomen radiated to left shoulder that embarrassed his breathing. Initially it managed conservatively with pain killer. On the 3th post-trauma day, the patient developed an increasing agonizing abdominal pain and consulted the emergency department at Al-Kadhimiya teaching hospital.

His story dated back to the last 6 months where he bothered by abdominal distention mainly after meal associated heaviness like pain over the left upper quadrant, in addition to intermittent low grade fever and unexplained bone aches. There was no jaundice. He reported weight loss of 6 kilogram but he didn't seek any medical advice.

Past medical history was unrevealing and no special drug history and not smoker.

The emergency surgical team received the patient as an acute abdomen. Initially the pulse is bounding with rate of 116/minute, blood pressure 100/60 mmHg, and respiratory rate 25 cycle /minute, shallow and thoracic in character in fully conscious anxious patient. Abdomen was tender with board like rigidity and negative bowel sounds. Immediate resuscitation offered including intravenous lines with administration of normal saline and meanwhile abdominal US was done for him which revealed the presence of large amount of clotted blood (thick fluid) with huge splenomegaly and moderate hepatomegaly. A decision for surgical operation was made. Two pints of blood prepared and consent was taken.

Exploratory laparotomy was made through a midline incision, old altered blood found in the peritoneal cavity, large spleen with non-bleeding laceration (Figure 1) and hepatomegaly with dilated mesenteric veins. Evacuation of the clotted blood was done with splenectomy (Figure 2) and liver biopsy.

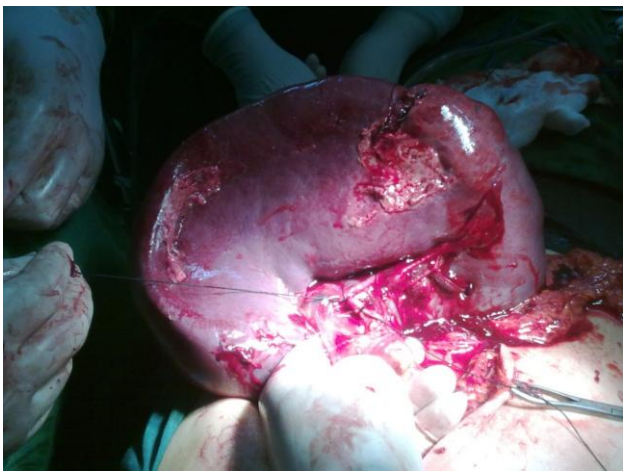

Figure 1: peritoneal cavity with altered blood and laceration in splenomegaly

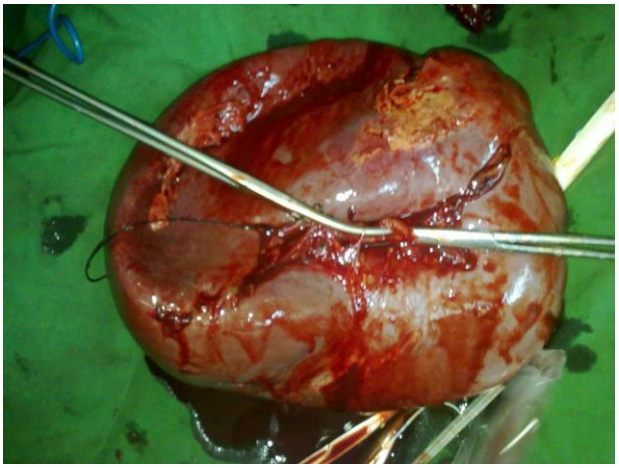

Figure 2: Splenctomy

The patient had a smooth postoperative recovery without major complications. Post operatively; re-evaluation investigations: Hb 8.3 g/dl, WBC 139 x 10^9/ml (differential count; N: 44%, L: 3%, M: 3%. E: 2%, B: 3% with presence of immature granulocyte as metamyelocyte 4%, myelocyte 37%, promyelocyte 1% and blast 3%) and platelet of 1723 x10^3/ml

Blood film confirms leucocytosis with basophilia, left shift and double peaks in granulocyte series, with marked thrombocytosis.

Bone marrow study including aspiration and biopsy revealed hyper cellular marrow with increase in M: E ratio. Erythropoiesis, graulopoiesis (in all stage of maturation) and megakaryopoiesies were hyperactive and blast cells formed only 3% with no increasing in fibrosis.

Peripheral blood sample in heparinised tube were aspirated for FISH study to look for Philadelphia chromosome (BCR-ABL proto-oncogen) and it revealed that t (9; 22) BCR-ABL fusion proto-oncogen presented in 93% metaphases.

Histological examination of the spleen and liver biopsy was that of chronic mylocytic leukaemia with liver infiltration.

Other laboratory assays reveal elevated serum uric acid 480 micromol/l, with normal liver function test and normal renal function. Post operative ultrasound was normal apart from splenectomy. Cardiac assessment was normal by ECG and echocardiography.

Initial treatment given in addition to antibiotics includes hydroxyurea 2000 mg /day with aspirin 100 mg /day and allopurinol 300 mg /day maintained for 10 days until preparation of imatinib mesylate therapy which had been used in dose of 400 mg /day. Initial re-assessment 3 weeks later reveled normalization of CBC as Hb. 13 g/dl, WBC 10 000/ml (differential count: N: 65%, L: 30%, M: 1%, B: 2% and E: 2% with no immature cells. Platelet of 800 000/ml.

Discussion

Splenic rupture is a rare clinical entity with grave consequences, if unrecognized and untreated. It mostly occurs in pathologically enlarged spleens but cases of spontaneous rupture in a histologically proven normal spleen have been reported [3].

Spontaneous splenic rupture has been reported as the presenting symptom in patients with CLL [4], lymphoma [5] and acute myeloid leukaemia [6].

The incidence of splenic rupture in leukemia is about 0.72% [2]. Although the mortality rate is high, prompt diagnosis and appropriate surgery can save the life of the patient [3-11].

Rupture of the spleen is usually associated with trauma [3-11]. Causes of pathological rupture [3-11] of the spleen have been reported as follows: [1] infections [i.e., viral (infectious mononucleosis), parasitic (malaria), and bacterial (abscess)], [2] congenital (i.e., cyst), [3] metabolic (i.e., Gaucher's disease), [4] degenerative (i.e., amyloidosis), [5] malignancy (i.e., leukemia).

Several possible mechanisms have been proposed for splenic rupture in patients with haematological disorders. Most commonly malignant cells of lymphoproliferative or myeloproliferative origin infiltrate the spleen directly. Their sheer volume exceeds the capacity of the relatively non-distensible splenic capsule, causing splenic rupture and splenic haemorrhage [7].

Three mechanisms of rupture of the spleen in leukemia [8-11] were described as follows: [1] mechanical effect of distension secondary to leukemic infiltration of the spleen, especially the capsule; [2] splenic infarct with capsular hemorrhage and subsequent rupture; [3] defects in blood coagulation.

Rupture probably results from a combination of these mechanisms rather than from any single mechanism

The choice of treatment for spontaneous splenic rupture is not only determined by haemodynamic stability, amount of blood products used but also by the underlying pathology [8].

Splenic artery embolisation may be used as an adjunct to non-surgical management of splenic injury [9].

There has been a shift towards non-operative management in haemodynamically stable patients to reduce risk of post splenectomy infection [10].

When surgery is undertaken, spleenoraphy, partial or total splenectomy may be performed depending on the extent of injury. Abdominal compartment syndrome may be another indication for emergency laparotomy in

patients with massive intra-abdominal or retroperitoneal haemorrhage [8-11].

There was evidence of a coagulation defect in this CML patient; platelet and von Willebrand's factor dysfunction which could presumably have played a role in the pathogenesis of splenic rupture [11].

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