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Genomic Biomarkers in Endometrial Carcinoma

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Abstract

Endometrial carcinoma is the second most common gynecological cancer in developing countries after cervical carcinoma and its incidence is increasing due to the rise in the rate of obesity. Diagnosis depend on invasive test (biopsy) with no routine screening investigation available for either general population or high-risk group, there are several types of biomarkers that can be used for diagnosis, prognosis and management but none are available for routine clinical practice. Following the discovery of the new gene-based classifications of endometrial cancer, the use of these gene-based biomarkers will be the cornerstone in the early diagnosis and management for endometrial carcinoma patients in the coming years.

Keywords Endometrial carcinoma, screening, PTEN, miRNA, P53, circulating tumor DNA, genomic classification

Citation Kareem NM. Genomic biomarkers in endometrial carcinoma. *Iraqi JMS*. 2019; 17(2): 100-102. doi: 10.22578/IJMS.17.2.1

List of abbreviation: ctDNA = Circulating tumor DNA, POLE EDM = Polymerase E mutated, MMR-D = Mismatch repair deficient, PTEN = The phosphatase and tensin homolog,

Endometrial carcinoma represents the most common gynecologic malignancy in developed countries and the second most common gynecologic malignancy in developing countries after cervical cancer ^(1,2). It is anticipated that the incidence of uterine cancer will increase to a higher rate worldwide in the following years due to the increasing rate of obesity. Most patients present in the post-menopausal years of age, the peak incidence at 70-74 years. The principal clinical presentation of endometrial carcinoma is post-menopausal bleeding. 81–83% of patients will be discovered at stages I–II ⁽³⁾. The 5 years survival rates decrease dramatically from Stages I (95%) reaching down to 14% in stage IV ⁽⁴⁾. Histologically there are two major categories of endometrial carcinoma; each has a different set of risk factors: type I

(endometrioid) and type II (non-endometrioid, e.g., serous, squamous, clear cell, undifferentiated and carcinosarcoma). The endometrioid types, which represent about 85% of the cases are typically estrogen dependent and low grade. Nevertheless, grade three tumors are more aggressive with overlapping clinical features with Type II endometrial carcinomas ⁽⁵⁾.

Screening

Currently there is no routine screening for the general population for endometrial carcinoma. Since Lynch syndrome carries a high risk of developing endometrial cancer reaching to 60% lifetime risk, regular follow up with ultrasound and endometrial biopsy are offered to Lynch syndrome female patients and their first-degree relatives starting from 35 years of age, but these measures have not been shown to result in an earlier finding and diagnosis of endometrial carcinoma ⁽⁶⁾.

Biomarkers

Biomarker can be defined according to The National Cancer Institute as 'a biological molecule found in blood, other bodily fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease' ⁽⁷⁾, the biomarkers can be used for diagnosis, prognosis, screening or treatment monitoring. many types of biomarkers had been researched focusing on predicting the probability of emergence of endometrial carcinoma from endometrial hyperplasia but none of them are available in practice ⁽⁸⁾.

Genomic biomarkers

P53

P53 is a tumor suppressor gene, which can act as a trigger to cellular responses that can lead to cell-cycle arrest, differentiation, apoptosis, senescence, inhibition of angiogenesis, and DNA repair ⁽⁹⁾. Many studies investigate the role of p53 in both endometrial hyperplasia and endometrial carcinoma like D'Andrilli et al. ⁽¹⁰⁾ who found that p53 gene mutation is present in the aggressive variant of endometrial carcinoma and undetectable in the hyperplastic endometrium.

Mirakhor Samani et al. ⁽¹¹⁾ who had investigated the expression of p53 in endometrial hyperplasia, endometrial carcinoma and normal endometrium and concluded that p53 overexpression is found in endometrial carcinoma and can be used for risk stratification and screening purposes.

PTEN

The phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that plays a vital role in preserving the chromosomal stability ⁽¹²⁾. Mutation of PTEN is the most common early genetic change in type I endometrial carcinoma, found in 83% of cases ⁽¹³⁾. Abd El-Maqsoud et al. ⁽¹⁴⁾ had suggested that PTEN expression has a role in early stages of endometrial carcinoma, however, a recent study done by Raffone et al. ⁽¹⁵⁾ showed that PTEN expression has a low diagnostic usefulness in differentiating between

endometrial hyperplasia and endometrial carcinoma and its use should be reconsidered.

Genomic classification and next-generation sequencing

Recently, The Proactive Molecular Risk Classifier for Endometrial Cancer had classified endometrial cancers into four genomic subtypes: polymerase E mutated (POLE EDM), mismatch repair deficient (MMR-D); p53 wild type and p53 abnormal. POLE EDMs had a favorable prognosis and tends to occur in thin and young women. MMR-D tumors were similar to POLE pathologically but with a worse outcome, this subtype may be related to Lynch syndrome so genetic test is mandatory in this category. The highest percentage of high grade, non-endometroid tumors were in the p53 abnormal category with the worst prognosis ⁽¹⁶⁾. These genomic and clinical classifiers may assist in risk-Stratify patients for chemotherapy or radiotherapy treatment and come up with a personalized follow up plans for each patient ⁽¹⁷⁾.

MicroRNAs

Small non-coding RNAs are contributing in different transcriptional processes, including carcinogenesis, and can be found in several body fluids ⁽¹⁸⁾, some miRNAs can be used to distinguish between early and advanced endometrial carcinoma ⁽¹⁹⁾. 18 urine cell-free miRNAs were investigated in a pilot prospective study of endometrial and ovarian cancers and showed a prominent suppression of MiR-106b in endometrial cancer ⁽¹⁸⁾. These urine microRNAs can be considered as potential biomarkers in gynaecological cancers.

Circulating tumor DNA

Circulating tumor DNA (ctDNA) has been documented as a useful biomarker in early diagnosis of cancer. They can detect both genetic and epigenetic mutations. They are observable in plasma, eliminated by the kidneys and so they can be detected in urine. ctDNA had been extensively studied in different cancers such as, prostate, breast, lung and colorectal. Regarding endometrial

carcinoma there are some continuous studies, the result of which are awaited ⁽²⁰⁾.

For the time being, there is no biomarker that can be used routinely in endometrial cancer diagnosis and prognosis assessment. Molecular biomarkers cannot be detected without tissue, which is obtained by surgical procedures causing morbidity and mortality. After the emergence of new genomic classification of endometrial carcinoma further studies is needed for a better management of endometrial cancer patients.

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Assessment of Serum Zinc Level in Patients with Atopic Dermatitis

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Abstract

Background	Atopic dermatitis (AD) is a common inflammatory skin disease with a chronic relapsed-remitting course with manifestations started at early childhood. AD is two types; extrinsic and intrinsic. Zinc has a crucial role in the immune system functions and antioxidant mechanisms, it also serves in the metabolism and cell growth through signaling several enzymes.
Objective	To assess serum zinc level in patients with childhood AD.
Methods	A case-controlled study was conducted in Dermatology outpatient's clinic in Al-Imamein Al-Kadhimein Medical City, from the period of September 2016- June 2017. Twenty patients with AD were enrolled in this study. AD severity was graded using the Scoring Atopic Dermatitis Index (SCORAD). The control group consisted of age-matched and sex-matched twenty healthy children were taken with weight above 80%. Fasting blood samples were taken from patients and controls between 8 AM and 10 AM.
Results	Of the twenty patients of AD, 13 (65%) were males and 7 (35%) were females. Their mean age was 4.58 ± 3.13 . Regarding the control group 9 (45%) were males and 11 (55%) were females, their mean age was 4.29 ± 2.46 . No statistical difference in serum zinc level between AD patients and control groups. Concerning zinc level in males and females, no statistical differences was found in AD patients, but highly significant difference was found in control group. Serum zinc level was highly significantly low in patients with moderate AD than in those with mild AD. There was negative correlation between serum zinc level and severity of AD.
Conclusion	There is a negative correlation between serum zinc level and severity of AD, also there is a gender variation in serum zinc level in normal children.
Keywords	Serum zinc level, atopic dermatitis
Citation	Farhood IG, Ahmed MA, Al-Bandar RT, Farhood RG. Assessment of serum zinc level in patients with atopic dermatitis. Iraqi JMS. 2019; 17(2): 103-107. doi: 10.22578/IJMS.17.2.2

List of abbreviations: AD = Atopic dermatitis, SCORAD = Scoring Atopic Dermatitis Index, Th = T helper cells

Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease with a chronic relapsed-remitting course with manifestations started at early childhood ⁽¹⁾. It affects 10% of children and 2% of adults and causes a high impact on quality of life since ⁽²⁾.

There are 2 types of AD; Extrinsic AD which exhibits immunological deviations consists of increased total IgE levels, multiple Type-I sensitizations to several inhalant and/or food allergens and a CD4 dominated cellular infiltrate in the skin ⁽³⁾. While; immunological phenomena are not present in patients with intrinsic AD and the cellular infiltrates are CD4, but also CD8 positive ⁽⁴⁾. Also, there are

physiological and biochemical defects of the skin barrier structure ⁽⁵⁾.

Zinc is one of essential trace mineral act as a key to nutrition and good health with zinc has a crucial role in the immune system functions and antioxidant mechanisms, it also serves in the metabolism and cell growth through signaling several enzymes. It is important for all cell proliferation. A sufficient daily intake of zinc is required for the proper immune function because there is no specific storage system ⁽⁶⁾.

This study was done to assess Serum zinc level in patients with childhood AD.

Methods

A case-controlled study was conducted in Al-Imamein Al-Kadhemein Medical City, in Dermatology outpatient’s clinic from the period of September 2016 to June 2017.

Twenty patients with AD were enrolled in this study, Patients with asthma, allergic rhinitis, any acute/chronic diseases were excluded. Also, those patients taking systemic corticosteroid, zinc supplement, antibiotics were excluded. Severity was graded using the

Scoring Atopic Dermatitis Index (SCORAD) ⁽⁷⁾. According to SCORAD, AD was classed as mild (SCORAD <25), moderate (SCORAD 25-50), or severe (SCORAD >50) ⁽⁷⁾. The control group consisted of age-matched and sex-matched twenty healthy children.

Fasting blood samples were taken from patients and controls between 8 AM and 10 AM.

Control group consists of twenty healthy children were taken with weight above 80% and having no acute or chronic problem at the time of collection of samples.

Results

Twenty patients were enrolled in this case-controlled study, 13 (65%) male patients and 7 (35%) female patients with atopic dermatitis. Their aged were ranged from 1-10 years with mean±SD 4.58±3.13.

Twenty other children enrolled as control group, 9 (45%) males and 11 (55%) females, their age ranged from 1-12 years with mean±SD 4.29±2.46 as shown in table (1).

Table 1. Comparison between patients with atopic dermatitis and control groups in regards to age and sex

Parameter		Patients N=20	Controls N=20	P value
Age (yr)	Mean±SD	4.58±3.13	4.29±2.46	0.743*
	Range	1-10	1-12	
Sex	Female	7	11	0.341**
	Male	13	9	

* unpaired ttest, ** Fisher exact test

We found no statistical difference in serum zinc level between patients with AD and control groups as shown in table (2).

There was no statistical difference in the level of serum zinc between males and female’s patients with AD but there are highly significant differences in the level of serum zinc

between males and females in control groups as in table (3).

Serum zinc level was highly significantly low in patients with moderate AD than in those with mild AD as in table (4).

There was negative correlation between serum zinc level and severity of AD as in table (5).

Table 2. Comparison of serum zinc level between patients with atopic dermatitis and control groups by unpaired ttest

Serum zinc	Patients N=20 Mean±SD	Controls N=20 Mean±SD	P value
(µg/dl)	85.82±7.35	86.02±7.38	0.932

Table 3. Comparison of serum zinc level between patients with atopic dermatitis and control groups in regards to sex by unpaired ttest

Serum zinc (µg/dl)	Patients N=20 Mean±SD	Controls N=20 Mean±SD	P value
Females	87.19±6.63	81.99±7.27	0.146
Males	85.08±7.87	90.93±28.97	0.052
P value	0.555	0.004	

Table 4. Comparison of serum zinc level between mild and moderate severity atopic dermatitis patients by unpaired ttest

Serum zinc	Mild N=12 Mean±SD	Moderate N=8 Mean±SD	P value
(µg/dl)	90.79±4.33	78.35±3.31	<0.001

Table 5. Correlation between serum zinc level with age and severity in patients with atopic dermatitis

Parameter	Serum zinc (µg/dl)	
	r	p
Age (yr)	0.376	0.102
Severity	-0.881	<0.001

Discussion

AD is a complex disease influenced by genetic predisposition and environmental factors with chronic nature ⁽⁸⁾.

Zinc is one of the most essential nutritional elements for human body with antioxidant importance since elevated oxidative stress (OxS) plays a role in the pathophysiology of childhood AD as well its importance in cell

proliferation and differentiation ⁽⁹⁾. Zinc has a critical role in immune system cells proliferation, apoptosis and differentiation. Also, it aids in T cell activation, T helper (Th) cells differentiation into their different subgroups (Th1, Th2, Th17, regulatory T cells (Treg)) ⁽⁶⁾. Normal value of serum zinc level in males = 70-125 µg/dl and in females = 68-115 µg/dl ⁽¹⁰⁾. Mild deficiency of zinc led to

reduction in Th1 functions, as measured by the production of (interferon gamma) IFN- γ , interleukin 2 (IL-2), and tumor necrosis factor- α (TNF- α). Thus, zinc deficiency in humans resulted in an imbalance between Th1 and Th2 cells ⁽¹¹⁾.

In the current study, serum zinc level was within the normal range in both patients and control group, and there was no significant difference in serum zinc level between both groups, which is consistent with other studies ^(12,13), and differ from the results of other studies which found low serum zinc level in patients with AD ^(14,15). There was no clear explanation for the differences in serum zinc level but it could be due to confounding factors for serum zinc quantification include diet, co-medications, co-morbidities, risk of external contamination during specimen collection or analysis, Improper specimen processing, hemolysis can also falsely increase zinc concentration & can contribute to falsely elevated concentrations ⁽¹⁶⁾.

It is well known that free zinc is mainly inside the cells and the required zinc can be provided by the plasma. About one percent of the total body content represented by the serum zinc pool, so zinc transporters and zinc binding molecules tightly controlled the intra cellular zinc level for important physiologic functions. Zinc transporter are found in plasma and found also on intracellular membranes. If there is any defect in the transport mechanism; low cellular zinc levels could be found even if it is normal in the plasma ⁽¹⁷⁾.

In the current study, there was no statistical difference in serum zinc level between males and female's patients with AD, which differs from other study were they found sex differences ⁽¹⁸⁾, and that could be due to differences in serum albumin concentrations and in lean body mass since concentrations of albumin and zinc in serum were strongly correlated because 80% of zinc in the circulation is bound to albumin ⁽¹⁹⁾.

Serum zinc level was highly significantly low in patients with moderate AD than in those with mild AD, low zinc level could affect the severity of AD as it causes membrane barriers' problem, which could increase trans epidermal water

loss, which lead to xerotic skin, and easier allergens penetration ⁽²⁰⁾.

This study showed a negative correlation between serum zinc level and severity of atopic dermatitis, and no similar result was found previously. This may require to measure erythrocyte zinc levels, which may suggest a probable intracellular zinc transport defect and AD progression.

The current study concluded that there is negative correlation between serum zinc level and severity of AD. There is gender variation in serum zinc level in normal children.

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Author contribution

Dr. Farhood: Study conception and design, acquisition of data, interpretation, reasoning and critical revision, Dr. Ahmed: statistical analysis and final revision of the article, Dr. Al-Bandar: collecting data, Dr. Farhood RG: Drafting of manuscript.

Conflict of interest

The author declares that there is no conflict of interest.

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Acute and Subacute Toxicity of Chloroform Extract of *Xanthium strumarium* Leaves

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Abstract

Background The incidence of cancer is increasing worldwide. *Xanthium strumarium* may possess anticancer activity, the plant extract for in vitro anticancer activity against a panel of three human cell lines (Breast MCF7, Renal TK10 and Melanoma UACC62), exhibit anticancer activity against these three human cell lines screened by National Cancer Institute.

Objective To investigate the safety of chloroform extract of *Xanthium strumarium* leaves, which showed potent cytotoxic activity against tumor cells.

Methods The leaves were dried and grounded into fine powder, and extracted with chloroform, that showed potent cytotoxic activity has been tested against two animal species mice and rats for testing its safety. Toxicity was evaluated in Swiss albino mice by feeding with serial doses of extract between 1.0 to 20.0 gm/kg orally and observed continuously for the first 4 hr and hourly for the next 24 hr, then every 6 hr for 48 hr (72 hr, acute toxicity). Rats were also fed with extract single dose of 5 gm/kg, the toxicity was carried out by assessing the effects on biochemical parameters, body weight and relative organ weights for both male and female rats.

Results LD₅₀ of chloroform extract was 3.07 gm/kg. The biochemical finding showed no significant differences compared to control. No significant weight changes occur throughout the study.

Conclusion Chloroform extract has low LD₅₀ (3.07 gm/kg), but the acute toxicity study showed no mortality or signs of toxicity, with non-significant changes in body weights, relative organ weights, and biochemical tests among treated groups compared to their controls.

Keywords *Xanthium strumarium*, chloroform extract, cytotoxic herb, acute toxicity

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List of abbreviations: None

Introduction

X*anthium strumarium* L. (Family: Compositae) a medicinal plant commonly found as a weed. It has several health promoting benefits including; antibacterial, antifungal, antimalarial, antiinflammatory, antinociceptive, antihypoglycaemic, diuretic, analgesic,

antioxidant, antitumor, anticancer, antimitotic, and insecticidal activities^(1,2).

Xanthium strumarium may possess antimitotic components. In a study, the plant was screened for its antimitotic activity using the microtubule-tubulin system isolated from mammalian tissue. The separated fractions obtained were identified and used for in vitro polymerisation studies. The whole as well as partially separated chemical constituents showed effective inhibition of tubulin

polymerization⁽³⁾. Also possesses anticancer activity. Two xanthanide sesquiterpene lactones, 8-epi-xanthatin and 8-epi-xanthatin-5 β -epoxide, isolated from the leaves demonstrated significant inhibition on the proliferation of cultured human tumor cells, i.e. A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (CNS) and HCT-15 (colon) in vitro. They were also found to inhibit the farnesylation process of human lamin-B by farnesyl transferase, in a dose-dependent manner in vitro⁽¹⁾. LD₅₀ is represent standard measures for acute toxicity, it stated in mg of herbal crude extracts per kg of the weight of tested animals. This dose considered the single dose that kill 50% of total tested animals. The lower LD₅₀ dose, the higher toxic substance⁽⁴⁾. LD₅₀ as a mean for identifying the required dose for in vivo experiment and latterly clinical trials; as the starting dose is 10% of LD₅₀⁽⁵⁾. Moreover, acute toxicity account for more than identifying the lethal dose but goes further to identify the changes that may the administered herb produce in the animals. Rats were used for acute toxicity while mice were used in LD₅₀ identification, in order to achieve 2 different species at least to be tested for identifying the acute toxicity⁽⁶⁾.

The aim of this study is to investigate the safety of chloroform extract of *Xanthium strumarium* leaves.

Methods

Extraction

Fresh leaves of *Xanthium strumarium* were collected in August 2016 from farms (Salamiyat county, Baghdad, Iraq) and authentication was done in Pharmacognosy and Medicinal Plants Department, College of Pharmacy, Mustansiriyah University prior of purchasing. The leaves were cleaned and shade dried at room temperature, then grinded into fine powder. The total of 600 g powder was divided into four equal parts and extracted via chloroform in a ratio of 1:4 W/V (150 g powder /600 ml solvent), for 24 hr by shaking water bath at 40 °C, then Whittman no.1 filter paper used for isolating the pure extract. Vacuum

rotary evaporator was used for concentrating and obtaining the final crude extract, that dried under stream of cold air, and weighed to determine the yield. Extraction process repeated three times and the extract kept in desiccators at room temperature prior to the experiment⁽⁷⁾.

LD₅₀ study

The toxicity study was carried out using seventy Swiss albino mice (35 male and 35 female) weighing 20-25 g each. The animals were randomly distributed into one control group and six treated groups (for each sex), containing five animals per group, provided with water and food and were allowed to adapt to the laboratory conditions for seven days before the experiment. After depriving the animals' food overnight, the control group received 0.3 ml of 2% Tween 80 solution orally while each treated group received orally chloroform extract of *Xanthium strumarium* leaves; prepared by dispersing 8.0 g in 10 ml volume of 2% Tween 80 in the doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg^(8,9). The animals were observed continuously for the first 4 hr and hourly for the next 24 hr and every 6 hr for the following 48 hr after administering of the extract, to observe any death or changes in general behavior and other physiological activities^(10,11). The study has been approved by the Institutional Review Board of College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Acute toxicity study

The acute oral toxicity was evaluated following the World Health Organization (WHO) guideline⁽¹²⁾. Twenty Albino rats (10 males and 10 females) were divided into one control group and one treated group (for each sex), containing five animals per group. The treated groups were orally given the extract in a single dose of 5 g/kg body weight, while the control groups received only water vehicle. The animals were monitored for apparent signs of toxicity for 14 days. The animals that died within this period were subjected to

necropsies. All rats were sacrificed on the 14th day of experiment.

Experimental design and analysis of data

The experiment design used for this study was Rationalized Complete Block Design (RCBD). The results were reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey test comparison t-test (2-tailed) was used to compare between treatments groups. The differences between the means are considered significant at the 5% confidence level. The statistical analysis was

carried out by using SSPS 16.0, the level of significance was set at P<0.05.

Results

LD₅₀ determination for chloroform extract of *Xanthium strumarium* leaves on mice

Table 1 shows the number of dead and survive mice for male and female mice. Different doses of chloroform extract have been administered for each group of mice. Figure 1 shows the LD₅₀, which represent the dose required to kill fifty percent of total treated mice.

Table 1. The number and percentage of dead mice in variant groups with different administered doses

Group	Dose of extract (g/kg)	No. of mice (male & female)	No. of dead mice	Dead mice cumulative (%)
Control	0	5 males	0	0
		5 females	0	0
1	1	5 males	2	40
		5 females	1	20
2	2.5	5 males	3	60
		5 females	2	40
3	5	5 males	3	60
		5 females	3	60
4	10	5 males	5	100
		5 females	4	80
5	15	5 males	5	100
		5 females	4	80
6	20	5 males	5	100
		5 females	5	100

Acute toxicity study for chloroform extract of *Xanthium strumarium* leaves on rats

After the rats were orally given a single dose 5 g/kg, neither mortality nor signs of toxicity were observed during 14 days of the acute toxicity experiment. The alterations of body weight and relative organs weight from the control would reflect the toxicity of the substance, there was no significant difference in relative organs weight between treated rats and their controls with the absence of any morphological changes. Table 2 shows the

body weight changes during the experiment period for male and female rats. Data expressed as mean ± SD, each group has five rats. The weigh for male and female rats were measured every seven days. Table 3 shows the relative organs weight for male and female rats. Table 4 shows the changes in serum profile for the treated rats in comparison to their controls. There were no significant differences between groups (male and female) relative to their controls (P>0.05).



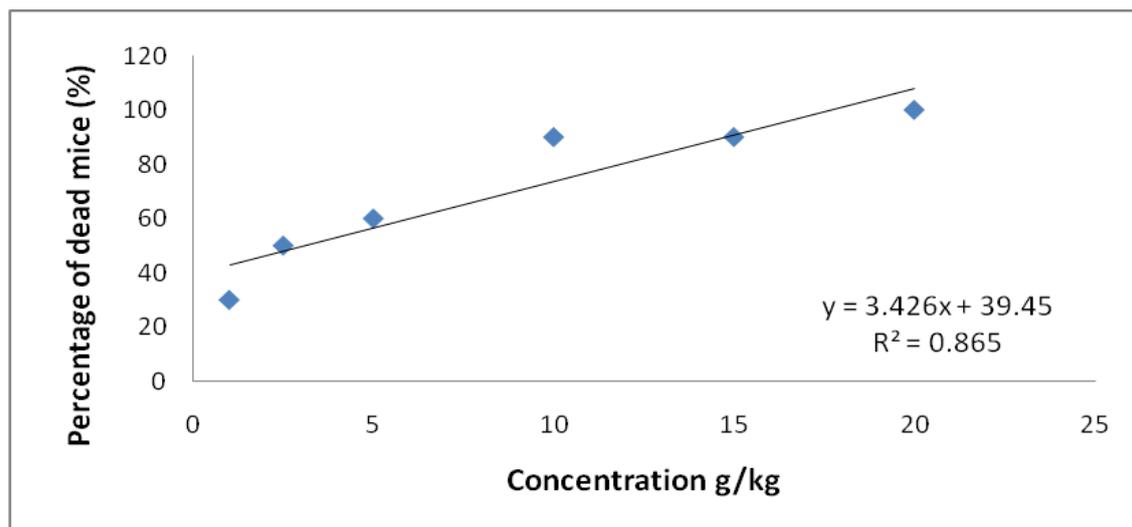


Figure 1. The LD₅₀ of chloroform extract of *Xanthium strumarium* leaves on mice. The LD₅₀ calculated through the equation $y = 3.426x + 39.45$ and it was 3.07 g/kg

Table 2. The animals' weight (g) of treated groups compared to their controls during fourteen days of the acute toxicity experiment, the data showed no significant weight reduction in animals ($P > 0.05$). The changes in weight represented by mean \pm SD, n=5

Time (day)	Weight (g)			
	Male	Male control	Female	Female control
Day 0	200.8 \pm 26.6	209.0 \pm 17.8	203.0 \pm 17.1	199.0 \pm 26.3
Day 7	192.0 \pm 12.5	212.6 \pm 17.1	201.0 \pm 15.9	199.0 \pm 18.7
Day 14	180.4 \pm 20.8	222.8 \pm 26.0	189.8 \pm 22.1	204.0 \pm 34.3

Table 3. Relative organs weight (g) for male and female rats and their controls after 14 days of acute toxicity experiment, n=5. There were no significant differences between relative organs weight of treated rats and their control ($P > 0.05$)

Organs		Treated group	Control group
Male Rats	Heart	0.635 \pm 0.072	0.677 \pm 0.064
	Lungs	0.929 \pm 0.032	0.918 \pm 0.075
	Liver	4.914 \pm 0.583	5.304 \pm 0.849
	Kidneys	0.856 \pm 0.103	0.915 \pm 0.040
	Spleen	0.534 \pm 0.062	0.528 \pm 0.083
Female Rats	Heart	0.510 \pm 0.060	0.559 \pm 0.054
	Lungs	0.908 \pm 0.030	0.920 \pm 0.034
	Liver	4.682 \pm 0.513	5.066 \pm 0.811
	Kidneys	0.864 \pm 0.092	0.882 \pm 0.099
	Spleen	0.462 \pm 0.039	0.509 \pm 0.032

Table 4. Serum biochemical tests for rats receiving single dose 5 g/kg of chloroform extract of *Xanthium strumarium* leaves and their controls after 14 days of acute toxicity experiment. the results represented as mean \pm SD, n=5. there were no significant differences between treated rats and their control (P>0.05)

Biochemical test	Male Rats		Female Rats	
	Treated group	Control group	Treated group	Control group
Blood Sugar (mg/dl)	97 \pm 10.36	112.6 \pm 5.59	91.4 \pm 9.34	112.8 \pm 6.83
Blood Urea (mg/dl)	32.2 \pm 4.45	27.4 \pm 5.59	30.6 \pm 3.57	25.8 \pm 4.81
Sr. Creatinine (mg/dl)	0.53 \pm 0.097	0.46 \pm 0.086	0.51 \pm 0.091	0.49 \pm 0.074
ALT (GPT) (U/L)	39.3 \pm 4.086	35.6 \pm 2.702	36.5 \pm 3.701	31.9 \pm 2.345
AST (GOT) (U/L)	39.2 \pm 7.905	27.7 \pm 9.055	37.5 \pm 5.594	33.6 \pm 5.403
ALP (U/L)	167.8 \pm 8.927	171 \pm 4.207	152.8 \pm 4.324	153.0 \pm 8.018

Discussion

The present study showed that chloroform extract of *Xanthium strumarium* leaves has LD₅₀ equals 3.07 g/kg. Other study showed that, LD₅₀ of ethanol extract of *Xanthium strumarium* leaves was 1.5 g/kg⁽¹³⁾. However, the difference is not high between the two studies, the difference may be attributed to the nature of lands where the plant has planted, the extraction process conditions, and the solvent used was ethanol while in this study chloroform was used.

Acute toxicity study, where rats received oral single dose of 5 g/kg aqueous solution of chloroform extract of *Xanthium strumarium* leaves, showed no mortality or signs of toxicity over the period of experiment. No significant changes in body weights of treated groups compared to their controls were observed (P>0.05), relative organs weight of treated groups was non-significantly changed compared to that of the control groups (P>0.05), and blood profile showed no significant changes in biochemical tests in comparison with normal controls (P >0.05). The variations of the animal weights and organs weight between tested animals and their

controls, reflect the toxic effect of substance⁽¹⁴⁾. Significant difference in organ weight between treated and control animals may occur in the absence of any morphological changes⁽¹⁵⁾. There was no mortality or signs of toxicity were found in mice received extract of *Xanthium strumarium* at dose levels of 500-2000 mg/kg, the initial and final weights of the animals were found to be similar to control⁽¹⁶⁾. Another study of toxicity performed with chloroform and hexane soluble fractions of *Xanthium strumarium* reveals that the administration of a very high dose (5 g/kg) for acute toxicity determination could not make any abnormal changes at gross as well as histopathological levels in the treated animals⁽¹⁷⁾. Further studies on this subject are highly recommended.

In conclusion, this extract is toxic substance since it has low LD₅₀ (3.07 g/kg), but the acute toxicity study showed no mortality or signs of toxicity, with non-significant changes in body weights, relative organ weights, and biochemical tests among treated groups compared to their controls.

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Author contribution

Dr. Alsabah: Collection of plant, extraction, performance of experiments. Dr. Abd: Supervision and writing of the manuscript. Dr. Al-Shammari: Supervision and editing of the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Reproductive Hormonal Assay of a Sample of Iraqi Obese Males

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Abstract

Background	The World Health Organization considered obesity as a medical condition that may lead to reduced life expectancy and/or increased health problems. While much of the focus on the impairments caused by obesity is on somatic health, recent data suggest that reproductive health may also be impacted.
Objective	To quantify the relation between obesity and the reproductive hormones.
Methods	This cross-sectional study was carried out at nutrition clinic in three teaching hospitals and one obesity clinic in a medical college in Baghdad. The body mass index (BMI) calculation, blood sugar, serum cholesterol, triglyceride, testosterone, prolactin, follicular stimulating hormone (FSH), and luteinizing hormone (LH) were measured.
Results	Ninety-five adult obese males participated in this study. Serum testosterone had significant negative correlation with BMI, weight, serum cholesterol, and serum triglyceride while serum LH had significant positive correlation with BMI (p value was 0.013), weight (p value was 0.027), and serum triglyceride (p value was 0.049).
Conclusion	Male obesity has significant effect on serum level of testosterone and LH.
Keywords	Obesity, reproductive hormones
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List of abbreviations: BMI = Body mass index, FPG = Fasting plasma glucose, FSH = Follicular stimulating hormone, LH = Luteinizing hormone, PRL = Prolactin, S.Chol = Serum cholesterol, S.TG = Serum triglyceride, T = Testosterone

Introduction

The obesity epidemic is a growing public health concern. Indeed, the American Medical Association classified obesity as a disease ⁽¹⁾. Previously, much of the focus on the impairments caused by obesity was on somatic health but the recent data suggested that reproductive health may also be impacted ⁽²⁾. Obesity can influence the normal concentrations of male sex hormones at different levels in form of reduced central production and an increased peripheral

degradation ^(3,4). The reproductive hormonal profiles of most obese men deviate from what is considered to be normal levels. Obese men tend to present with elevated estrogen and low testosterone (T) and follicular stimulating hormone (FSH) levels ⁽³⁾. Obese men exhibit decreased levels of total T and increased estrogens levels. Decreased T and T/estrogen ratios have been documented among infertile obese men compared with infertile non-obese men and fertile obese men ⁽⁵⁾. Grossly obese men may show an unequivocal reduction of free T levels, where luteinizing hormone (LH) and FSH levels are usually low or inappropriately normal, suggesting that the dominant suppression occurs at the hypothalamic-

pituitary level⁽⁶⁾. Several reports showed that obese men exhibit significant decrease in androgen level, which was correlated with the degree of obesity^(6,7). It has been shown that body mass index (BMI) was positively related to estradiol levels and inversely related to total T level⁽⁸⁾. There was also a strong inverse relation between BMI and a lower T/LH ratio among men with a BMI ≥ 35 kg/m²⁽⁹⁾.

The effects of increased BMI in Iraqi men on the reproductive hormones level have not been subjected to the same degree of research as Iraqi females. Therefore, this study was designed to assess the pattern of reproductive hormones among obese males, specifically: (T, prolactin (PRL), FSH, LH) and to investigate the correlations between obesity and the reproductive hormones.

Methods

A cross-sectional study was carried out at nutrition clinic in three teaching hospitals in Baghdad (Al-Imamein Al-Kadhimein Medical City, Al-Kindy Teaching Hospital and Al-Yarmouk Teaching Hospital) and at Obesity Clinic in Al-Kindy Medical College for the period from 20th of February 2016 till 5th of July 2017. All adult males who visited the clinics for obesity were asked to participate in the study. Consecutive sampling was used to collect the sample after taking their informed consent.

Ninety-five adult males (18 to 65 years of age) with BMI of greater than or equal to 30 were included. Those with history of hypertension or on antihypertensive drugs, those who were previously diagnosed to be aspermic or azoospermic, and/or those who were diagnosed to have endocrine diseases or on treatment for these diseases were excluded from the study.

Socio-demographic characteristics of each participant were obtained, focusing on age, residency (rural or urban), marital status (single, married, widow, or divorced), occupation (type of occupation), and smoking (current and ex-smoking). Physical examination was done to all participants stressing on height and weight measurements (to calculate the BMI), and blood pressure measurement were done. The weight

was measured (to nearest 0.5 Kg), in erect position without shoes, coats, or overalls with an electronic scale. Height was measured by using tape height measure which is suitable to measure a person's height with an approximation of ± 1 mm. BMI was calculated as body weight/height² (Kg/m²). Participants were classified as class 1 obesity if their BMI is (30-34.9 kg/m²), class 2 obesity if their BMI is (35-39.9 kg/m²), and class 3 obesity if their BMI is (40 kg/m² or more)⁽¹⁰⁾. Blood pressure was measured using mercury sphygmomanometer in sitting position.

Venous blood samples were withdrawn from each participant and sent for fasting plasma glucose (FPG), serum cholesterol (S.Chol), serum triglyceride (S.TG), serum T, serum PRL, serum FSH, and serum LH. Participant was considered diabetic if his FPG ≥ 7.0 mmol/l⁽¹¹⁾. S.Chol was considered of desirable level when it was < 200 mg/dl, borderline high when it was 200-239 mg/dl, and high when it was ≥ 240 mg/dl. S.TG was considered normal when it was < 150 mg/dl, borderline high when it was 150-199 mg/dl, and high when it was ≥ 200 mg/dl⁽¹²⁾. Normal ranges for the reproductive hormones were considered as follows: serum T (2.8-11 ng/ml), serum PRL (2.5-15 ng/ml), serum FSH (1.4–15.4 mIU/ml), and serum LH (1.24–7.8 mIU/ml)⁽¹³⁾.

Data entered and analyzed using SPSS (Statistical Packages for Social Sciences) program, version 18. Descriptive data were expressed as means and standard deviations for continuous measurements and as frequencies and percentages for categorical measurements. Differences of Reproductive hormones according to obesity classes were compared using analysis of variance (ANOVA) test. Relationships between reproductive hormones and obesity parameters were studied by Pearson correlation test. $P < 0.05$ was set as statistically significant.

Results

Ninety-five obese men participated in this study with mean (\pm SD) BMI was (39.23 \pm 4.41) kg/m². One fifth of them (20%) were of class 1 obesity,

43 (45.3%) were of class 2, and 33 (34.7%) were of class 3 obesity.

Socio-demographic, clinical, and laboratory characteristics of the participants were shown in table 1.

Table 1. Socio-demographic, clinical, and laboratory characteristics of the participants

Variable		No	%
Age (years) Mean±SD (Range)		32.62±7.02 (21-46)	
Residency	Rural	7	7.4
	Urban	88	92.6
Marital Status	Single	43	45.3
	Married	52	54.7
Occupation	Employed	30	31.6
	Unemployed	54	56.8
	Student	11	11.6
Smoking	Current	36	37.9
	Ex	18	18.9
	Never	41	43.2
Diabetes	Yes	18	18.9
	No	77	81.1
Cholesterol (mg/dl)	(Mean±SD)	265.83±85.59	
	High	60	63.2
	Borderline	4	4.2
	Normal	31	32.6
Triglyceride (mg/dl)	(Mean±SD)	275.35±92.05	
	High	68	71.6
	Borderline	16	16.8
	Normal	11	11.6
Testosterone (ng/ml)	(Mean±SD)	2.83±2.13	
	Normal	25	26.3
	Low	70	73.7
Prolactin (ng/ml)	(Mean±SD)	13.65±7.47	
	High	7	7.4
	Normal	79	83.1
	Low	9	9.5
FSH (mIU/ml)	(Mean±SD)	4.69±2.28	
	Normal	95	100
LH (mIU/ml)	(Mean±SD)	3.61±1.52	
	High	2	2.1
	Normal	93	97.9

There was a significant difference in the mean of T, FSH, LH among different classes of obesity (p value <0.05) while no significant difference in the mean of PRL (p value >0.05) as shown in table 2.

The BMI, body weight, and S.TG had significant negative correlation with T, significant positive

correlation with LH, nonsignificant correlation with PRL, and nonsignificant correlation with FSH. S.Chol had significant negative correlation with T, nonsignificant correlation with PRL, and nonsignificant correlation with FSH and LH as shown in table 3.

Table 2. Differences of Reproductive hormones according to obesity classes

Hormones	Obesity Class 1	Obesity Class 2	Obesity Class 3	F	P value
Testosterone (ng/ml) (Mean±SD)	3.33±2.40	3.18±2.15	2.08±1.78	3.27	0.042
Prolactin (ng/ml) (Mean±SD)	15.71±7.15	13.38±7.0	12.81±8.2	0.96	0.387
FSH (mIU/ml) (Mean±SD)	3.50±1.15	5.32±2.22	4.56±2.58	4.64	0.012
LH (mIU/ml) (Mean±SD)	2.37±0.53	4.21±1.65	3.56±1.44	11.76	0.000

Table 3. Correlation of reproductive hormones with obesity parameters (r, P value)

	Testosterone	Prolactin	FSH	LH
BMI	-0.316, (0.002)	-0.046, (0.661)	0.172, (0.096)	0.253, (0.013)
Weight	-0.292, (0.004)	-0.010, (0.924)	0.154, (0.135)	0.227, (0.027)
Cholesterol	-0.209, (0.042)	-0.110, (0.289)	0.066, (0.525)	0.134, (0.195)
Triglyceride	-0.235, (0.022)	-0.082, (0.428)	0.065, (0.529)	0.203, (0.049)

(P values between parentheses)

Discussion

There was scarcity of previous studies about the prevalence of obesity among Iraqi people. However, WHO had estimated that the prevalence of obesity among adult Iraqi people in 2008 to be 29%. The prevalence of obesity among females was 36% while among males was 22%. In 2014, the prevalence among females was 14.9% and among males was 10.8%⁽¹⁴⁾. The Iraqi Ministry of Health, in association with WHO, declared in 2015 that the prevalence of obesity among adult Iraqi people became 33.5%. The prevalence among females was 42.6% and among males was 25.6%⁽¹⁵⁾.

The mean age group of participants in this study was 32.62 years, 92.6% of them were from urban area, 54.7% were married and the rest were single. More than half of them 56.4% were unemployed, and 37.9% of them were currently smokers. Owing to the scarcity of literature on this subject we were unable to compare these socio-demographic characteristics with other studies.

The present study showed also that there were significant differences in the mean level of T, FSH, and LH among the different classes of obesity. It is previously known that obesity has a negative influence on the level of T⁽¹⁶⁾. A significant negative correlation between BMI and T was demonstrated in this study. It has

been proposed that obesity may lead to suppression of hypothalamic pituitary function and inhibit the production of FSH and LH, thereby resulting in reduced testicular function and testosterone production and lower levels of intratesticular and circulating testosterone; Zohdy et al. found a significant negative correlation between BMI and serum total T⁽¹⁷⁾. Also, the researchers from Reproductive Biology Associates reported that high BMI in men is correlated with reduced testosterone levels⁽¹⁸⁾. Al-Hameid et al. showed that obesity is associated with a significant decrease in T level⁽¹⁹⁾. Glass et al. reported significant negative correlations between total serum T and percentage ideal body weight, and normal level of serum LH and FSH among the obese subjects⁽²⁰⁾.

This study showed also that there was a significant positive correlation between BMI and LH level and non-significant correlation between BMI and FSH. Hofny et al. had also reported that BMI had a significant positive correlation with LH and a non-significant correlation with serum FSH in obese infertile males compared with obese fertile males⁽²¹⁾. Al-Hameid et al. also found that obesity is associated with a significant increase in serum LH and FSH levels⁽¹⁹⁾. While, Jensen et al.

reported that BMI had no effect on serum FSH or LH in men ⁽²²⁾.

The association between BMI and serum PRL level was also investigated in the present study. It was found that serum PRL level was non-significantly correlated with the BMI. Although the increased body weight may be associated with prolactinoma and that weight loss occurred with normalization of prolactin levels. A Nigerian study had found non-significant associations between BMI and serum levels of PRL, T, or LH ⁽²³⁾.

A significant negative correlation between serum T level and both serum TG and chol levels was demonstrated in this study. Also, a significant positive correlation between serum LH and serum TG level but non-significant correlation between serum TG and FSH and prolactin were found. Adipose tissue is one of the tissues where conversion of androgens to estrogens took place, therefore, obese men usually have increased estrogen levels which inhibit the production of FSH and LH. Hagiuda et al. found a negative association between serum testosterone levels and serum TG levels and no significant association between the TG and LH or FSH levels ⁽²⁴⁾.

In conclusion, the association found between BMI and some reproductive hormones may be of help to broaden the understanding of the effect of obesity on male reproductive physiologic characteristics since this study showed that there was significant negative correlation between BMI and serum T and significant positive correlation between BMI and serum LH.

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Author contribution

Dr. Abdul-Rahman: Collection of study cases, performing and doing the tests of the research.
Dr. Abdul-Ameer: Interpretation the results done under her supervision.

Conflict of interest

None declared.

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Frequency of Metabolic Syndrome in Subfertile Female Population in Mosul

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Abstract

Background	Metabolic syndrome is associated with obesity, which is a common condition in subfertile women population. For that reason, the infertile women will be more predisposed for cardiac diseases.
Objective	To know the prevalence of the metabolic syndrome among subfertile women in Mosul, a city in the north of Iraq.
Methods	Seventy subfertile females aged >17 years were selected randomly. Adult Treatment Panel-III (ATP-III) guidelines was used for diagnosing metabolic syndrome. The diagnosis was done if any three of the following were present: central obesity, raised triglycerides ≥ 150 mg/dl, low high-density lipoprotein (HDL-C) cholesterol, blood pressure $\geq 130/\geq 85$ mmHg, and diabetes or fasting serum glucose (FSG) ≥ 100 mg/dl.
Results	Metabolic syndrome was diagnosed in 16 (22.85%) women. The prevalence was 15.8% in the population younger than 30 years and 55.5% in ages more than 30 years. Fifty percent of women with class II obesity had metabolic syndrome. The most common abnormality was the abnormal waist circumference (100%). Patients having metabolic syndrome had three components of the syndrome and none had four or five components at the same time.
Conclusion	The prevalence of metabolic syndrome is high among subfertile women. Focus of cardiovascular prevention should be undertaken for these subjects.
Keywords	Metabolic syndrome, infertility, polycystic ovary syndrome, diabetes, obesity
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List of abbreviations: ATP-III = Adult Treatment Panel-III, BMI = Body mass index, CO = Celsius or Centigrade, FSG = fasting serum glucose, HDL-C = High-density lipoprotein cholesterol, 11 β -HSD = 11 β -hydroxysteroid dehydrogenase type 1, HPA = Hypothalamic-pituitary-adrenal, IL-6 = Interleukin-6, QC = quality control, rpm = round per minute, SD = standard deviation, TG = triglycerides, TNF- α = Tumor Necrosis Factor - α

Introduction

Subfertility (or commonly referred as to infertility) is a failure of conception after 12 months of unprotected regular intercourse, could be primary in which the women has never get pregnant before or could be secondary when the women has delay in conception after a previous pregnancy ⁽¹⁾. The

association between infertility and obesity is a well-known relation as one of the most important causes of infertility is the polycystic ovary syndrome which is characterized by central obesity ⁽²⁾.

Metabolic syndrome, is an association between at least three of the five following medical conditions: abdominal obesity, hypotriglyceridemia, hyperglycemia, hypertension and low high-density lipoprotein (HDL) levels ⁽³⁾. The prevalence of the metabolic syndrome has varied markedly between different studies; because of the different

criteria for the definition of the syndrome ^(4,5). The syndrome is common in the United States ⁽⁶⁾ and its prevalence is increasing in Asia ⁽⁷⁾. Metabolic syndrome increases the risk of developing cardiovascular disease and type 2 diabetes ⁽⁸⁾.

The Ministry of Health in Iraq, reported that death from cardiovascular diseases and diabetes constitute more than 40% of causes of death among females in the 15-49 years age ⁽⁹⁾. The aim of this study was to determine the prevalence of the metabolic syndrome in subfertile female in Mosul by the use of the ATP-III guidelines ⁽¹⁰⁾.

Methods

Study Population

The study design is a cross sectional study. The place of the study is the private clinic of the author between 2010 and 2011 in Mosul, the capital of the Iraqi governorate Ninevah. The anthropometric parameters were assessed. Random sampling was used and a total of 70 women aged between 17-43 years were included in the study. Consent was taken from all the subjects after they were given verbal information about the study. Approval of the Ethical Committee of Mosul and Ninevah Colleges of Medicine was achieved. The study participants were without medications for hypertension, diabetes, or dyslipidemia and without clinical diabetes.

Measurements

Waist circumferences were measured with flexible tape at the level of umbilicus between the lowest rib and the iliac crest in duplicate to the nearest mm.

The blood pressure was measured two times on the left upper arm and the average used. Systolic (Korotkoff phase I) and diastolic (Korotkoff phase V) blood pressure was measured in sitting position with the use of random zero sphygmomanometer. Hypertension was diagnosed when the average systolic blood pressure was more than 130 mmHg or diastolic blood pressure more than 85 mmHg.

Biochemical investigations were analyzed at the laboratory postgraduate studies in the Department of Biochemistry of Mosul College of Medicine. Overnight fasting blood samples were obtained from all subjects included in this study subjects by antecubital venipuncture.

Five milliliters (5 ml) of venous blood sample from each patient were collected in a plain tube, allowed to clot for 15 minutes in a water bath at 37 °C. Serum was separated by centrifugation at 3000 rpm for 15 minutes to ensure complete separation, and then the serum was divided into 2 parts: 0.5 ml was taken in a plain tube for glucose estimation which was measured at once by standard kit method. The remaining was used for the estimation of lipid profile parameters, particularly serum TG and HDL-C.

The sample was stored at -20 °C until determination was done on daily basis. For accuracy and reproducibility internal quality control (QC) of pooled serum was used within the run and within the batch throughout the work.

Fasting serum glucose (FSG) was estimated using a kit supplied by Bio-con Company, Germany (by Glucose-oxidase-peroxidase colorimetric enzymatic method). FSG more than 100 mg/dl was considered abnormal in this study.

Serum triglycerides (TG) was also estimated by enzymatic method by Fossti in 1982, using a kit supplied by Biomerieux Company, France ⁽¹¹⁾.

Serum high density lipoprotein cholesterol (HDL-C) was determined by enzymatic method followed by Lopez in 1977 using a kit supplied by Biomerieux Company, France ⁽¹²⁾.

Statistical analyses

The data are presented as frequencies and percentages. Risk ratio is calculated at confidence interval of 95%.

Results

Of the 70 women involved in the study there were 16 having the criteria of metabolic syndrome (22.85%). The mean age of the study group was 27.2 years (SD 6.56), range between

17-43 years. Table 1 showed the distribution of age in the study population.

Table 1. The distribution of age in the study population

Age groups in years	Number	Percentage
<20	10	14.3%
21-30	38	54.3%
31-40	18	25.7%
>40	4	5.7%
Total	70	100%

Table 2 showed the frequency of metabolic syndrome according to age group. The prevalence was 15.8% in the population

younger than 30 years and 55.5% in ages more than 30 years. Risk ratio is 4.41 (1.46 at confidence interval of 90%).

Table 2. The frequency of metabolic syndrome according the age groups

Age group in years	Number	Percentage
Less than 20 (n=10)	0	0%
21-30 (n=38)	6	15.8%
31-40 (n=18)	10	55.6%
41-50 (n=4)	0	0%
Total (n=70)	16	22.8%

The mean BMI was 27.6 kg/m² (SD 4.85), range from 19-38. Table 3 showed the distribution of

BMI. About 75% of the study population was overweight or obese class I and II.

Table 3. The distribution of BMI among the study group (n=70)

BMI in kg/m ²	No.	%
Not overweight BMI <24.9	18	25.7%
Overweight BMI 25-29.9	32	45.7%
Class I obesity BMI 30-34.9	12	17.1%
Class II obesity BMI 35-39.5	8	11.4%
Class III obesity BMI >40	0	0%

Fifty percent of the eight women with class II obesity have the metabolic syndrome (table 4). The prevalence of metabolic syndrome increased with increasing BMI. Risk ratio was 2.59 (1.74 at 90% confidence interval).

The most common abnormality was the abnormal waist circumference (100%), then the low high density lipoprotein (87.5%), hypertension (56.3), high fasting serum glucose (43.8%), and the least is the high triglycerides (12.5%) (Table 5).



Table 4. The frequencies of metabolic syndrome in different BMI groups

BMI	Number of cases with metabolic syndrome	Percentage
Not overweight (n=18)	2	1.1%
Overweight (n=32)	6	18.7%
Class I obesity (n=12)	4	30.0%
Class II obesity (n=8)	4	50.0%
Class III obesity (n=0)	0	0%
Total (n=70)	16	22.8%

Table 5. The frequencies of abnormalities in women with metabolic syndrome (n=16)

Abnormality	Number	Percentage
Abnormal waist circumference	16	100%
Low high density lipoprotein	14	87.5%
High blood pressure	9	56.3%
High fasting serum glucose	7	43.8%
High triglycerides	2	12.5%

Twenty women have two abnormalities (28.5%), eight had abnormal waist circumference and hypertension and 2 had low

high density lipoprotein and high triglyceride level. Twenty-four women had one abnormality (34.2%), (table 6).

Table 6. The frequencies of other abnormalities

Abnormalities	Two abnormalities (n=20)	One abnormality (n=24)
Low high density lipoprotein	2	20
Waist circumference more than 88 cm	8	2
High triglyceride level	2	0
Hypertension	8	2
High fasting serum glucose	0	0

Discussion

The prevalence of metabolic syndrome among United States adult population was 21.8%, about the same prevalence of this study ⁽⁶⁾. This may reflect similarities of eating habits and low physical activity between the two communities. Because of the population growth; the total people of metabolic syndrome would increase in Mosul.

Infertility may predict metabolic syndrome and the development of cardiometabolic disease ⁽¹³⁾. Disruptions in the hypothalamic-pituitary-adrenal (HPA) axis in women with infertility is implicated in the development of metabolic syndrome ⁽¹⁴⁾.

The prevalence of metabolic syndrome increases with age and affects women more than men ⁽¹⁵⁾. In this study also the main age

group affected by the metabolic syndrome is (31-40) year age (table 2).

In this study, 1.1% of women have metabolic syndrome without obesity (table 4). Elevated liver 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) activity may be relevant to the metabolically obese, normal-weight individual⁽¹⁶⁾. This enzyme has a role in the development of metabolic syndrome via intracellular steroid reactivation of inert circulating 11-dehydrocorticosterone (cortisone in humans) into active corticosterone (cortisol), and so increasing tissue glucocorticoids and causes hypertension⁽¹⁷⁾. This enzyme can also enhance hepatic lipid deposition⁽¹⁸⁾.

The association of metabolic syndrome with obesity has been shown in other studies. Weiss et al. found that 49.7% of severely obese has metabolic syndrome⁽¹⁹⁾.

The most common abnormality is waist circumference which is due to visceral adiposity. Visceral adiposity plays a role in the pathophysiology of insulin resistance⁽²⁰⁾. Insulin resistance is thought to be an underlying feature of metabolic syndrome⁽²¹⁾. In a study done in Baghdad, it was found that insulin resistance is caused by inflammatory cytokines (interleukin-6 'IL-6' and Tumor Necrosis Factor α 'TNF- α ')⁽²²⁾. TNF- α is also associated with endometriosis which may exacerbate the condition of subfertility in these patients⁽²³⁾.

The presence of hypertriglyceridemia, low HDL-C concentrations, and high TG/HDL-C ratios almost never occurred as isolated disorders, and were nearly always associated with insulin resistance because insulin affects TG and HDL-C metabolism⁽²⁴⁾. Low level of serum HDL-C cholesterol is a risk of ischemic stroke⁽²⁵⁾.

This study concluded that metabolic syndrome is a common disorder in women especially in association with infertility. Metabolic syndrome is a predictor of cardiovascular disease, for that reason more attention has to be made to prevent the development of this disease in infertile women

Author contribution

Dr. Jarjees: sample collection and analysis of the results and writing the paper. Hasan and Al Dabbagh did the biochemical tests.

Conflict of interest

The authors have no conflict of interest to declare.

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Validity of MRI Measurements in Lumbar Spinal Canal Stenosis

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Abstract

Background	Lumbar spinal canal stenosis results from compression of spinal cord and/or nerves at any level of lumbar vertebra. The relationship between clinical features of the patients and the degree of stenosis is not clear and there is no accepted "gold standard" for the diagnosis of lumbar stenosis.
Objective	To evaluate the relationship between the degree of radiologically confirmed stenosis and the severity of Oswestry disability Index and to assess the most valid parameter for the diagnosis of the lumbar stenosis.
Methods	A cross-sectional study conducted on randomly selected patients with lumbar stenosis at Magnetic Resonance Image Unit of Al-Imamein Al-Kadhimein Medical City in Baghdad from May to September 2018. All patients filled Oswestry disability Index questionnaire and underwent examination using 1.5 Tesla magnetic resonance unit (Avanto, SIEMENS).
Results	A total of 41 patients were included (51.46±12.62) years of age. The measurements of spinal canal including the cross-sectional area of dural sac at intervertebral levels, stenosis ratio, and depth of lateral recesses are found to be correlated significantly with the level of disability assessed by Oswestry disability Index. At all levels, neither the cross-sectional area of the lateral recesses nor Ligamentous interfacet distance correlated significantly to the level of disability.
Conclusion	Magnetic resonance image measurements of spinal canal correlated to the level of disability. Stenosis ratio and cross-sectional area of dural sac at intervertebral disc were more sensitive measurements for lumbar stenosis than other parameters.
Keywords	Oswestry disability Index, Spinal stenosis, magnetic resonance image
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List of abbreviations: AP = Anterior-Posterior, CSA = Cross-sectional area, LID = Ligamentous interfacet distance, LRD = Lateral recess depth, LSCS = Lumbar spinal canal stenosis, MRI = Magnetic resonance image, ODI = Oswestry Disability Index, SR = Stenosis ratio

Introduction

Spinal stenosis refers to the compression of the neural elements in the spinal canal, lateral recesses, neural foramina, or any combination of these locations secondary to soft tissue or bony abnormalities^(1,2). Soft tissue abnormalities that can lead to spinal stenosis include hypertrophy of the ligamentum flavum, bulging disc(s) and

ossification of the posterior longitudinal ligament. While bony causes include; congenitally narrow spinal canal, osteophytes, facet osteoarthritis, or spondylolisthesis. A spinal canal that was borderline normal in size may become stenotic when any of these processes superimposes to further narrow the canal⁽³⁾.

The evaluation of patients with known or suspected lumbar spinal stenosis is one of the primary indications for magnetic resonance image (MRI) of the lumbar spine^(1,2,4). MRI is

considered the best single imaging modality of the spine for its ability to demonstrate all of the spinal components; bone, discs, ligaments, fatty tissue, dura, cerebrospinal fluid, neural tissue, and blood vessels with superb contrast resolution ⁽⁵⁾, and for its accurate measurement of the dimension of the spinal canal and spinal cord in various planes ⁽⁶⁾. MRI findings may correspond to the severity and duration of the compression ⁽⁴⁾.

A variety of both radiological and anatomical measurements of normal lumbar spinal canal were performed to define the lumbar spinal canal stenosis (LSCS) and to correlate the severity of lumbar spinal stenosis symptoms with the extent of narrowing of the spinal canal dimensions. Although some studies focused on cross-sectional area (CSA) of the dural sac, transverse diameter, or dural sac anterior-posterior (AP) diameter for the diagnosis of LSCS ^(7,8). Generally, the relationship between the clinical feature of the patients and the degree of a radiologically confirmed stenosis is not clear and there is no accepted “gold standard” for the diagnosis of LSCS ⁽⁹⁾. The Oswestry Disability Index (ODI) also known as the Oswestry Low Back Pain Disability Questionnaire) is an extremely important tool that researchers and disability evaluators use to measure a patient's permanent functional disability. The test is considered the ‘gold standard’ of low back functional outcome tools ⁽¹⁰⁾. Therefore, the aim of this study was to evaluate the relationship between the degree of radiologically confirmed stenosis and the severity of ODI and to assess the most valid measurement for the diagnosis of the LSCS.

Methods

Design and setting

This a prospective cross-sectional study carried out in MRI unit of Radiology Department in Al-Imamein Kadhimein Medical city in Baghdad during the period from May to September, 2018.

Study population

Forty-one adult selected symptomatic patients were included in the study. The exclusion criteria were: previous lumbar vertebral fracture, or surgery of the spine, Spinal tumors, pregnancy, gross spinal pathology (spondylolisthesis), recent trauma, and vertebral abnormalities. The presenting symptoms of the patients were lower back pain, neurologic claudication, unilateral or bilateral sciatic pain, and/or numbness, consequently.

Data collection

The data collected by researcher from the patients directly and filled in a prepared questionnaire. The questionnaire included the followings: demographic characteristic of each patient, grading scale to quantify disability, and lumbar vertebral canal anthropometric measurements.

The Clinical grading was done using ODI scoring. It was considered the “gold standard” to quantify disability in a patient with low backache ⁽¹⁰⁾. Every patient answered the ODI questionnaire. ODI comprised of 10 questions, these questions give the physician information about how the pain affect the ability of the patient to overcome in everyday life. The method of Scoring is as follows: (0-20%): minimal disability; (21-40%): moderate disability; (41-60%): severe disability; (61-80%): crippling back pain; and (81-100%): bed-bound ⁽¹¹⁾.

Quantitative MRI image evaluation for LSCS were calculated as following: CSA of dural sac at each mid-vertebral level (L1, L2, L3, L4, and L5) and CSA of dural sac at for each level of the intervertebral discs; CSA of left and right lateral canals; AP diameter of dural sac; Transverse diameter of dural sac; ligamentous interfacet distance (LID); and lateral recess depth (LRD) for each level of the intervertebral discs (L1-L2, L2-L3, L3-L4, L4-L5, and L5-S1). On a diagnostic workstation, the measurements performed using a program measured the parameters in centimeters (cm) as shown in figure 1.

MRI examination

All patients underwent MRI examination using 1.5 Tesla MR unit (Avanto, SIEMENS, German). Each patient was placed in supine position. T2-weighted axial and sagittal images were obtained (TR/TE 5700/99 ms; for axial scan; FoV read: 280 mm; FoV phase: 100.0%; FOV: AP: 350 mm; RL: 263 mm; FH: 350 mm; slice thickness, 4.0 mm; flip angle, 150°.

Image analysis

Two readers including a single skilled radiologist with 10-year experience participated in the evaluations of each patient in the current study.

- AP diameter was measured on the axial plane: “distance between middle of vertebral body and middle of basis of spinous process at border of dural sac”
- LID was measured on axial MRI as “the distance between the inner surfaces of ligamentum flavum on a line connecting the joint disc of facet joints”.
- LRD was measured on the axial plane as “distance between the superior articular

facet and the top part of the pedicle”. as shown in figure 1.

- The stenosis ratio (SR) is defined as the ratio of the CSA of the spinal canal at the intervertebral discs to the CSA at the next mid-vertebral level above. It has been used as index for measuring the severity of the stenosis ⁽¹²⁾.

In the measurement of the CSA of the lateral recess, if there is interruption of the lateral recess due to disc bulge, summation of the patent zones of the lateral recess were calculated.

Grouping of SR according to degree of stenosis LSCS performed using quartile analysis with the SR as follows: (no lumbar stenosis) between 0.75 and 1, (mild stenosis) between 0.50 and 0.75, (moderate stenosis) between 0.25 and 0.50, and (severe stenosis) between 0 between 0.25. Compromise of the nerve root in the lateral recess was grouped as follows: LRD >0.5 cm (no stenosis); 0.3-0.5 cm (relative stenosis); and <0.3 cm (definitive stenosis).

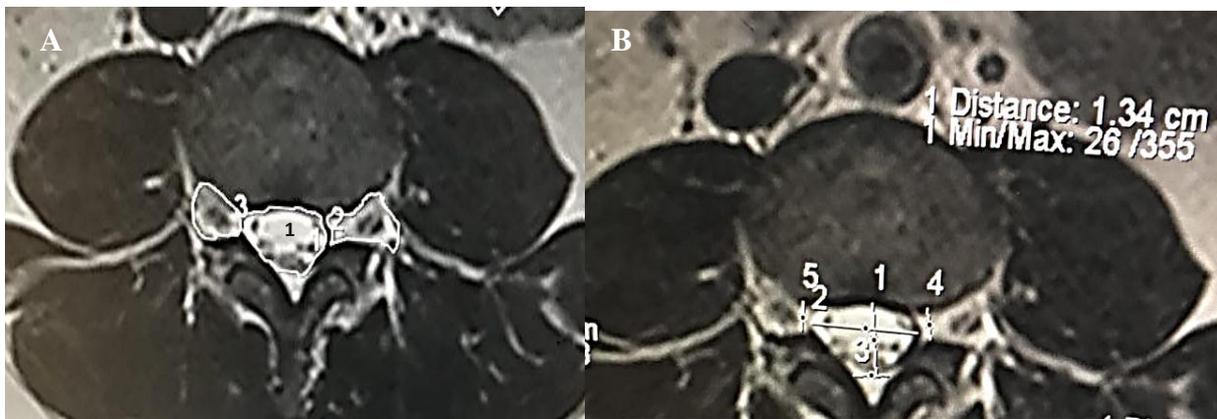


Figure 1. A- spinal canal areas measurement: 1. CSA of dural sac; 2,3. Cross-sectional area of lateral recesses. B- 1. AP diameter of dural sac; 2. Transverse diameter of dural sac; 3. Ligamentous interfacet distance; 4,5. Depth of the lateral recesses

Ethical considerations

Verbal informed consent obtained from all patients

Statistical analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) Version 22.0 for Windows. Mean and standard deviation

(SD) were calculated for all variables. The chi square test was used for categorical scales and Pearson correlation was used for continuous variables. ANOVA has been used to find the significance of study parameters between three or more groups. SR ratio as index for measuring the degree of stenosis were calculated. P-values <0.05 were considered statistically significant.

Results

During the study period, 41 selected patients who underwent LSS MRI at the MRI department of Al-Imamein Kadhimein Medical City were included in the study. Of them 14 (23.1%) were male and 27 (65.9%) were

female. The age of the patients ranged from 29 to 75 years with mean age \pm SD was 51.46 \pm 12.62 years.

Of those 41 patients, a total of 205 intervertebral discs were analyzed for lumbar stenosis. The CSA of the dural sac at mid-vertebral level varied between 0.8 and 3.0 cm², and the CSA of the dural sac at each disc level varied between 1.36 and 3.97 cm² in supine position. The mean and SD of CSA of each level were shown in table 1. The mean and SD of AP diameter, transverse Diameter, CSA of the lateral recesses, and LRD at intervertebral discs levels were shown in table 2.

Table 1. Range, minimum, maximum, Mean and SD of the CSA of the dural sac at mid-vertebral and intervertebral disc levels in cm

Level	Range	Minimum	Maximum	Mean	SD
L1	1.97	2.00	3.97	2.83	0.49
L1-L2	1.57	1.40	2.97	2.24	0.35
L2	1.90	1.79	3.69	2.61	0.48
L2-L3	1.63	1.19	2.82	1.93	0.43
L3	2.02	1.82	3.84	2.37	0.43
L3-L4	1.77	.87	2.64	1.73	0.41
L4	1.57	1.56	3.13	2.19	0.40
L4-L5	2.16	.84	3.00	1.62	0.53
L5	2.50	1.36	3.86	2.3	0.53
L5-S1	2.05	0.80	2.85	2.00	0.62

Table 2. Mean and SD of AP diameter, Transverse Diameter, CSA of the lateral recesses, and LRD at intervertebral disc levels of the lumbar spine in cm

	AP Diameter	Transverse Diameter	CSA of left lateral recess	CSA of right lateral recess	Left LRD	Right LRD	LID
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
L1-L2	1.40 \pm 0.18	2.11 \pm 0.24	0.98 \pm 0.31	0.93 \pm 0.29	0.59 \pm 0.18	0.65 \pm 0.2	0.81 \pm 0.12
L2-L3	1.28 \pm 0.21	2.02 \pm 0.26	0.92 \pm 0.33	0.85 \pm 0.31	0.45 \pm 0.15	0.50 \pm 0.19	0.80 \pm 0.13
L3-L4	1.21 \pm 0.22	1.89 \pm 0.23	0.86 \pm 0.4	0.87 \pm 0.4	0.33 \pm 0.17	0.37 \pm 0.15	0.78 \pm 0.15
L4-L5	1.14 \pm 0.29	1.79 \pm 0.25	1.02 \pm 0.52	0.95 \pm 0.48	0.27 \pm 0.15	0.30 \pm 0.15	0.89 \pm 0.22
L5-S1	1.25 \pm 0.26	1.90 \pm 0.36	1.10 \pm 0.62	1.06 \pm 0.55	0.36 \pm 0.16	0.39 \pm 0.18	1.04 \pm 0.29

AP: anterior-posterior; CSA: cross-sectional area; LRD: lateral recess depth; LID: Ligamentous interfacet distance. Of the 290 evaluated levels, 59 revealed moderate and 80 revealed severe central stenosis

Regarding the disability score of the ODI, out of the 41 patients, 11 patients (26.8%) showed mild disability; 8 patients (19.5%) showed moderate disability, 12 patients (29.3%) showed severe disability; 6 patients (14.6%) were crippled and 4 patients (9.8%) were bedridden.

In terms of validity, the disability score of the ODI was highly correlated with the CSA of the dural sac at the intervertebral discs of L1-L2, L2-L3, L3-L4, and L4-L5 (P-value = 0.01, 0.04, 0.01, and 0.02) consequently. There is no

significant correlation between the disability score of the ODI and CSA of lateral recess or LID at any level, P-value > 0.05.

Of the 205 evaluated levels, 58.5% showed no stenosis, 35.6% showed mild central stenosis, and 5.9% showed moderate central stenosis, none of the evaluated levels shows severe central stenosis. This study shows a highly significant correlation between stenosis ratio and the disability score of the ODI (P-value is 0.015), as shown in table 3.

Table 3. Relation of stenosis ratio to ODI at intervertebral disc levels of the lumbar spine

	No stenosis		Mild stenosis		Moderate stenosis		Total
	No.	%	No.	%	No.	%	
Minimal Disability	42	35	12	16.4	1	8.3	55
Moderate Disability	15	12.5	21	28.8	4	33.3	40
Severe Disability	35	29.2	23	31.5	2	16.8	60
Crippled	17	14.2	9	12.3	4	33.3	30
Bed-bound	11	9.1	8	11	1	8.3	20
Total	120	100	73	100	12	100	205

The chi-square statistic is 18.95. The p-value is 0.015. The result is significant at p < 0.05

In these 41 patients, a total of 410 lateral recesses were analyzed for nerve root compression. Of the 410 evaluated lateral recesses, 30.7% showed no stenosis, 42% showed relative stenosis, and 27.3% showed

definitive stenosis. This study shows a significant correlation between the grade of nerve root compression and the disability score of the ODI (P-value is 0.041), as shown in table 4.

Table 4. Relation of the severity of lateral stenosis to ODI at intervertebral disc levels

	No stenosis		Relative stenosis		Definitive stenosis		Total
	No.	%	No.	%	No.	%	
Minimal Disability	45	35.7	43	25	23	20.5	111
Moderate Disability	20	15.9	39	22.7	21	18.7	80
Severe Disability	28	22.2	51	29.7	41	36.6	120
Crippled	20	15.9	19	11	20	17.9	59
Bed-Bound	13	10.3	20	11.6	7	6.3	40
Total	126	100	172	100	112	100	410

The chi-square statistic is 16.02. The p-value is 0.041. The result is significant at p < 0.05

The disability score of the ODI was significantly associated with the AP diameter of the intervertebral discs of L2-L3 (P-value 0.004) and L3-L4 (P-value 0.03). There is no significant

correlation between the disability score of the ODI and AP diameter at the other levels (P-value >0.05), as shown in table 5.

Table 5. Relation of AP diameter to of the ODI at intervertebral disc levels of the lumbar spine in cm

	Minimal Disability		Moderate Disability		Severe Disability		Crippled		Bed-bound		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
L1-L2	1.45	0.11	1.27	0.20	1.27	0.20	1.37	0.05	1.41	0.16	0.13
L2-L3	1.40	0.17	1.21	0.18	1.38	0.17	1.20	0.29	1.02	0.06	0.004*
L3-L4	1.33	0.16	1.05	0.23	1.30	0.16	1.18	0.30	1.08	0.26	0.03*
L4-L5	1.15	0.40	1.19	0.33	1.20	0.29	1.09	0.18	0.99	0.05	0.78
L5-S1	1.24	0.33	1.34	0.17	1.22	0.25	1.27	0.23	1.24	0.36	0.9

*Significant

There is no significant correlation between the disability score of the ODI and transverse diameter of the intervertebral discs at all levels

except at L3-L4 level show significant correlation (P-value > 0.04), as shown in table 6.

Table 6. Relation of transverse diameter to the ODI at intervertebral disc levels of the lumbar spine in cm

	Minimal Disability		Moderate Disability		Severe Disability		Crippled		Bed-bound		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
L1-L2	2.16	0.30	2.09	0.22	2.14	0.25	2.00	0.12	2.10	0.28	0.78
L2-L3	2.10	0.19	1.97	0.35	2.04	0.26	1.87	0.12	2.08	0.40	0.48
L3-L4	2.03	0.29	1.85	0.14	1.92	0.15	1.67	0.29	1.84	0.18	0.04*
L4-L5	1.86	0.17	1.73	0.43	1.82	0.25	1.70	0.21	1.81	0.15	0.74
L5-S1	2.03	0.39	1.94	0.29	1.85	0.34	1.90	0.49	1.62	0.30	0.41

*Significant

Discussion

Several quantitative radiological criteria have been used to define LSCS. Measurement of AP diameter and the CSA of spinal canal with variable levels are the most frequently applied criteria for central LSCS; depth of the lateral recess for lateral stenosis⁽¹⁰⁾.

Genevay et al.⁽¹³⁾ in their study noticed that the researchers had a variety of combinations of clinical symptoms, signs, and radiological criteria to study LSCS. However, the degree of

narrowing of the spinal canal that considered symptomatic for LSCS is not clear, but it is still needed to ensure appropriate care for the patients and successful treatment plan⁽¹⁴⁾.

Regarding the choice of ODI instrument to measure the level of disability, as described previously by other studies, it has been proven to be the 'gold standard' to quantify disability in a patient with low backache⁽¹⁵⁾ as it is simple, condition specific, reliable, and valid instrument for the assessment of disability in

patients with lower back pain with the benefit of easy comprehension and compliance for the patient. It takes less than five minutes to complete and one minute to be scored, without the need for training, equipment or any cost requirements; it comprises a wide range of function, pain and role limitation⁽⁹⁾.

This study shows that the disability of the patients assessed by ODI correlates significantly with the CSA at the intervertebral disc at 4 levels (L1-L2, L2-L3, L3-L4, and L4-L5) (P-value = 0.01, 0.04, 0.01, and 0.02) respectively. This was in concordance with several studies⁽¹⁶⁻²⁰⁾. Ragupathi et al.⁽¹⁶⁾ noted a significant association between CSA of dural sac and the disability of the patients assessed by ODI. Kanno et al.⁽¹⁷⁾ noted a significant correlation between the CSA of dural sac in axial loaded MRI and severity of clinical symptoms in patients with LSCS. This study disagrees with Schizas et al.⁽²¹⁾ and Sirvanci et al.⁽⁸⁾ studies, they found no correlation between CSA of dural sac and ODI (measured on axial MRI), also disagrees with Lohman et al. study which show no relation between CSA of the spinal canal measured on Computed Tomography and clinical symptoms⁽¹⁵⁾. The current study shows non-significant correlation between CSA of dural sac at the L5-S1 level and ODI, this may be due to the fact that the anatomical and functional aspects of this level of spinal column differs significantly from other levels of the lumbar spine. Functionally, it is consider as junction between the lumbar and sacral vertebrae, act as a joint separating lumbar and sacral vertebrae, anatomically, L5 is significantly different in morphology, with its body being much deeper in front than behind, which allows for articulation with the sacrovertebral prominences. The spinous process is smaller, there is a wider interval between the inferior articular processes, and the transverse processes are thicker and spring from the body as well as the pedicles⁽²²⁾.

The current study shows a non-significant correlation between the disability score of the ODI and the ILD of the intervertebral disc levels. This agrees with Pawar et al. study⁽²³⁾. Moreover, this study shows a non-significant

correlation between the disability score of the ODI and the CSA of the lateral recesses at any level.

The current study shows that the disability of the patients assessed by ODI correlates significantly with the severity of central stenosis (P-value 0.015) and the severity of lateral stenosis (P-value 0.041) of the lumbar spine. This agrees with Hurri et al. study,⁽²⁴⁾ but disagrees with Sirvanci et al.⁽⁸⁾ and Schizas et al. studies⁽²¹⁾ they found no correlation between severity of lumbar stenosis (measured on axial MRI) and ODI. Although various authors had reported a non-significant correlation between radiologically detected stenosis and severity of clinical findings, patients with narrower lumbar spinal canals expected to be more liable to develop symptoms of LSCS. Sirvanci et al.⁽⁸⁾ shows the correlation with only moderate to severe grade of central stenosis however, the current study shows the correlation with only mild to moderate grade of central stenosis as none of the patients present with severe central stenosis through-out the study period.

This study shows a significant correlation between the disability score of the ODI and the LRD for all levels with (P-value 0.041). This agrees with Pawar et al. study⁽²³⁾ who noted a significant correlation between LRD of all levels (except L1 on right side and L1 and L2 on left side) and clinical symptoms.

This study shows significant correlation between the disability score of the ODI and the AP diameter of the intervertebral discs for just two levels; L2-L3 (P-value 0.004) and L3-L4 (P-value 0.03), non-significant correlation at the other levels (P-value > .05), and non-significant correlation of the average of AP diameter of all intervertebral disc levels with the disability score of the ODI (P-value >0.05). This agrees with Kumar et al.⁽²⁵⁾ study, they noticed a significant correlation between AP diameter of intervertebral disc of 2 levels (L4-L5, L5-S1) with clinical symptoms. This study disagrees with Ragupathi et al.⁽¹⁶⁾ study, they noticed a significant correlation between AP diameter with ODI scoring at all intervertebral disc levels, also disagrees with Geisser et al.⁽²⁶⁾, they

noted a non-significant correlation of the AP diameter at all intervertebral disc levels. This study shows a significant correlation between the disability score of the ODI and the transverse diameter of the intervertebral disc for just one level L3-L4 (P-value 0.04), and non-significant correlation at the other levels (P-value >0.05). This disagrees with Kumar et al. (25) study, they noticed a significant correlation between transverse diameter at intervertebral disc of 3 levels (L2-L3, L3-L4, and L4-L5) with clinical symptoms, also disagrees with Ragupathi et al. (16) study, they noticed a significant correlation between transverse diameter with ODI scoring at 4 intervertebral disc levels.

Strength of the study

The strength of the current study is to use combined samples of patients diagnosed with LSCS, compromise those who were planning for surgery, and those who were not. Therefore, it could validate the measures that used in assessing ODI in patients with LSCS. The other important aspect of the study is that: the combined results of central and lateral stenosis would correlate better to the ODI and this may explain the disagreement with other studies (9,21).

Limitation of the study

There are some limitations to this study. Firstly, low back pain related disability is systemic symptom, may be due to a variety of causes. It could be due to LSCS as well as renal calculi or infections. The other limitation is pain tolerance may change with each patient, as some patients can tolerate even more severe pain while other may not be able to tolerate a minimal pain.

Conclusion

Although the CSA of dural sac of 4 levels, SR of all levels, LRD of all levels, and AP diameter of 2 level were all significantly associated with LSCS. SR and CSA of dural sac at intervertebral disc were more sensitive parameter for LSCS than other parameters. There is significant correlation between MRI measurements of spinal canal and levels of disability measured

by ODI in patients with LSCS, the degree of stenosis correlates to the severity of disability scored measured by ODI.

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Author contribution

Dr. Al-Jaberi and Dr. Kanaan: Conception and design of study, acquisition of data and revising the manuscript critically for important intellectual content. All three authors participated in analysis and/or interpretation of data, drafting the manuscript.

Conflict of interest

There are no conflicts of interest.

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Breast Cancer in a Sample of Yemeni Female Patients: Forensic Dermatoglyphic Traits and Clinico-Pathological Features

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Abstract

- Background** Breast cancer has a major impact on health of women worldwide and Yemen is not in exception. Fingerprints play an important role, which is highly individualistic and could be recognized as a powerful tool in diagnosis of various diseases, furthermore, their medico-legal importance.
- Objective** To study the clinico-pathological aspects of breast cancer and the role of fingerprints as screening test.
- Methods** Prospective study of 68 female patients with breast cancer came to modern histopathology laboratory - Aden during the period from January - June 2018. All the patients suffering from breast lumps were referred for Fine Needle Aspiration Cytology diagnosis as well as fingerprints of their both hands were taken.
- Results** Most of the participants (45.6%) aged between 40-49 years old, females from urban areas consisted 57.4%, and about 51.5% of females in this study were illiterate. Females who had 1-3 child consisted 33.8%. Regarding the breast lump, 54.4% of females had tumor size 2-5 cm, while skin change and fixed tumor to skin presented in same rate (14.8%). No pain and no nipple discharge present in 85.2% and 91.2% respectively in females' study. The females with palpable axillary lymph nodes and right breast side tumor consisted the same percentage (58.8%). Regarding the result of fine needle aspiration cytology, the invasive ductal carcinoma presented with high rate (85.3%), while stage II present with 36.8%. In relation to the finger print patterns; the loop patterns presented high rate in the little digit with 30.4%, while whorls patterns presented in index digit in 33.6% of patients. The loops and whorls patterns revealed significant association with breast cancer with a P-value of 0.005 and 0.028 respectively.
- Conclusion** The social, behavioral, and hereditary factors play an important role in the development of breast cancer in addition to reproductive history, beside that the fingerprints are genetically determined factors that can be used as simple and cost-effective screening test for breast cancer.
- Keywords** Breast carcinoma, fingerprints, Dermatoglyphic, forensic, Yemeni female.
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List of abbreviations: BC = Breast cancer, FNAC = Fine needle aspiration cytology, IDC = Invasive ductal carcinoma, ILC = Invasive lobular carcinoma (l) WHO = World Health Organization

Introduction

Breast cancer has a major impact on health of women worldwide. In both high and low resource countries, it is

considered the most common malignancy and the second leading of cancer death in women and responsible for over one million of the estimated 10 million neoplasm diagnosed worldwide each year in both sexes. It is also the primary cause of cancer death among

women globally, responsible for about 375,000 deaths in the year 2000 ^(1,2).

The World Health Organization (WHO) estimates that more than 60% of new cancer cases occur in low- and middle-income countries of Africa and Asia as well as central and south America ⁽³⁾.

Yemen is not an exception, in Sana'a / Yemen, a study of the patterns of malignancies among 1,491 patients found that, breast cancer (BC) ranked first among Yemeni women and formed 8% of all cancers ⁽⁴⁾, on the other side; remote epidemiological studies in south eastern areas of Yemen, reported BC as the most common cancer among women in Aden city and in south eastern areas of Yemen (between January 2002 and December 2006), according to the report of Aden Cancer Registry Center, 334 cases of females had breast cancer, and presented with 16.6% as first ranked cancer among over all sites and 30.3% in females ⁽⁵⁾.

BC is a heterogeneous disease caused by interactions of both inherited and environmental risk factors that lead to progressive accumulation of genetic and epigenetic changes in BC cells ⁽⁶⁾. Quality of life has become an important outcome measure in the treatment of cancer patients during the last decade. The adoption of western lifestyles and changes in diet has led to an increase in the number of overweight and obese women, as well as changing reproductive patterns, such as an earlier menarche, delayed childbearing, low

parity, and decreased breastfeeding. These factors have been collectively described as "Westernization" and may have a significant impact on BC risk and prognosis ^(7,8). Arabs share common demographic features that include high rates of consanguinity, large family size and rapid population growth. There is a high frequency of autosomal recessive disorders and an increased frequency of homozygosity for autosomal dominant traits which have made certain disorders more prevalent in Arabs ⁽⁹⁾.

Identification of women at increased risk for the development of BC and the earliest possible diagnosis of patients with BC should improve the results of BC treatment. Genetic predisposition is one of the most intriguing factors associated with increased risk for breast cancer. Extensive studies identified the genetic link of breast cancer, and available evidence suggestion that family history of BC might be associated with a specific fingerprint ⁽¹⁰⁻¹³⁾.

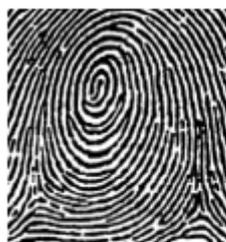
Fingerprint (dermatoglyphic/dactylography) is an impression of the friction ridge on all parts of the palms of the hands and soles of the feet; it came from two Greek words derma means (skin) and glyphs means (curves) ⁽¹⁴⁾. Dermatoglyphic is highly individualistic and makes up the basis form for personal identification in forensic examinations; Galton classified dermatoglyphic depending upon their primary patterns as loops, whorls, arches, and compound as seen in figure (1) ⁽¹⁵⁾.



Left Loop



Right Loop



Whorl



Arch

Figure 1. Different patterns of fingerprint

These dermal ridge differentiation takes place early in fetal development, between 13th to 19th weeks of intrauterine life. The medico-legal importance of these patterns is unique and remain unchanged throughout life ⁽¹⁶⁾, these patterns may be affected by environmental factors in the first trimester of pregnancy, but after birth the patterns of fingerprints remain constant. The study of dermatoglyphic plays an important role and it's considered as a window of various diseases, and could be recognized as a powerful tool in the diagnostic features of certain psychological, medical, genetic, congenital malformation, and chromosomal disorders ⁽¹⁷⁻¹⁹⁾, and may be useful to study the genetic patterns in person and that used as a guide in the future screening of BC and represent a noninvasive anatomical marker of BC ^(11,12,20).

However, reports of dermatoglyphic patterns studies in patients with BC have been done by few workers as studies by Sridevi et al. (2010), Natekar et al. (2006), and Abbasi et al. (2006) have shown that finger print patterns were also affected in breast cancer, which is the commonest neoplastic disease in women, with a lifetime risk of 11-12% in the general population ⁽²¹⁻²³⁾. In humans, the mammary buds begin to develop during the 6th week, as solid down growths of the epidermis, into the underlying mesenchyme. These changes occur in response to an inductive influence from the mesenchyme. These dermal ridges develop in relation to the mammary buds take place early in fetal development, which are also formed by the 6th week of gestation and they reach their maximum sizes between the 13th to 19th weeks of intrauterine life, this means that the genetic message which is contained in the genome - normal or abnormal - is decoded during this period and it is also reflected by dermatoglyphics ⁽²⁴⁾.

The objective of the current study was to study is clinico-pathological aspects of BC and the role of fingerprints as screening test.

Methods

This prospective study of 68 female patients with BC came to Modern Histopathology Laboratory from Aden and neighboring southern governorates of Yemen, during the period of January to June 2018, all the patients presented with breast lump were referred to Modern Histopathology Laboratory for fine needle aspiration cytology (FNAC) diagnosis. During the procedure of FNAC, patients have been asked after taking oral informed consent to fulfill data questionnaire including fingerprints.

Data were classified into different statistical variables including the demographic data (age, address and education level), risk factors (smoking, exercise, Khat chewing, fat diet, obesity, oral contraceptive pills, family history, and breast feeding), reproductive life (parity, age at menarche and age at menopause), clinical finding (lump, pain, size, location, breast side, consistency, fixation, skin changes, nipple discharge and palpable lymph nodes).

The fingerprint patterns were taken from female patients with BC at the Modern Histopathology Laboratory – Aden during the procedure of FNAC: The materials used in this study were as follows:

- A clean plain glass plate (3x5 inch) with blue ink.
- White papers.
- Good lighting and hand magnifying lens.
- Detergent with towel for cleaning the ink from the hand.

To take finger prints, the following method was used: First, press and roll the finger firmly on the ink area, then press thoroughly to print record card (white paper). Next, label each print "left" and "right" for the hands, afterwards, label each fingerprint with "T" for thumb, "I" for index, "M" for middle, "R" for ring and "L" for little finger. Finally, all prints were analyzed by using magnifying lens. Finally, the FNAC result matched with histopathology result of the patients. All data were reviewed and analyzed by computer facility, using Microsoft and SPSS. Collected data were

entered into an SPSS program and presented in frequency and percentage while Pearson's Chi-squared test used to determine relations between categorical variables, and the level of statistical significance was taken as $P < 0.05$, and presented in statistical tables.

Results

Sixty eight cases of female patients of BC were studied during a period of 6th months from January to June 2018 attending to Modern

histopathology Laboratory – Aden for FNAC diagnosis, 31 (45.6%) of females presented in age group 40-49 years old, while those females with age group 20-29 and ≥ 60 presented in equal rate (10.3%), but the BC of females at age < 20 years old was not presented in the study. About 57.4% of female came from urban areas, while illiterate females consisted the high percentage of BC with 51.5%, as shown in table (1).

Table 1. Demographic characteristics of breast cancer patients

Character	No. (68)	%
Age		
<20	None	0.0
20-29	7	10.3
30-39	12	17.6
40-49	31	45.6
50-59	11	16.2
≥ 60	7	10.3
Address		
City (Urban)	39	57.4
Rural	29	42.6
Education		
University	10	14.7
Secondary school	10	14.7
Primary school	13	19.1
Illiterate	35	51.5

In table 2, none of patients were smokers and all experience inactive life style. Obese females and those who took oral contraceptive pills consisted 25% and 27.9% respectively, and 64.7% of females breast feed their children. According to obstetrics and gynecology history; females with 1-3 child, menarche at age 13-14 years and menopause at age 46-50 years were presented in 33.8%, 55.9% and 38.2% respectively.

Regarding the characteristics related to BC, more than half of female participants (54.4%) had tumor sized 2-5 cm, the outer upper

quadrant and hard consistency presented in 45.6% and 67.6% respectively, while central quadrant and soft consistency presented in equal rate (7.4%). Majority of BC (85.2%) was not fixed without skin change and with no pain. Nipple discharge was presented only in 8.8% of participants female while no nipple discharge presented in 91.2% of female patients. Palpable axillary lymph nodes present in 58.8% of study females. Regarding the breast side tumor (58.8%) present in the right side while 35.3% in the left side, as illustrated in table (3).

Table 2. Distribution of patients by risk factors

Character	No. (68)	%
Habits		
Smoking	None	0.0
Exercises	None	0.0
Fat diet	12	17.6
Qat chewing	10	14.8
Obesity	17	25.0
Oral CCP	19	27.8
Family history	10	14.8
Breast feeding		
Yes	44	64.7
No	24	35.3
Parity		
Nullipara	15	22.1
1-3	23	33.8
4-6	19	27.9
>6	11	16.2
Age at Menarche		
<12	12	17.6
13-14	38	55.9
>15	18	26.5
Age at menopause		
None	35	51.5
46-50	26	38.2
>50	7	10.3

Regarding the FNAC results, which were confirmed by histopathology study found that the invasive ductal carcinoma (IDC) presented with high rate 85.3%, while medullary carcinoma presented with low rate 2.9%. in relation to the clinical stage study, stage II presented in 36.8% of study females, while stage I and III presented in close rate (26.5% and 27.9%) respectively and the rest of study females (8.8%) present with stage IV, as shown in table (4).

Regarding the patterns of fingerprints in females with BC, the loop patterns presented

were higher (135, 43.5%) than other patterns (whorls (113, 36.5%) and arch (62, 20%). Regarding the digit, the loop patterns (30.4%) present in the little digit higher than other patterns, the whorls patterns presented in the index digit were higher than other patterns with 33.6% and arches patterns presented in thumb digit with 27.4%. The three patterns (loops, whorls and arches) had significant association with BC (p-value = 0.005, 0.028, and 0.011 respectively), as illustrated in table (5).

Table 3. Clinical finding of breast cancer patients

Character	No. (68)	%
Tumor size		
>2 cm	17	25.0
2-5cm	37	54.4
>5 cm	14	20.6
Quadrant		
Outer upper	31	45.6
Outer lower	12	17.6
Inner upper	12	17.6
Inner lower	8	11.8
Central	5	7.4
Consistency		
Hard	46	67.6
Firm	17	25.0
Soft	5	7.4
Fixation		
Fixed	10	14.8
Non fixed	58	85.2
Skin changes		
Yes	10	14.8
No	58	85.2
Pain		
Yes	10	14.8
No	58	85.2
Nipple discharge		
Yes	6	8.8
No	62	91.2
Palpable axillary Lymph nodes		
Yes	6	8.8
No	62	91.2
Breast side		
Right	40	58.8
Left	24	35.3
Bilateral	4	5.9

Table 4. Histopathology and clinical stage result of breast cancer patients

Character	No. (68)	%
Histology		
Invasive ductal carcinoma (IDL)	58	85.3
Invasive lobular carcinoma (ICL)	8	11.8
Medullary carcinoma	2	2.9
Clinical stage		
In situ	None	0.0
Stage I	18	26.5
Stage II	25	36.8
Stage III	19	27.9
Stage IV	6	8.8

Table 5. Distribution of Patterns of finger print in different fingers of female with breast cancer

Digits	Fingerprint patterns					
	Loops		Whorls		Arches	
	No (%)	p-value	No (%)	p-value	No (%)	p-value
Thumb	31 (23)	0.169	20 (17.7)	0.580	17 (27.4)	0.011
Index	23 (17)	0.228	38 (33.6)	0.028	7 (11.3)	0.784
Middle	37 (27.4)	0.113	19 (16.8)	0.428	12 (19.4)	0.216
Ring	3 (2.2)	0.169	20 (17.7)	0.580	15 (24.2)	0.051
Little	41 (30.4)	0.005	16 (14.2)	0.145	11 (17.7)	0.784
Total	135 (100)		113 (100)		62 (100)	

Note: 6 cases missed because had compound fingerprint patten

Discussion

Most epidemiological studies have evaluated risk factors for BC in western populations. The epidemiology of BC in most Asian populations is less well understood. Recent studies from several East Asian countries have shown that women in these countries increasingly share risk factors for BC with women from western countries⁽⁷⁾. In Arab countries, BC accounts for 14% to 42% of all cancers in women⁽²⁵⁾, and in Yemen BC was the first ranked cancer among overall cancer sites (16.6%) and female cancers (30.3%)⁽⁵⁾. Although the high incidence of BC in Yemen, there paucity of literature about the breast and certain epidemiological risk factors. Many studies, from many parts of the world, have looked at the prognostic value of age at diagnosis in patients with BC. After controlling

for race, stage and treatment, it has been found that mortality due to BC is greatest in younger women. About 45.6% of female patients in this study were in age group 40-49 years old, with mean age 44.2±1.68 years old. This finding nearly similar to that seen in Kingdom of Saudi Arabia (KSA) with 43.6±8.3 years old by Elkum et al. (2014)⁽²⁶⁾, Najjar and Easson (2010), reported the mean age in Arab countries of BC was 48±2.8 years old⁽²⁷⁾. BC in this study is at age <30 years (10.9%), this finding is more or less similar to that reported by Mehdi et al. (2016) in Omani women (6.2%)⁽²⁸⁾, Egyptian women (8.19%)⁽³⁹⁾. More than half of our patients were from urban areas (57.4%) unlike data published in Qatar were most of the patients were from rural areas (88.7%)⁽³⁰⁾.

More than half of our patients (51.5%) in this study were low educated, and 19.1% had finished primary school, while only 14.7% were highly educated (secondary school and university certificates). In Bangladesh, 87.67% of women with BC were at lower level of education ⁽⁷⁾ and according to study done in Qatar one third of women with BC at university level ⁽³⁰⁾, while in KSA, 36.7% were illiterate, and those with higher level of education more than 12 years account only 12.4% ⁽²⁶⁾. Education is an important factor of awareness against disease and highly educated females have a good awareness of early signs of BC.

A number of studies have observed that the association between smoking and the risk of developing BC may depend on the years of smoking, the lifetime amount of smoking, and the age at initiation, all females in this study were nonsmokers. Another study reported that half of the women had never smoked, and 20% reported actively smoking one year before BC diagnosis. Although not statistically significant, the women who quit smoking after their BC diagnosis had 33% lower risk of death as a result of BC than did women who continued to smoke after diagnosis ⁽³¹⁾.

Obesity is now a common health problem worldwide. It is a lifestyle risk factor associated with not only high risk of cardiovascular and metabolic disease, but also with high incidence and poor prognosis of many malignant tumors. The correlation between general obesity and poorer prognosis of BC may be mediated by increased circulating estrogen levels from excess adiposity through aromatase activity and reduced levels of sex hormone-binding globulins ⁽³²⁾. In this study, 25% of the cases with BC were obese. Many studies showed correlation between obesity and BC, one of them in KSA; when 38.1% of women with BC were also obese ⁽²⁶⁾.

The relationship between oral contraceptive and cancer incidence is controversial, 27.9% of patients enrolled in this study with BC gave a history of previous oral contraceptive pills use. In Tehranian et al. (2010) study, 38.8% of Iranian women with BC gave a history of previous oral contraceptive pills use and in other study 70.2% of Iranian women with BC

gave a history of previous oral contraceptive pills use in comparison to control (52.2%) ⁽³²⁾, and these may signify that the use of oral contraceptive pills doubled the incidence of BC ⁽³³⁾.

In BC, family history is a key risk factor of BC ⁽²²⁻²⁴⁾. Women with a strong family history of BC could inherit genetic alterations that modify their risk of disease. Several studies have demonstrated a relationship between BC and other cancer cases in the family, with the prevalence of cancer cases ranging from 5 to 10 % ⁽³⁴⁾. In this study only 14.7% of the patients with BC had a family history of BC. The family history of BC (first degree relatives) had strong relation with BC or another cancer with 9.5% and 13.3% respectively as reported in a study done in Pakistan ⁽³⁵⁾, while high prevalence (33.3%) of family history and BC was seen by Ribeiro et al. ⁽³⁴⁾.

An association between lactation and protection from BC has also been postulated for a long time. The hypothesis that prolonged lactation protects against the development of BC is one of the oldest and the most enduring hypotheses concerning the etiology of this neoplasm. Age at menarche and BC risk are probably indirectly associated, research estimate that the risk of BC can be reduced 10-20% for each year menarche is delayed, the results of a large study revealed that for each two-year delay in one set of menstruation, BC risk was reduced by about 10% ⁽³⁶⁾. In this study, most of the cases (55.9%) were started menstruation between the age of 13-14 years old and only 17.6% started menarche at earlier age. Nearly similar figure of age at menarche of the women with BC in Karachi Pakistan (51.9%) ⁽³⁷⁾. The age of 13 years at first menarche was seen in (33.1%) of Iranian women with BC ⁽³¹⁾. In this study, most of the patients (51.5%) with BC were at pre-menopause; in a study conducted in different Arab countries to determine the age at diagnosis of BC in Arab nation, they found that the medium age was 44.5 years which was the pre-menopausal age ⁽²⁷⁾. More than half of patients in this study present with breast lump of size range from 2-5 cm at the diagnosis (54.4%), also the large size of BC at the time of diagnosis was seen in Arab

Palestine (33.6%) compared to Jewish women (28.6%)⁽⁶⁾. Upper outer quadrant and the right breast were the most common sites of BC involvement (45.6%) and (58.8%) respectively and bilateral was uncommon (5.9%), also the right side was the commonest in Egyptian women (52.7%) and bilateral involvement seen in (0.3%)⁽²⁹⁾. Histologically, most of the patient shows invasive ductal carcinoma (85.3%) and only (11.8%) was invasive lobular carcinoma, invasive ductal carcinoma was the commonest histological type of BC seen in Yemen by Harhra and Basaleem (2012)⁽³⁸⁾, and in Egyptians women invasive ductal carcinoma account (81%) and invasive lobular carcinoma usually uncommon and account (13%). In this study, most of the patient was at stage II clinically and pathologically (36.8%), 33.6% Arab Palestine women with BC are at stage II similar to Jewish (31.7%)⁽⁶⁾. Stage III was the most common clinical stage seen by Harhra and Basaleem study (2012) done in Yemen (43.3%)⁽³⁸⁾.

It is suggested that many genes, which take part in the control of finger and palmar dermatoglyphic development, can also give indication to the development of pre-malignancy and malignancy. The specific BC predisposing genes are BRCA1, BRCA2 and p53. studies by Bowcock (1997), Easton et al. (1993), Shattuck et al. (1995) and Petty et al. (1997)⁽³⁹⁻⁴²⁾, all corroborate the finding that mutations in BRCA1 account for BC in 50 % of families. In this study, the loop patterns of fingerprints were presented with 43.5% high than other patterns, and taken in consideration the distribution of patterns on digit we found the loop presented high in little finger accounting 30.4%, while whorls presented high in index finger with 33.6% and arches presented in thumb with 27.4%. All the three mentioned patterns had significant association with BC with p-value = 0.005, 0.028, and 0.011 respectively. A study done by Abbasi et al. (2006) in Iran reported those women with BC had whorls pattern as a common fingerprint⁽²³⁾, while other study done by Srivdevi et al. (2010) and Natekar et al. (2006) founded the loop is the most common fingerprints in their participants^(21,22).

This study concluded that the social, behavioral, and hereditary factors play an important role in the development of BC. Yet the reproductive history is an important factor in the development of BC that signify the role of the hormones in the pathogenesis of BC, in addition to that, the genetic factors are still important and not well studied factors in BC development. The medico-legal importance of these patterns is unique and remain unchanged throughout life, and the fingerprints are genetically determined factors that can be used as simple and cost-effective screening test for BC. Further study of fingerprints among high risk families matched with BRCA1 and BRCA2 genes is recommended to the validity of fingerprints as screening test of high-risk families.

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Author contribution

Dr. Bin Thabit: performed the Introduction, data collection of (FNAC) and discussion. Dr. Abdullah: participated in the Data collection of (Printing the female patients' fingerprints) and method. Dr. Alnoban: Interpretation of results and statistical analysis.

Conflict of interest

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4-Hydroxy-2-nonenal, Induced Nitric Oxide Synthase Status in Hypertension with Kidney Disease Patients

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Abstract

Background	The oxidative stress is one of the main reasons for cardiovascular diseases and also one of results of these diseases, its development (like kidney disease).
Objective	To identify the effect of oxidative stress nitric oxide and reactive oxygen species on cardiovascular diseases.
Methods	The study involved 56 subjects comparable in age and sex divided into two groups; 28 hypertensives subjects with kidney disease and 28 apparently healthy subjects as control group. The following analysis was done: 4-Hydroxy-2-nonenal (4HNE), Induced nitric oxide synthase (iNOS) and albumin.
Results	There was a significant increase in (4HNE) between patients' group and control group. iNOS was significantly higher in patients as compared to controls while there were no significant differences found in albumin between patient and control group. There is a positive relationship between oxidation results from hypertension and their developments. There is a positive correlation between BMI and disease.
Conclusion	Based on this study is important on ideal weight, because obesity considered main factors for heart disease and hardening of the arteries. In addition, the effect of oxidative stress, which leads to high blood pressure and thus chronic kidney disease.
Keywords	Chronic kidney disease, hypertension, 4-Hydroxy-2-nonenal (4HNE), Induced nitric oxide synthase (iNOS), albumin, creatinine, reactive oxygen species
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List of abbreviations: BMI, Body mass index, BP = Blood pressure, CKD = Chronic kidney disease, iNOS = Induced nitric oxide synthase, ROS = Reactive oxygen species

Introduction

Blood pressure (BP) is the force created by the heart as it pushes blood into the arteries through the circulatory system. Every time the heart contracts or beats, the blood is pumped out and creates a wave of pressure in the arteries. Macrophages had been supposed to be the source of the most reactive oxygen species (ROS) in the vessel's

wall ⁽¹⁾. However, it has become clear that all the cells in the vessel wall produce ROS in different quantities and in response to diverse stimuli ⁽²⁾. High BP is one of the main factors causing the disease, which contributes to the deterioration of kidney function ⁽²⁾. The presence of kidney disease is a medical reason by the subscriber to find and underappreciated from high BP resistance. Therefore, treatment of hypertension has become the most important intervention in the management of all type of chronic kidney disease (CKD) ⁽³⁾. CKD

(also called chronic kidney failure) is a condition that affects the function of the kidney, which can progress over time to kidney failure. When the kidneys fail, dialysis or a kidney planting is needed to support life. Many diseases can cause CKD ⁽⁴⁾. The most common are diabetes and high BP. High BP puts more pressure on the blood vessels throughout the body, including the kidney filters (nephrons). Hypertension is the number two cause of kidney failure ⁽⁵⁾. In addition, increased oxidative stress causes tissue damage by different mechanisms including promoting lipid peroxidation, DNA damage, and protein modification ⁽⁶⁾. ROS is a group which are either free radicals or molecular species capable of generating free radicals, examples include singlet oxygen, superoxide, peroxides, and hydroxyl radical. In a biological context, ROS which is formed as a naturally byproduct of the natural metabolism of oxygen and have important roles in cellular signal and homeostasis, but through times of environmental stress (like, UV or heat exposure) ROS levels can raise dramatically ⁽⁷⁾, and this may lead to severe damage to cell structures, Cumulatively, this is called oxidative stress. ROS are also created by exogenous sources like ionizing radiation ⁽⁸⁾. When there is an imbalance between the generation of ROS and the antioxidant defense system so that the last one becomes overwhelmed, oxidative stress happens ⁽²⁾. Oxidants and free radicals are produced inevitably through most physiological and metabolic processes, and the human body has an anti-oxidant defense mechanisms, these different mechanisms according to the type of cells and tissues, and they may behave aggressively or synergy ⁽⁸⁾. Oxidative stress causes many pathophysiological conditions in the body ⁽⁹⁾, including neurodegenerative diseases such as Alzheimer's disease, gene mutations, chronic fatigue syndrome, atherosclerosis and inflammatory diseases ⁽¹⁰⁾. In physiological conditions, low level ROS play a role in the protection the organism, while high levels of

ROS may cause damage to the structures of the cell, nucleic acids, lipids, proteins or DNA damage ⁽¹¹⁾. 4-hydroxy-2-nonenal (4HNE), a high toxicity product of lipid peroxidation, is an inhibitor of mitochondrial respiration. 4HNE exerts its influence on respiration by inhibiting α -ketoglutaratedehydrogenase (KGDH) ⁽¹²⁾. A study by Campos et al. in 2015 observed an increase in 4HNE with hypertension and kidney disease ⁽¹³⁾. Nitric oxide synthases (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine ⁽¹⁴⁾.

Albumin is the most abundant protein in the circulatory system and act as antioxidant ⁽¹⁵⁾, the antioxidant activity of albumin due to its ability to bind bilirubin, homocysteine and lipids ⁽¹⁶⁾.

The objective of the study was to investigate the relationship between of some oxidative stress markers and kidney disease associated with hypertension.

Methods

The present study comprised of 56 subjects divided into two groups control group (28) and hypertensive with kidney disease group (28) aged between 22-65 years. These patients were hospitalized at Educational Laboratories in the Al-Yarmouk Teaching Hospital. Blood sample were collected and centrifuged at [4000 xg] for 5 min after clotting. The resultant serum were separated and stored at [-20] °C until used. Estimation of serum albumin was done using kit provided by Bio Systems Company.

Also, Creatinine in the blood has been estimated using several provided by Randox. Serum 4HNE is typically quantified from serum samples with the most popular method being a colorimetric assay based on biotin double antibody sandwich technology. The serum INOS is typically quantified from the serum test samples employed by the quantitative quantification enzyme immunoassay technique.

Statistics

The Statistical Analysis System-SAS- (2012) was used to determine of various factors in studied parameters.

Results

Fifty-six sample patients comprising of 28 patients and 28 apparently healthy were

included in the present study, table 1 shows the means and standard deviation of age, body mass index (BMI), duration of disease, 4HNE, in addition to albumin levels and creatinine levels for the control and patients' group.

Table 1. Characteristics of the Hypertension (HT) and control group (mean ± SD)

Characteristic	Hypertension with kidney group n=28	Control group n=28	P-Value
Age (year)	47.60 ± 11.71	23.82 ± 5.02	<0.01
Body Mass index (Kg/m ²)	28.17 ± 3.90	25.18 ± 2.89	<0.01
Systolic blood pressure (mmHg)	19.03 ± 3.23	11.96 ± 0.66	<0.01
Diastolic blood pressure (mmHg)	10.83 ± 1.25	8.63 ± 0.56	<0.01
4HNE (ng/L)	273.71 ± 96.75	136.46 ± 24.55	<0.01
iNOS (IU/ml)	15.58 ± 14.44	6.42 ± 4.93	<0.01
Albumin (g/dl)	3.90 ± 0.48	4.73 ± 0.25	<0.01
Creatinine (mg/dl)	8.01 ± 5.88	0.75 ± 0.09	<0.01

The highly significant difference (p<0.01) of 4HNE in HT with CKD patient as shown in figure 1 may be due to high oxidative stress which generally causes damage to the membrane

polyunsaturated fatty acids which leading to the generation of 4HNE as shown in figure 2 and 3 and this result agrees with study done by Usberti et al. in 2002 ⁽²¹⁾.

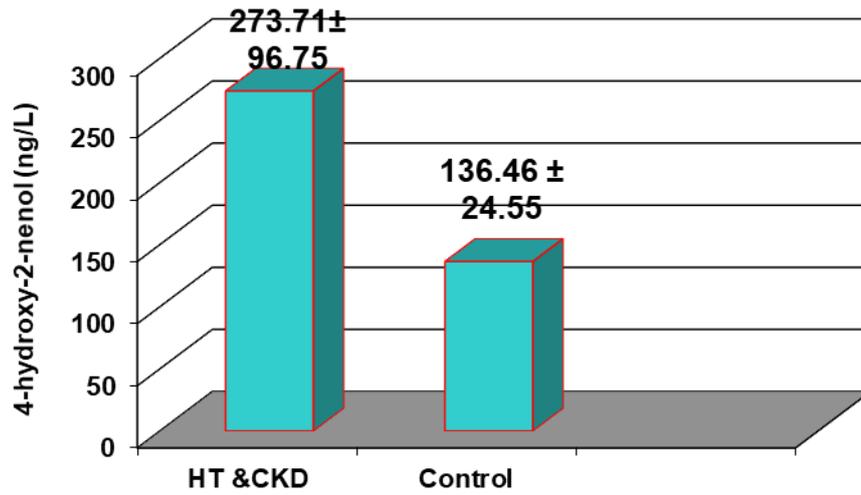


Figure 1. Mean concentration (ng/L) of oxidative marker 4HNE for studied groups

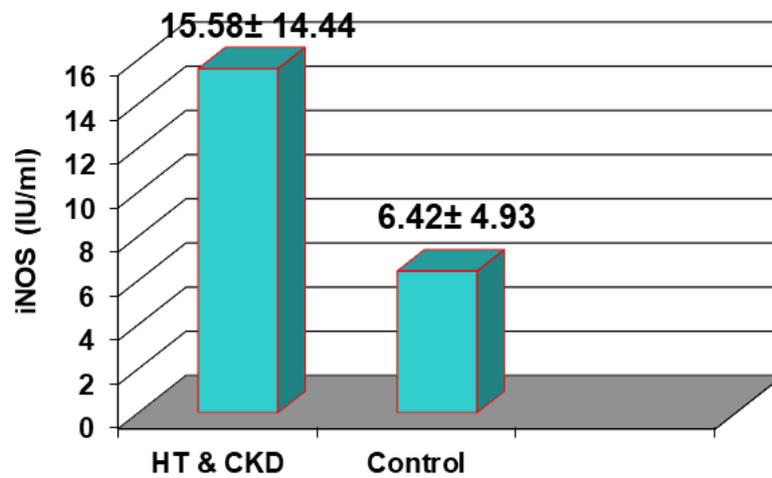


Figure 2. Mean concentration (IU/ml) of iNOS in the studied groups

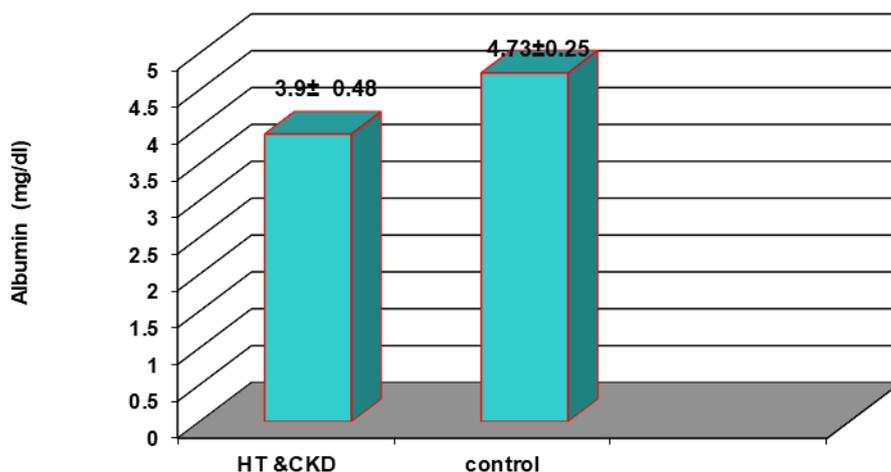


Figure 3. Mean concentration (g/dl) of albumin in the studied groups

Discussion

There is a significant difference ($p < 0.01$) in age when comparing patients' groups with control group. The significant difference in BMI ($p < 0.01$) between HT patients with CKD patients and control group reveal the positive correlation between obesity and disease. This finding was similar to Hall. et al in 2014 who found a significant increase in BMI in hypertensive with kidney disease patients⁽¹⁹⁾. Obese patients are more able to be hypertensive than lean patients, and weight gain is usually associated with increases in arterial pressure⁽²⁰⁾.

The results of this study showed a significant difference in iNOS between hypertension with kidney patient and control groups ($p < 0.01$). In this study, results were agreed with Abd El Gawad et al. in 2011⁽²²⁾ who found a significant difference in iNOS levels in hypertensive patients. There is a positive correlation between serum iNOS levels and systolic, diastolic and mean blood pressure. Several studies have demonstrated high iNOS level in patients with hypertension⁽²³⁾.

Impaired endothelial dependent vasodilatation associated with abnormal iNOS may be an important factor in the development and progression of atherosclerosis and hypertension. In addition, endothelial dysfunction, in hypertensive patients may

initiate vascular inflammation that leads to cytokine-induced activation of inducible NOS which favors the formation of peroxynitrite contributing to cytotoxicity and tissue injury⁽¹¹⁾.

Excessive or inappropriate NO production by iNOS reacts with superoxide anions in the plaque to yield reactive oxidant species such as peroxynitrite contributing to cytotoxicity and tissue injury. Another possible outcome for NO coming from iNOS could be the activation of matrix metalloproteinase and induction of apoptosis in smooth muscle cells and macrophages.

Albumin contains a free sulfhydryl group, and this forms a disulfide with many compounds like cysteine, homocysteine, or glutathione, Albumin is able to scavenge hydroxyl radicals⁽²³⁾, the decrease in albumin in patient is agreed with results of Menon et al. in 2005 in hypertension⁽¹⁷⁾ who suggested that result is may be due to its function as antioxidant activity. On the other hand, the non-oxidized albumin is decrease in addition to negative acute phase protein, so inflammation is considered the main cause of a decline in the serum albumin⁽²³⁾. The results showed a significant in creatinine levels in patients' groups when compared to control group and this agree with AL-Hamdani⁽²⁴⁾. There were significant differences between patients and

control in creatinine concentration. This elevation of serum creatinine concentration may be due to the decline in creatinine clearance due to the lack in the GFR⁽²⁵⁾. Creatinine as a waste product of creatine produced in muscles, and is converted about 1% of the total pool muscle creatine and creatinine daily through the loss of water is enzymatically spontaneous. Since released in the blood at a constant rate, and since it matched closely to the secretion of glomerular filtration rate⁽²⁶⁾. The elevation of serum creatinine concentration may be attributed to the lack in creatinine clearance due to the decline in the GFR.

The weak negative correlation between 4HNE and albumin in hypertension with kidney patient and control group agreed with Aldini et al. in 2006 who found a negative correlation between 4HNE and albumin⁽²⁷⁾. There is an evidence for a significant antioxidant activity of the represent the major and predominant circulating antioxidant in plasma known to be exposed to continuous oxidative stress.

There is also negative correlation between iNOS and albumin in patient and control group this result shown Poteser and Wakabayashi in 2004 who found a negative correlation between iNOS and Albumin⁽²⁸⁾. There is a negative correlation between 4HNE and creatinine in patient and control group was observed in this study which agreed with Kobori et al. who found a negative correlation between 4HNE and creatinine⁽²⁹⁾. A negative correlation between iNOS and creatinine in patient and control group was observed in this study which agreed with Park et al. who found a negative correlation between iNOS and creatinine⁽³⁰⁾.

Based on this study is important on ideal weight, because obesity considered main factors for heart disease and hardening of the arteries. In addition, the effect of oxidative stress, which leads to high blood pressure and thus chronic kidney disease.

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Author contribution

Hammed: conducted the sampling, work and writing. Dr. Jawas and Dr. Ali supervised the work.

Conflict of interest

The authors declare no conflict interest

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The Effects of Gender and Hand Dominancy on Motor Unit Number Estimation in A Sample of Healthy Iraqis Using Two Different Methods

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Abstract

- Background** Motor unit number estimation (MUNE) is a unique electrophysiological technique that was developed to determine a numeric estimate of the number of innervating axons. This technique can be used to determine the approximate number of motor neurons in a muscle or group of muscles.
- Objective** To test the MUNE values according to subject's gender and hand dominancy in the normal population.
- Methods** Healthy volunteers who had neither neuromuscular nor systemic/ metabolic disease, with normal neurological examination were studied. Ninety hands of 56 healthy volunteers (11 males and 45 females) with ages ranging from (24-58) years were included in the study. All had normal median nerve conduction studies. Manual incremental (INC) method and adapted multiple points stimulation (AMPS) method were performed for MUNE.
- Results** Gender did not have an effect on the scores according to the two studied methods ($p=0.054$ by INC method and $p= 0.700$ by AMPS method). Hand dominancy also show no statistically significant difference of the scores of MUNE according to both studied methods ($p=0.091$) by INC method and ($p=0.051$) by AMPS method.
- Conclusion** Incremental stimulation and adapted multiple point stimulation are reliable and easily applicable methods with same reproducibility in estimating motor units with no significant effect for subject gender or hand dominancy.
- Keywords** Motor unit, motor unit number estimation (MUNE), incremental stimulation (INC) method, adapted multiple point stimulation (AMPS) method
- Citation** Abdul Muneem SD, Kaddori HG. The effects of gender and hand dominancy on motor unit number estimation in a sample of healthy Iraqis using two different methods. *Iraqi JMS*. 2019; 17(2): 153-160. doi: 10.22578/IJMS.17.2.9

List of abbreviations: AMPS = Adapted multiple point stimulation, APB = Abductor pollicis brevis muscle, CMAP = Compound motor action potential, CTS = Carpal tunnel syndrome, EMG = Electromyography, EP = Evoked potentials, INC = Incremental stimulation, MCP = Metacarpophalangeal joint, MNCS = Motor nerve conduction study, MU = Motor unit, MUNE = Motor unit number estimation, SNCS = Sensory nerve conduction study

Introduction

A motor unit (MU) is defined as a single anterior horn cell or brain stem motor neuron, its peripheral axon (which

travels in a cranial or peripheral nerve), and each of the muscle fibers innervated by that axon. they are considered as the final common pathway of the motor system⁽¹⁾.

The numeric determination of numbers of MUs is described as motor unit number estimation (MUNE)⁽²⁾. MUNE provides determination of functional MU numbers quantitatively as the

most parallel to the real numbers and can be performed in various techniques ⁽¹⁾.

The thought of estimating MUs was firstly mentioned by McComas in 1967. Estimating the functional MUN in a human muscle group in vivo has been facilitated by using computer-aided MUNE techniques. There have been at least 10 different MUNE techniques reported up till now and each of which has some advantages and disadvantages. There have not been so many studies directly quantifying the effect of hand dominancy or even the effect of gender on MUNE although these electrophysiological techniques have been available ⁽²⁻⁵⁾.

This study aimed to determine the effect of subject gender and hand dominancy on estimated MUN in Abductor pollicis brevis (APB) muscle of healthy subjects using incremental stimulation (INC) and adapted multiple point stimulation (AMPS) methods

Methods

A cross sectional study was conducted at the Neurophysiology Unit at Al-Imamein Al-Kadhimein Medical City during a 4-month period (Nov. 2017 to Mar. 2018). The study was approved by the Institute Review Board of the College of Medicine, Al-Nahrain University. An ethical consent was taken from each participant to be enrolled in the study.

Subjects

The study was performed on 90 hands of 56 healthy volunteers with a mean age of 36.22 ± 7.32 years (age range = 24-58 years). Eleven subjects were males (19.6%) and 45 were females (80.4%). Forty-nine subject (87.5%) were right-handed and only seven were left-handed (12.5%). The study excludes any subject with history of wrist fracture or surgery, carpal tunnel syndrome, cervical radiculopathy, hereditary or acquired peripheral neuropathy.

Healthy volunteers with neither Neuromuscular disease nor any systemic/metabolic disease were included in the study. All subjects were considered to be

moderately active for their respective age groups. Before the study, systemic and neurological examinations of subjects were made and if they had normal findings, conventional neurophysiological studies of median and ulnar nerves were performed and if these were also normal, MUNE studies by INC stimulation and AMPS techniques were carried on.

Instrumentation

The following were used for all electrodiagnostic testing:

Computerized Electromyography/ Evoked potentials (EMG/EP) machine (Cadwell, 8-channel electromyograph) supplemented with different types of electrodes including grounding electrode used to protect the subject against electrical hazard and to reduce stimulus artifacts and interference, stimulating surface electrodes was used to stimulate the nerves through the skin and surface recording electrodes.

Electrophysiological studies

For each subject, conventional neurophysiological studies (sensory, motor and F wave studies) were performed to exclude peripheral neuropathy. MUNE of both APB muscles using both INC & AMPS methods were performed.

Sensory nerve conduction study

An antidromic method was used for sensory nerve conduction study (SNCS) determination, in which, the nerve was proximally stimulated from the trunk and the evoked activity was distally recorded from a finger. The parameters studied were the sensory latency (SL), sensory nerve action potential (SNAP) amplitude from peak to peak and sensory nerve conduction velocity (SNCV) measured by dividing the conduction distance (d) by the SL and measured in meter/second (m/sec) ⁽⁶⁾.

Motor nerve conduction study and F-wave

The motor nerve was simulated at two points along its course, by applying stimuli at the

distal and the proximal sites of the nerve and recording from the muscle innervated by that nerve. The parameters studied were distal motor latency (DML). Motor nerve conduction velocity (MNCV) measured by dividing the distance between the two stimulation points over the difference between the latencies of the recorded responses ensuring both

compound muscle AP (CMAP) configurations must be similar in addition to F wave latency measured from the stimulus artifact to the beginning of the evoked potential ⁽⁶⁾.

Motor-unit number estimation

MUNE values are calculated from the ratio:

$$\frac{\text{maximal compound muscle action potential (CMAP) amplitude or area}}{\text{average single motor unit potential (MUP) amplitude or area}}$$

MUNE test was performed using a manual INC method. MUNE was recorded from a surface-active recording electrode placed over the motor point of the APB with the reference over the metacarpal phalangeal (MCP) joint of the thumb. The median nerve was stimulated at wrist by Cadwell electrical stimulator, at 8 cm proximal to the active electrode. For accurate nerve stimulation, the exact site of stimulation was marked by pin at wrist prior to stimulation. A built-in MUNE analysis program in the Cadwell EMG device was used, and the maximum M response was first obtained by increasing stimulation intensity and a maximal CMAP is recorded. Next, the display sensitivity is raised to 100–200 $\mu\text{V}/\text{div}$ to help visualize low amplitude steps in the response envelope. The stimulus intensity is lowered to 3–10 mA in order to activate the first axon, indicated by an all-or-none response. By small increases in stimulation intensity, an envelope of responses is obtained with 8–10 discrete steps before the increments in the envelope become indistinguishable. The number of steps is divided into the peak to-peak amplitude of the envelope to determine the average amplitude of each step.

This average value represents the average single MUP (S-MUP), and is used to calculate the MUNE value (figures 1 and 2) ⁽⁷⁾.

Adapted multiple point stimulation (AMPS) method

Multipoint incremental MUNE method with the Shefner modification is a noninvasive, easy to perform method with high reproducibility ^(7,8).

By stimulating the nerve at many sites, this technique yield data from MUs with different morphologies. The recording electrode was placed on the belly of the APB muscle and a maximum CMAP was obtained by applying a supramaximal stimulus.

Median nerve of the right and left hand was studied. Recording electrodes were placed on the median nerve innervated APB muscle, using the standard belly tendon method. The MUNE program recorded the maximum CMAP amplitude obtained. Then display sensitivity was raised to 100–200 $\mu\text{V}/\text{div}$ to help visualize low amplitude steps in the response envelope and the stimulus intensity was lowered to 3–10 mA in order to activate the first axon, indicated by an all-or-none response. Again, by small increase in stimulation intensity, an envelope of responses is obtained with 3-5 discrete steps before jumping to the next site.

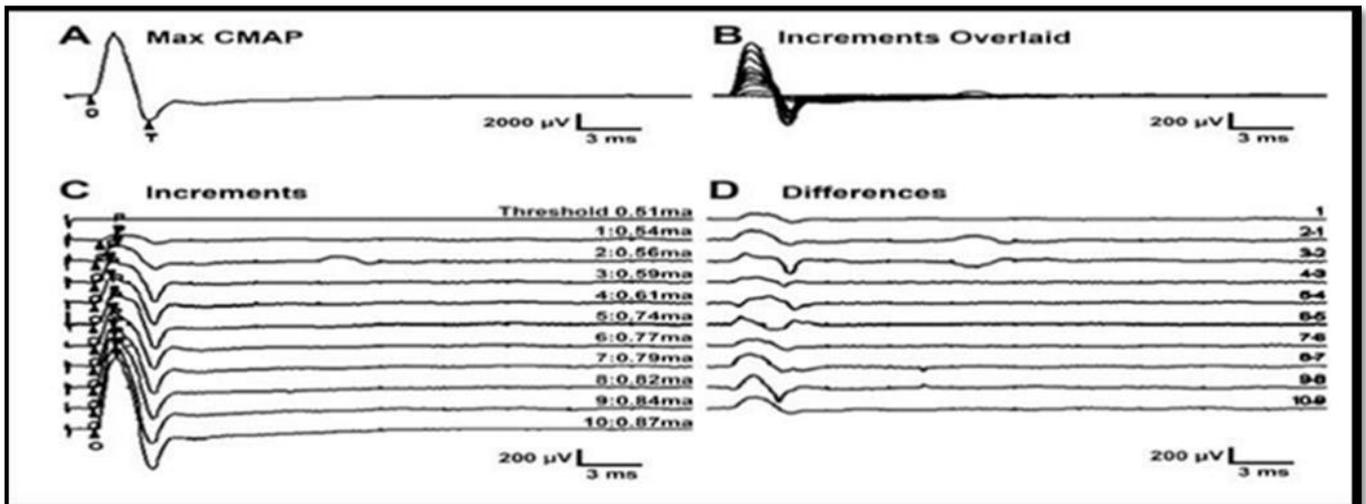


Figure 1. Scheme of incremental stimulation. A. with maximal compound muscle action potential, CMAP max. B. 10 stacked incremental evoked potentials. C. Individual evoked potentials and D. single motor unit potentials, SMUPs⁽⁶⁾

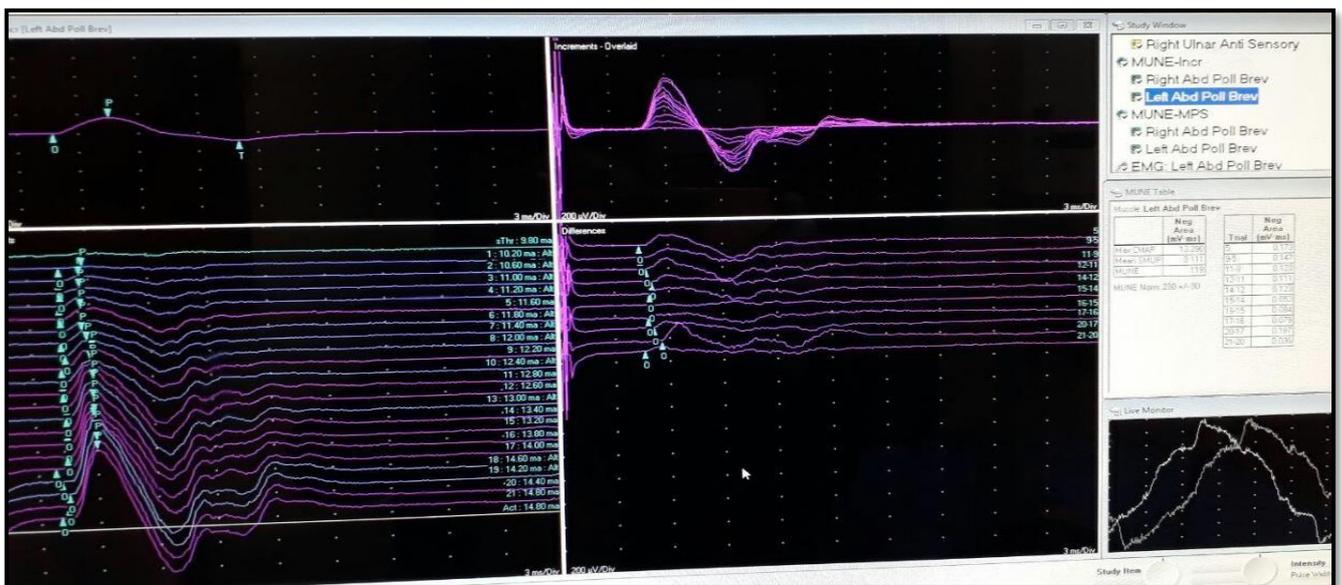


Figure 2. A photographic picture. of Cadwell EMG screen during incremental MUNE study

Using standard 3-site motor conduction program traces were obtained and superimposed. Three stimulus locations were used for the median nerve; 2 cm proximal to the wrist crease, 4 cm proximal to the first stimulation site, and in the cubital fossa. Optimum stimulus location was determined using a submaximal stimulus and moving the stimulator to evoke the greatest response.

Three responses were obtained at each stimulation site with each response of 25 µV incremental amplitude. The negative peak amplitude of the third response was recorded. Stimulation at the second and third location was identical to the first location. Once the sample collection was complete, we reviewed all tracings for potential repeating MUs (so that they were not included more than once). Using

standard 3-site motor conduction program traces were obtained and superimposed (figure

3) ⁽⁵⁾ The test last about 30 minutes for each subject.

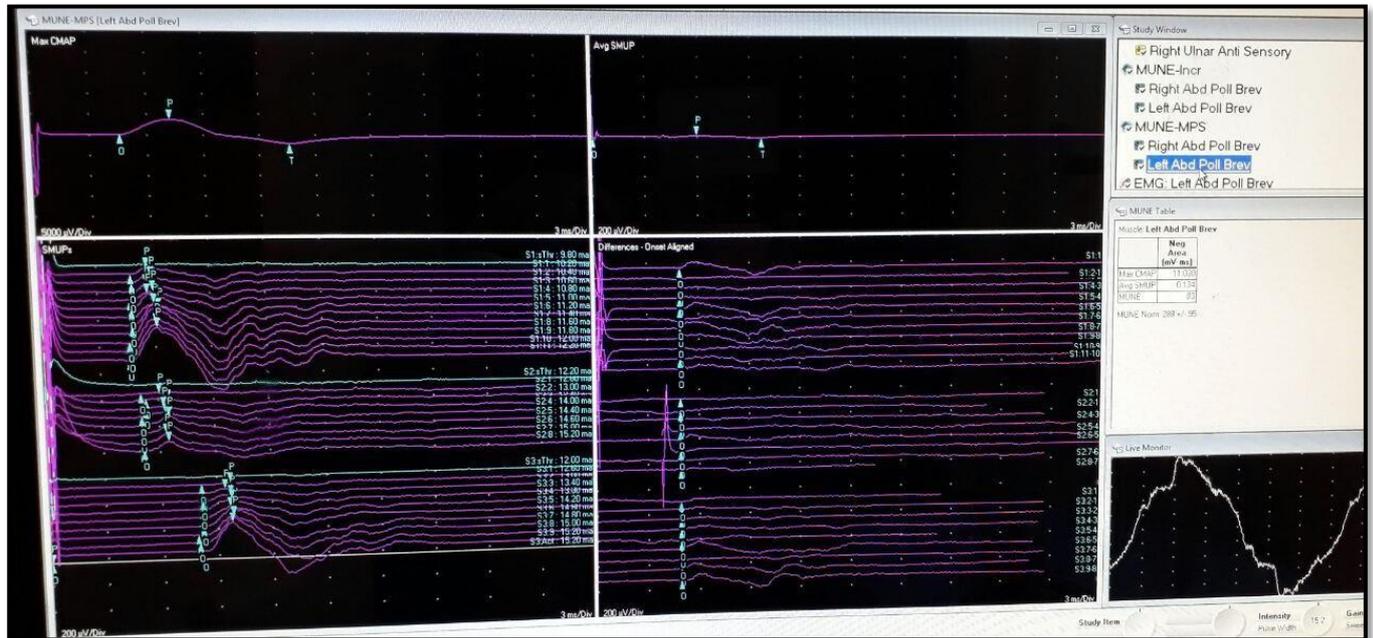


Figure 3. Photographic picture. of Cadwell EMG device during Adapted multiple point stimulation MUNE study

Statistical analysis

The statistical analysis was obtained using statistical package of social sciences (SPSS) version 23 software and Microsoft Office Excel 2016.

All data were expressed as mean \pm SD. Paired t-test was used to compare the dominant and non-dominant side and between males and females. P-value of 0.05 or less was considered significant.

Results

This study found that MUNE values of APB muscles by both techniques are higher in male group than in female (but not statistically significant).

Considering the hand dominance, we found that the non-dominant hand APB muscle contains higher MUNE value that is 214.74 ± 50.18 by INC. method and 272.35 ± 68.49 by AMPS method for non-dominant APB and 196.18 ± 49.2 by INC. method and 244.82 ± 54.27 by AMPS method for dominant APB muscle- as shown in tables 1 & 2.

Table 1. MUNE value of the APB muscles in female and male subjects by INC and AMPS methods (unpaired t test)

MUNE method	Females N=67 Mean ± SD	Males N=23 Mean ± SD	P value
INC method	197.38±47.15	226.44±56.11	0.054
AMPS method	254.11±63.58	259.67±51.69	0.700

MUNE = motor unit number estimation, INC= incremental stimulation method, AMPS = Adapted multiple point stimulations method, ± SD = standard deviation.

Table 2. MUNE value of APB muscles of the dominant and non-dominant hands (unpaired t test)

MUNE method	Dominant hand N=56 Mean±SD	Non-dominant hand N=34 Mean±SD	P value
INC method	196.18±49.2	214.74±50.18	0.091
AMPS method	244.82±54.27	272.35±68.49	0.051

MUNE = motor unit number estimation, INC= incremental stimulation method, AMPS = Adapted multiple point stimulations method, ± SD = standard deviation.

Correlation of two MUNE methods

The above data denotes - no significant deference in MUNE values between INC and AMPS techniques. As seen in figure 4, a strong -

positive correlation between values obtained by both methods was found ($r=0.769$ and $p < 0.001$).

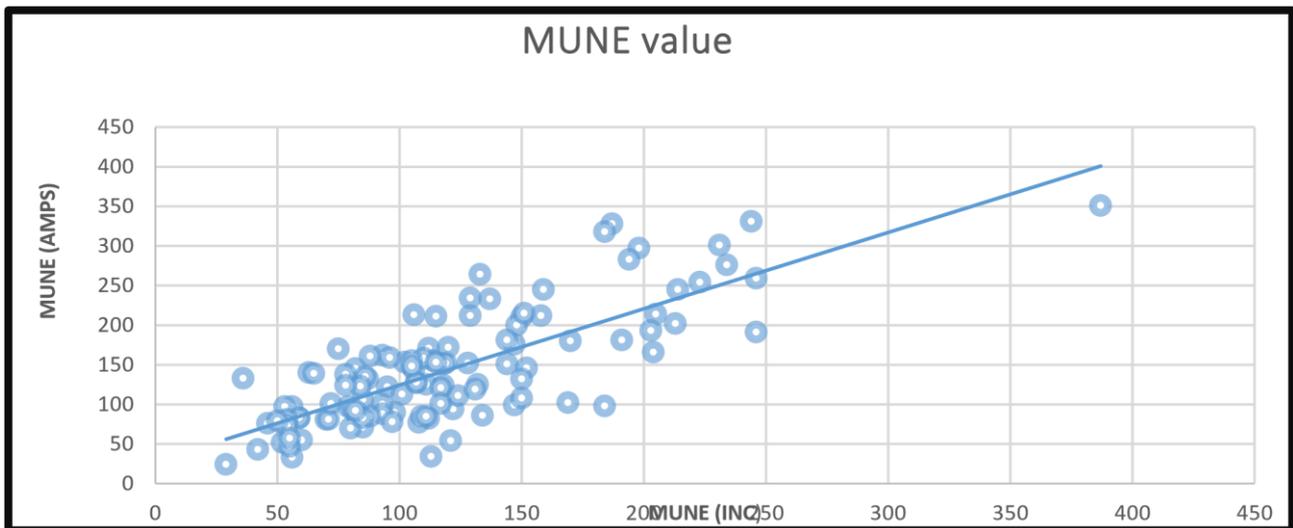


Figure 4. Correlation of INC and AMPS methods for MUNE

Discussion

For now, there have been many available MUNE techniques share in common studying

the features of MUs in an attempt to determine quantitatively the functional MUN - as the most similar to the real numbers. All the

techniques have been developed based on manual incremental method ⁽⁹⁾.

The aim of MUNE methods is to calculate the number of fibers in a muscle almost correctly and relative to the real numbers. Certainly, the most accurate way of this is to estimate fibers histologically. Studies dealt with correlation between histological and electrophysiological studies are scarce ^(9,10). In general, according to the test reliability, the necessary period for completing study, easiness, obtaining data parallel to disease and sufficiency in determining progression of disease, none of these different methods is superior to each other ⁽¹¹⁾.

Manual INC and AMPS methods were used in this study. Results of the study revealed quite similar values in standard conditions between the two methods. although practically we prefer to use incremental stimulation method since it easier to stimulate median nerve at wrist in comparison with three stimulation sites for median nerve in AMPS.

Furthermore, the study demonstrated that gender has no significant effect on MUNE values (though the results show that MUNE in males higher than that of females but statistically not significant). This may be explained by the more muscular components of male body or hormonal differences, increasing sample size is mandatory to study this difference. These results are similar to that reported by Yerdelen et al. in 2006 who state that gender had no effect on MUNE value although his results show higher values in male than females but statistically not significant ⁽¹⁾.

Also, we found that MUNE values of median innervated APB muscle by both techniques are not statistically significantly different according to hand dominance (even though the results show higher values in non-dominant hands but still not significant). The current data were comparable with those reported by Li et al. who state that there was no statistically significant difference between both dominant and non-dominant hand regarding first dorsal interossei (FDI) and thenar muscles regardless his results show high motor unit number index

(MUNIX), lower motor unit size index MUSIX in non-dominant hands for both muscles ^(12,13).

Handedness is associated with brain lateralization. Asymmetrical excitability in the corticospinal system has been observed in many researches with transcranial magnetic stimulation ^(14,15). Contradictory evidence was also reported showing no significant influence of handedness on motor evoked potentials ⁽¹⁶⁾. The neural mechanisms underlying handedness is not fully understood yet. Regardless of neural origins of handedness, this study examined the MUNE estimations in dominant and non-dominant hand muscles. The MUNE values from bilateral hand muscles were within the range of previously reported reference values ^(3,13,17). No significant difference in MUNE values was observed between the dominant and non-dominant hands for the thenar muscles, implying that the population of MUs or spinal motor neurons may not be associated with handedness ⁽¹³⁾.

In contrast to investigations on the lateral asymmetry in the central nervous system, aspects related to lateral asymmetry of the peripheral nervous system have been relatively less studied. By applying the MUNE technique, a previous study observed that the number of motor units of the APB muscle was higher for the non-dominant hand than the dominant hand muscles ⁽¹⁸⁾. So higher sample size study is recommended to clarify this association.

The non-significant difference in MUNE results obtained by INC and AMPS methods in this study denotes that both are similarly reproducible and are equally effective and none of them was superior to the other which is in accordance with the other researchers as Xu et al. who stated that both MUNE methods are similarly reproducible and are equally effective in estimating motor units and their reduction with aging and ALS patients ⁽¹⁷⁾.

In conclusion the present study revealed that the gender of subjects or hand dominance did not affect MUNE value, both INC and AMPS techniques are easily applicable, similarly reproducible and are equally effective MUNE methods.

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Authors contribution

Dr. Abdul Muneem: collection of data, analysis of them. Interpretation and discussion of results done by both authors.

Conflict of interest

The authors declare no conflict of interest.

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Large Intraarticular Ganglion in Knee Joint: A Case Report

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Abstract

Ganglion is defined a cystic lesion composed of myxoid matrix having jelly like consistency and is lined by pseudomembrane. Large ganglionic cyst in Hoffa Pad is quite uncommon and only few are mentioned in literatures. A 25-year old female presented with history of gradually worsening anterior knee pain and swelling for 10 months duration, she was disabled due to repeated attacks of knee pain. Magnetic resonance imaging (MRI) shows large intra-articular multilocular cyst. The decision was to do open excision of the cyst in order to decrease the recurrence rate. Histopathological finding shows multi-lobulated cysts with glassy fibrous wall and clear jelly like consistency, which confirm diagnosis. Postoperative period was uneventful and she was doing well during follow up after four months. Large ganglionic cyst developed in Hoffa fat pad of the knee should be considered in deferential diagnosis of intraarticular mass causing pain around the knee. The decision was open excision depending on MRI finding to avoid incomplete resection and prevent recurrence.

Keywords Knee, Ganglion Cyst, Hoffa Fat Pad, MRI

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List of abbreviations: ACL = Anterior cruciate ligament, CT = Computerized tomography, HFP = Hoffa fat pad, MRI = Magnetic resonance imaging, PCL = Posterior cruciate ligament, U.S.G = Ultrasonography

Introduction

Ganglion is a cystic lesion filled with gelatinous fluid containing hyaluronic acid and other mucopolysaccharides surrounded by dense network of collagen fibers and fibrocytes ⁽¹⁾.

Ganglion usually arise from tendon sheaths, joint capsule or muscles; can be solitary or multi lobulated ⁽²⁾.

Ganglionic cyst may be seen in all the joints with variable frequency. Most common site of ganglion is dorsum of the hand ⁽³⁾, and it is rare in knee joint ^(4,5).

In the knee, joint intraarticular ganglion arises near lateral meniscus, anterior cruciate ligament (ACL), posterior cruciate ligament (PCL); they are rarely arising from Hoffa Fat Pad

(HFP). Only few cases had been reported in literatures ^(4,6).

The HFP also known as infrapatellar fat pad is bounded superiorly by the inferior pole of the patella, anteriorly by joint capsule and patellar tendon, posteriorly by joint cavity, and inferiorly by prominence of tibia. It is attached to anterior horns of both menisci and to the tibia inferiorly, projecting in the intercondylar notch superiorly via two alar folds which fused together forming the infrapatellar plica.

The size of this fat pad varies according to individual shape and it is important for lubrication especially during flexion. Infrapatellar plica or ligamentum mucosa run from the intercondylar notch anteriorly through the fat to the ACL ^(7,8).

Intraarticular ganglionic cyst in the knee had been reported nearly 0.2-1% on MRI and 0.6% on arthroscopy ⁽⁹⁾.

Dagher & Hassan, *Ganglion in Knee Joint: A Case Report*

The ganglionic cyst can be associated with an intraarticular pathology and called (asymptomatic), because other disorders responsible for complain of the patient incidentally, detected cysts without any other abnormality are called (symptomatic) ^(10,11).

Case presentation

A 25-year-old female presented with worsening of left knee pain for 10 months, which started after blunt trauma to knee. Immediately after trauma there was no swelling and patient was able to walk. She was disabled due to repeated attacks of knee pain.

Clinical examination showed palpable well detected mass at lateral aspect of her knee which increased during extension and decreased during flexion of the knee.

There was no effusion in the joint. Range of movement was limited in extreme, no collateral and cruciate ligament injury and MacMurray test for meniscus injury was negative.

X-ray and hematological investigations were both normal.

On ultrasonography (U.S.G) examination, a well-defined large lobulated cystic lesion in anterolateral aspect of left knee joint not connected to the joint (Figure 1).



Figure 1. Ultrasonography of ganglion cyst in the left knee joint of the patient

Magnetic resonance imaging showed well defined large lobulated lesion (3x5x3.5) cm, which was arising from HFP extend lateral with close relation to anterior horn of lateral

meniscus, picture suggest a ganglion cyst of Hoffa.

The rest of knee joint was normal with no meniscal tear (Figure 2).



Figure 2. Magnetic resonance imaging of left knee of the patient with intraarticular knee joint ganglion

The ganglionic cyst in this case was large so the decision was to take the patient to open excision to avoid an incomplete removal of the large cyst and to minimize the risk of recurrences compared with arthroscopic resection.

Operative finding

Under spinal anesthesia using tourniquet. A 5cm incision in the lateral Para patellar region, to approach the cyst.

A multilobulated cyst of 5.5 cm excised from Hoffa not attached to synovium or menisci, the cyst was completely resected and wound closed in layers.

Histological evaluation

Histological finding showed cystic lobules with a glassy fibrous tissue wall and clear jelly like consistency.

There were no cells inside, which confirm diagnosis.

Post operatively the patient was doing well and went back to her activities without complaint. She was reevaluated four months after operation with full range of movement, there was no palpable swelling.

Discussion

Caan was the first who describes knee joint ganglion in 1924 ⁽¹²⁾.

Brown did 6500 arthroscopic knee surgeries, he found in 38 of them intra articular ganglion cyst ⁽¹³⁾.

Etiology of ganglion is not understood ^(14,15), currently two theories about the pathogenesis of ganglion cyst. First theory attributes the presence of the ganglion cyst being products of mucinous degeneration of connective tissues ⁽¹⁶⁾. The second theory consider the cyst as a cause of herniation of synovium through a defect in the capsule of the joint or the tendon sheath similar to those of wrist joint ^(16,17). For both theories the relationship to previous trauma is uncertain and has not been documented.

Intraarticular ganglionic cyst usually asymptomatic and are often hard to diagnose clinically due to lack of specific symptoms and signs.

Symptomatic patients may present with knee pain aggravated with activity, increased during posture change; locking, clicking, or popping sensation and decreased range of motion.

Because HFP is relatively spacious, it may take time for the mass to develop into large size enough to cause symptoms.

Many tumors and tumors like conditions may affect the HFP. MRI is the technique of choice in diagnosis and evaluating these conditions.

The ganglion cyst of Hoffa should be differentiated from the following; lipoma, synovial cysts, meniscal cyst, parameniscal cysts, synovial myxoma, pigmented villonodular, hemangioma, aneurysm, sarcoma, chondromatosis of synovial membrane⁽¹⁸⁾.

Plain x-ray is useful to detect loose bodies or other bone abnormalities.

Ultrasonography; the ganglion appears as an echoic and the lesion either unilocular or multilocular.

Computed tomography (CT) not very helpful, MRI is the most important tool in diagnosis and assessing the size, and the location of the cyst and it helps to exclude neoplasm and any pathology around the knee⁽¹⁹⁾.

Histopathology reveals a very dense capsule with a layer of connective tissue and thick fluid material.

Microscopically it shows pseudocystic spaces with multifocal areas of mucoid degenerations. There are many types of methods in treatment of ganglionic cyst of the knee joint; spontaneous resolution of the cyst had been reported⁽²⁰⁾.

Ultrasound and CT guided percutaneous aspiration has excellent results⁽²¹⁾. Bisicchia et al. reported the recurrence of infrapatellar fat pad cyst after ultrasound guided aspiration⁽²²⁾. Arthroscopic resection of the ganglion cyst is preferred for small lesion restricted to synovium^(23,24).

In case of Hoffa pad, it is difficult to reach cyst arthroscopically, although Yang et al. reported an endoscopic resection of ganglion cyst in

infra patellar fat pad extending in the subcutaneous layer⁽²⁵⁾.

Bisicchia et al. reported the recurrence of infrapatellar fat pad cyst after ultrasound guided aspiration⁽²²⁾. Also, Amin et al. reported no recurrence after open surgery⁽⁴⁾, so it could be a choice if the patient refuses arthroscopic or open surgery.

Saha et al. described that arthroscopic resection of subcutaneous extension in infrapatellar fat pad ganglionic cyst may lead to leaving residual tissue behind which may cause recurrence⁽²⁶⁾.

Nikolopoulos et al. described that when there is large cyst treatment should be by open and thorough resection⁽²⁷⁾.

So, as a conclusion, intraarticular ganglionic cyst should be in differential diagnosis in cases of unexplained progressive knee pain or mechanical locking. MRI is investigation of choice for identification of ganglion in knee joint. The size, the shape and location can accurately identify by MRI helping in planning for modalities of treatment.

To achieve complete excision, open surgical resection is indicated for large Hoffa ganglion cyst, on other hand arthroscopic excision is best for small lesion within the synovium.

Author contribution

Dr. Dagher received the patient and managed him with examination, laboratory, radiological, imaging and histopathological investigations.

Dr. Hasan was involved in the design, writing of the manuscript and contribute in the preparation of discussion with other similar cases and papers.

Patient consent

Agreement was taken from the patient for publication of this case and accompanying image, availability of data and material.

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