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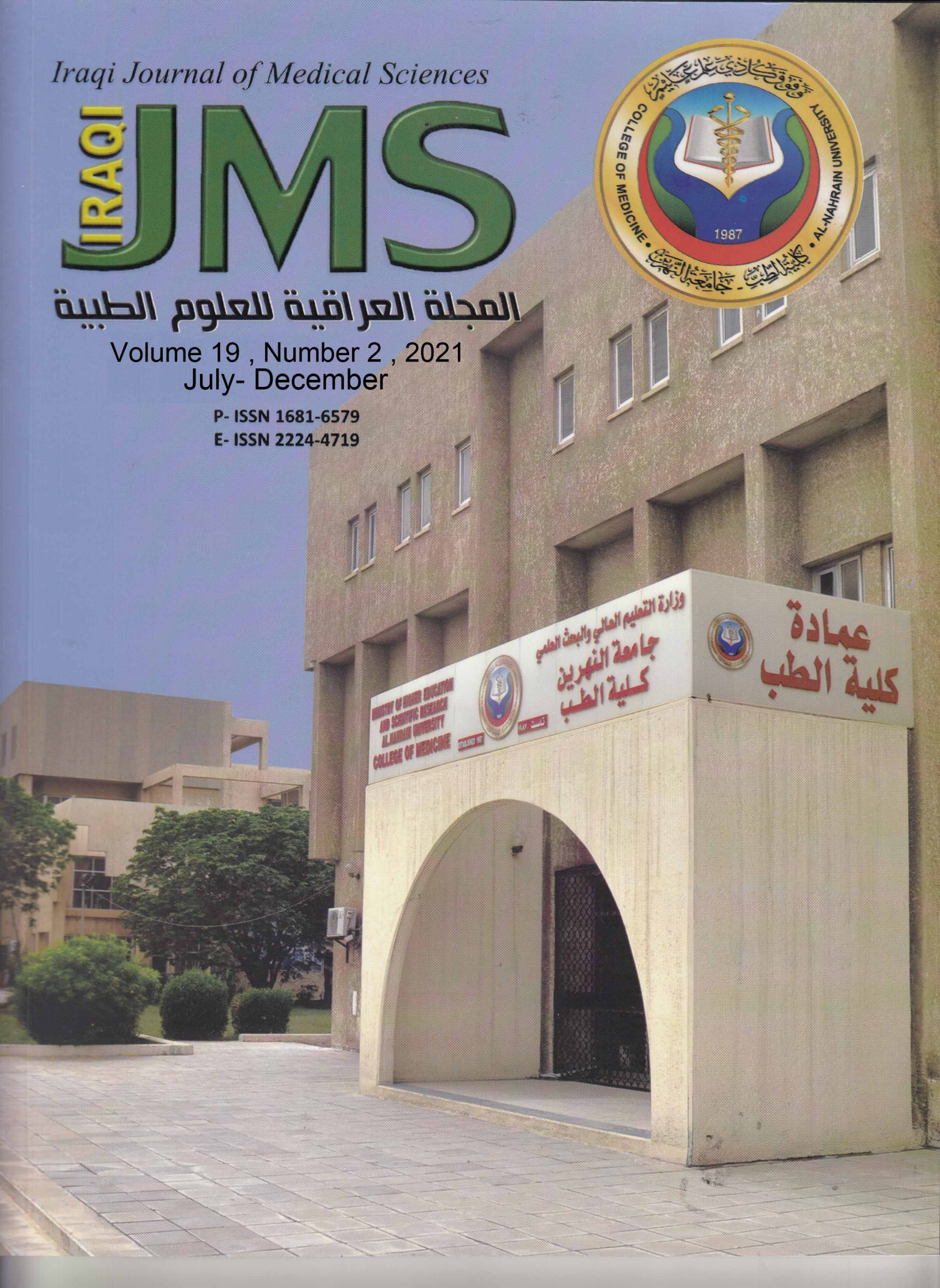

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CONTENTS

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Points of Consideration in Applying to Surgery Training Programs

Faiz L. Tuma *FACS, FRCSC*

Central Michigan University College of Medicine, USA

Abstract

Surgery training continues to be challenging and competitive. Training programs developed multiple strategies to select the best candidates. Applicants continue to improve their chances to join the highly ranked programs. Various skills and strategies have been adopted to optimize their application process. However, many applicants are still in need to prepare for the application process and identify the best strategy to present themselves. In this editorial, we provide practical points to consider when planning to apply and join a surgery training program.

Keywords : Surgical training, Application strategies, Surgery programs, Residency application

Citation Tuma FL. Points of consideration in applying to surgery training programs. Iraqi JMS. 2021; 19(2): 134-136. doi: 10.22578/IJMS.19.2.1

Introduction

Acceptance into a general surgery program may become a natural outcome to a well-planned preparation in the few years before applying. In other words, preparing to apply to general surgery should start as early as possible and be planned for adequately. Surgery programs have several selection strategies for surgery candidates [(1)]. Hence, well and advanced planning with hard work is crucial for training career success. Stories from senior professionals and academic experts confirm the importance of planning and hard work in shaping careers. The aim of this article is to provide introductory points for surgery applicants to consider in their preparation for their candidacy.

The first and most important point is the genuine desire and intent to become a surgeon. This should be based on thoughtful and comprehensive planning and knowing self-competencies. It is crucial to know if you want to be a surgeon, and why. The stronger and original motives behind your decision the more likely you will be a perseverant and eventually successful candidate, trainee, and practicing surgeon. Most successful candidates prepare for the program from their early medical school time. There are common elements that most programs look for in the candidates such as high interest, exam scores, research activities, and interest in education. However, several other factors are variably considered by various programs. Factors like assertive personality, school of graduation, future place of practice, likeability, perseverance, tolerance.

Criteria for high ranking

Few important points to consider when applying:

1- Exposure to surgery

Candidates are preferred to have sufficient exposure to surgery indicating that they chose this specialty based on adequate awareness. Early exposure to and learning experience are

known to inform specialty selection [2,3]. It would look weird to express interest in surgery without being involved in surgical services. Elective surgery during clerkship improves exposure and career development [4]. Performance and grade in the rotation are generally looked at with attention. An honor degree in the surgical rotation is expected. It would raise a question mark if the grade is below honor.

2- Letters of reference

These are routine requirements of a good candidate. They are preferred to be from surgeons with a key role in surgery training such as clerkship director, program director, chairman, or known educators to validate the letter content. The letter should be directed specifically to surgery programs with specific performance highlights. Avoid generic letters that may not give the impression of genuine evaluation.

3- Research experience

Most programs welcome a candidate who can participate in research activities in various roles [5]. Be prepared to describe your role and participation in research. Having your name in a publication or abstract is minimal. It is preferred to have your name as a first author in a study with various research skills such as knowledge in statistics, scholarly writing, and submitting manuscripts. These additional skills may help the program in improving their scholarly activities which almost all programs need.

4- Interest in education

As a good resident, you are expected to teach medical students and junior residents. In addition, interest in education will likely lead to good learning skills. Interest in education cannot be claimed by words. Taking a couple of courses or workshops in education or having some knowledge of education principles will give a better impression about the interest in education.

5- Future planning

Candidates who have a future vision of their subspecialty planning give a better impression. The early involvement of medical students in specialty exposure and planning enhances career selection [6]. However, candidates should have a flexible mind and plan to use the residency to refine their ultimate plans of subspecialty.

6- Interview

This is a very important part of the application. It plays a major role in ranking. Interviewees make a final impression based on how candidates present themselves. There are several ways to prepare for the interviews. Unfortunately, many candidates come with scripts of what to say in the interview. Even though this reflects preparation efforts, but it does not help the interviewees to know the candidate's personality. Therefore, use all preparation points as materials to create your thoughts around rather than scripts to say during the interview. It is important to be clear, well-spoken, honest, humble but confident, spontaneous with good communication skills - both verbal and nonverbal. Tips and training on interviewing can be extensive and are beyond the scope of this article.

7- USMLE score

USMLEs scores have significant implications on a candidate's application [7]. Many programs use the United States Medical Licensing Examination (USMLE) score as a filter of the initial applications. Programs receive hundreds of applications each year. They offer only about 10% of the applicants an interview. Therefore, the USMLE score is used as the first filter.

Program perspectives

Surgical programs and their respective societies have embraced strategies to recruit successful candidates [1]. Therefore, it is important to understand what perspectives the programs have in the selection process. As a potential resident:

a) You will work within the program intimately for 5 years, so it is crucial to be a nice person who is easy to get along with.
b) You will provide patient care, so reliability, trustworthiness, and efficiency are important as that will save the team significant efforts/time and prevent serious problems.
c) The program must have the highest board exam success rate. Therefore, programs want to be sure that you will pass the final board exam without difficulty. Programs should have a minimal 65% passing rate.
d) All programs have research and publication requirements. They will appreciate someone with research and publications skills, not just having your name on a couple of published articles.
e) All attending faculty like to have teachable/learning resident who shows improvement after teaching and feedback. Attain this learning attitude and if you have a story to show that, use it in the interview.

Conclusions

It is crucial to verify the motives and desire behind considering training in surgery. Once decided, prepare as early as possible. Build your case as a reliable, contributing, achieving, successful, and professional resident and future surgeon that can be easily identified as such.

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Biochemical Estimation of Total Sialic Acid, Lipid-Bound Sialic Acid and Fucose in Serum Patients with Nasal and Paranasal Sinus Malignancies

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Abstract

Background It is known that measurement of serum total sialic acid (TSA), lipid bound sialic acid (LBSA) and L-Fucose levels may be altered and associated with different types of disease included malignant tumors, therefore, the evaluation of these compounds in serum cancer patients may elucidate the possibility of using this as a diagnostic marker.

Objective To explore the clinical application of TSA, LBSA and L-Fucose serum levels in patients with different site of nasal and para nasal sinus malignancies and compare with a group of healthy controls.

Methods Blood samples obtained from 16 patients with nasal-paranasal sinus malignancies confirmed cases (12 males, 4 females) (age range 45-73 years) and 28 healthy individuals (18 males, 10 females) (age range 38-67 years) participated in this study. Serum TSA and LBSA levels were determined by using colorimetric methods and the serum Fucose level estimation was done based on the method as adopted by Winzler using cysteine reagent.

Results Results showed that serum levels of TSA, LBSA and L-Fucose were significantly higher in cancer patients compared to normal healthy control ($P<0.001$) and more increased in patients with ethmoid and frontal sinuses cancer group.

Conclusion Estimation of TSA, LBSA and L-Fucose is suggestive to be a reliable marker as well as can use an effective diagnostic biomarker of cancer patients.

Keywords Sialic acid, lipid-bound sialic acid, Fucose, nasal, paranasal sinus cancer, spectrophotometer

Citation Mohammed AK, Mahdi NR, Ahmed FS. Biochemical estimation of total sialic acid, lipid-bound sialic acid and fucose in serum patients with nasal and paranasal sinus malignancies. Iraqi JMS. 2021; 19(2): 137-146. doi: 10.22578/IJMS.19.2.2

List of abbreviations: EFSC = Ethmoid and frontal sinuses cancer, LBSA = Lipid bound sialic acid, LWNCC = Lateral wall of the nasal cavity cancer, NCC = Nasal cavity cancer, TSA =Total sialic acid

Introduction

The incidence and mortality rate of cancer is still unacceptably high; this stark fact itself is the strong argument for further research in the field of cancer biology. Immense increase in knowledge of the altered characteristics of malignant cells has shown that cell surface glycoconjugate are considered to be important in relation to cancer because many of the altered properties of cancer cells expressed at the cell surface. Cell surface is transformed during carcinogenesis and is vital for uncontrolled growth and malignant behavior of the neoplastic cells. The only traces of sialofucosyl glycopeptides, which is characteristic of tumor tissue, are found in the serum of healthy subjects, whereas it is found in high concentration in malignant transformed cells [(1)]. Glycoconjugate molecules such as sialic acid and Fucose level are imported constituents of

cell membrane and also reported to be associated with tumor progression [2-7].

Majority of studies have been documented increase levels of glycoprotein in serum/plasma in cancer patients with lung cancer [8], urologic cancer [9], melanoma [10], breast cancer [11], thyroid cancer [12], and liver metastasis [13].

Numerous studies have documented that tumor cells modulate their surface by increasing fucosylation levels (addition of L-Fucose at the terminal end of the oligosaccharide chain) to escape recognition, which contribute to several abnormal characteristics of tumor cells, such as decreased adhesion and uncontrolled tumor growth [14] and found to be a powerful immune modulator as it is distributed in macrophages, which are important for immune function [15].

Alterations in serum Fucose levels had been detected in patients with different types of malignancies [16]. Hence, monitoring serum/tissue Fucose levels could be a promising approach for the early detection, diagnosis, and prognosis of various cancer types.

The present study aimed to evaluate total sialic acid (TSA), lipid bound sialic acid (LBSA) and Fucose as monosaccharide in serum patients suffered from nasal and paranasal sinus cancer patients and their role as a biomarker in diagnosis and compared with normal group.

Methods

A retrospective study of medical records was performed for 16 patients with different site of nasal and paranasal sinus malignancies referred to ear, nose and throat (E.N.T) and Maxillofacial Department in the Basra General Hospital between April 2014 and October 2018 were enrolled in this study. Provisional diagnosis of tumors was made on the basis of clinical examination and was confirmed by biopsy. Ethical approval was not required as this is a retrospective study and clinical date were identified before analysis.

The patients were divided into the following categories according to American Joint on Cancer Tumor staging based on TNM (T=tumor, N=node invasion. M=metastasis) [17].

Group (1): Nasal cavity cancer (NCC), consist of 6 patients.

Group (2): Lateral wall of the nasal cavity cancer (LWNCC), consist of 6 patients with maxillary sinus.

Group (3): Ethmoid and frontal sinuses cancer (EFSC), this group included 4 patients with intracranial extension.

The diagnosis of these tumors was carried out by E.N.T Maxillofacial Surgeons, moreover, the disease had to be measured in two dimensions by a computed tomographic scan (CTS). Based on incisional biopsy most cases included in this study were adenocarcinoma type, moderately differentiated grade (I) and out of 16 patients 7 had grade (II).

Group (4): Comprised of 28 healthy donors as a healthy person (18 males, 10 females age of 38-67 years). The control was judged to be healthy by reviewing their medical histories and no evidence of disease.

Serum preparation

Whole blood samples were collected from each patient and control and allowed at room temperature for 10 minutes, then centrifuged at 2500 rpm for 20 minutes.

The serum was separated and store at -25°C until analysis.

Estimation of TSA and LBSA

Serum TSA and LBSA values was measured by Svennerholm [18] and Katopoids et al. [19] methods respectively.

Estimation of serum L-Fucose

The serum Fucose assay in all samples using method of Dische and Shettles [20] as adopted by Winzler [21].

Statistical analysis:

The differences among mean serum tumor marker levels found in healthy group and subgroup of patients with cancer were tested using student's test. Sensitivity was calculated as the percentage of individuals in the groups with cancer who had levels of the tumor

markers above the cut-off level (2SD) standard deviation. Specificity was calculated as the percentage of individuals in the cancer groups who had levels of the tumor markers within the normal.

Results

Healthy subjects

Comparison of serum TSA, LBSA and Fucose was done in both sexes; the date showed that mean serum levels of TSA and LBSA in male and female was (78.61±6.69), (73.53±8.42), (27.63±6.6) and (23.98±4.08) mg/dl respectively, while the mean serum level for Fucose was (5.49±0.61) and (5.16±0.96) for male and female respectively. Comparing the TAS, LBSA and Fucose levels show little differences between male and female, so that the total mean average for both sexes was (76.06±7.53). (25.63±5.31) and (5.31±0.61) respectively (Table 1).

Table 1. Comparison of serum TSA, LBSA and Fucose levels for healthy control group

Sex	Serum TSA Mean±SD (mg/dl)	Serum LBSA Mean±SD (mg/dl)	Serum Fucose Mean±SD (mg/dl)
Male (n=18)	78.61±6.69	27.63±5.64	5.49±0.61
Female (n=10)	73.53±8.42	24.98±6.08	5.16±0.96
Total (n=28)	76.06±7.53	26.30±5.84	5.31±0.61

TSA: Total sialic acid, LBSA: Lipid bound sialic acid

Cancer patients

Separate calculations were done for each group of patients to investigate whether the changes in serum TSA, LBSA and Fucose levels of patients are conversely related to the location of cancer.

Date analysis revealed significant differences in serum TSA level in different patients' groups when compared with healthy control (Table 2). In the normal healthy sera sample the overall mean TSA level was found to be (76.06±7.53) mg/dl, while the mean sera levels among those in cancer patients was found to be (91.28±4.67) mg/dl, this increase of 17% was statistically significant (P<0.001). The mean values of TSA levels for patients with NCC and LWNCC was found to be 15% higher than that the healthy controls (P<0.001). On the other hand, the most pronounced changes were found in the level of serum patients with EFSC, this 23% increase was statistically significant as compared with the healthy control (P<0.001).

Table 2. Comparisons of serum TSA for the patients diagnosed as NCC, LWNCC and EFSC with healthy control

Clinical condition	Range (mg/dl)	Mean±SD (mg/dl)	*P* value
Control (n=28)	64.99-87.88	76.06±7.53	-
NCC (n=6)	85.18-99.88	90.87±3.04	P<0.001
LWNCC (n=6)	82.19-98.11	89.19±4.55	P<0.001
EFSC (n=4)	85.12-104.18	93.78±6.48	P<0.001
Total (n=16)	82.19-101.18	91.28±4.67	P<0.001

TSA: Total sialic acid, NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

Table 3 shows the data of mean values of serum LBSA found in patients with malignancies and in healthy controls. Likewise, the mean value of overall serum LBSA level in 16 patients under study was found to be 16% increase than that of healthy control (P<0.001). The most pronounced was found in the levels of serum LBSA for the patients with Ethmoid and frontal sinuses cancer (EFSC), this 18% increase was statistically significant (P<0.001).

Table 3. Comparisons of serum LBSA for the patients diagnosed as NCC, LWNCC and EFSC with healthy control

Clinical condition	Range (mg/dl)	Mean±SD (mg/dl)	*P* value
Control (n=28)	16.99-33.88	26.30±5.84	-
NCC (n=6)	29.55-39.89	34.23±5.15	P<0.001
LWNCC (n=6)	32.29-42.22	35.04±3.51	P<0.001
EFSC (n=4)	31.62-46.61	41.44±5.97	P<0.001
Total (n=16)	29.55-46.61	36.91±4.86	P<0.001

LBSA: Lipid bound sialic acid, NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

Table 4 shows mean±SD values of serum TSA and LBSA in histopathological grade I and II in malignancies patients. Significant differences in values of serum TSA and LBSA in grade I and grade II when compared with healthy group and the increase of mean value of TSA with stage of malignancy was more prominent than LBSA.

Table 4. Comparison of TSA and LBSA levels within different histopathological grades of patients diagnosed as NCC, LWNCC, EFSC and healthy control

Group	TSA mean±SD (mg/dl)	LBSA mean±SD (mg/dl)
Control (n=29)	76.06±7.53	26.30 ±5.84
NCC		
Grade (I) (n=4)	86.17± 2.65	32.03±2.21
Grade (II) (n=2)	98.74± 0.81	38.22±1.66
LWNCC		
Grade (I) (n=5)	84.80±1.85	35.03±0.92
Grade (II) (n=1)	98.20	42.22
EFSC		
Grade (I) (n=1)	85.12	31.62
Grade (II) (n=3)	97.04±2.31	45.30±0.16

TSA: Total sialic acid, LBSA: Lipid bound sialic acid, NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

Sensitivity of the TSA is shown in (Figures 1 and 2). The magnitude of the sensitivity TSA is varied between 16% for patients with LWNCC one case of 6 and 75% for patients with EFSC three cases of 4 have elevated levels above cut-off level for the healthy controls plus 2SD

(91.12 mg/dl), while the value reach to 33% for the patients with NCC (two cases of 6), this increase in sensitivity might have contributed to the reflection type of tumor and disease stage.

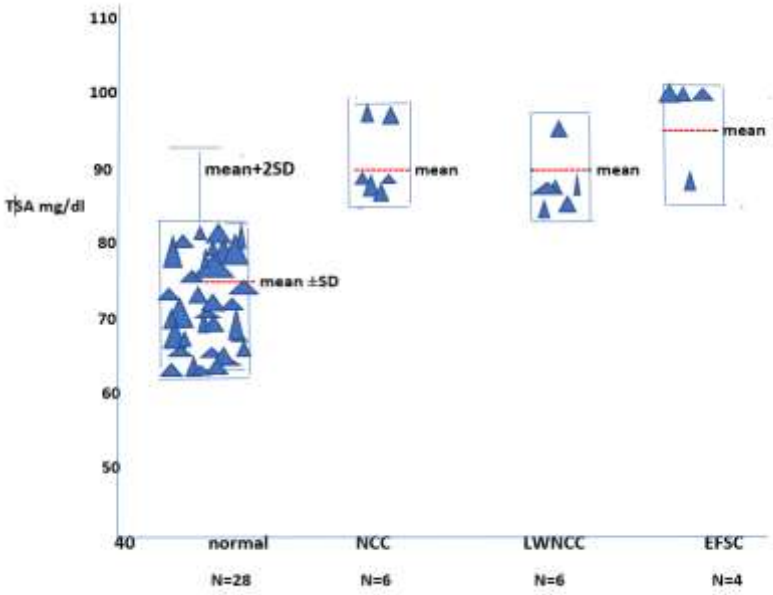

Figure 1. Levels of TSA in serum from healthy control and cancer patient. NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

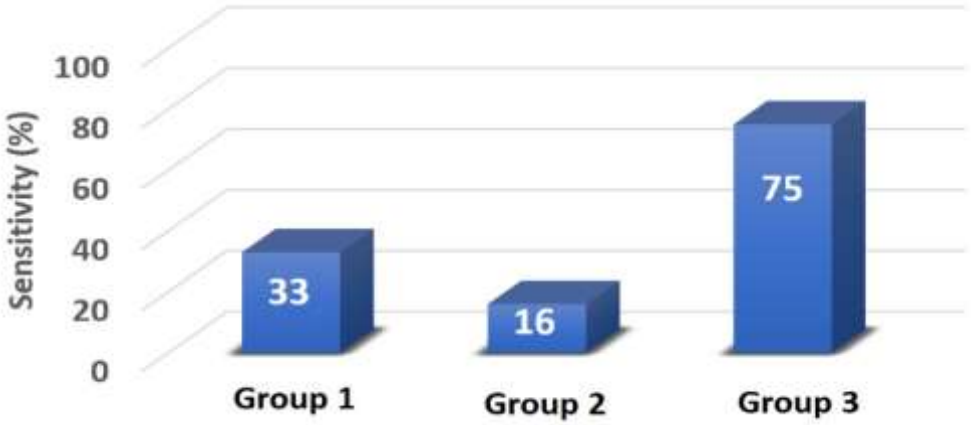

Figure 2. Sensitivity of TSA in cancer patients. Group (1): Nasal cavity cancer (NCC). Group (2): lateral wall of the nasal cavity cancer (LWNCC). Group (3): Ethmoid and frontal sinuses cancer (EFSC)

To assess the alteration in sensitivity of the LBSA separate calculation was done and show in (Figures 3 and 4). Nevertheless, only one of 6 cases of NCC, three of 6 cases of LWNCC and

three of 4 cases of EFSC are just above 2SD (37.99 mg/ml). It is obvious from the results that the extent of increased sensitivity varied between 17% for cases with NCC and 75% with cases with EFSC, in contrast the value reach 50% for LWNSC cases.

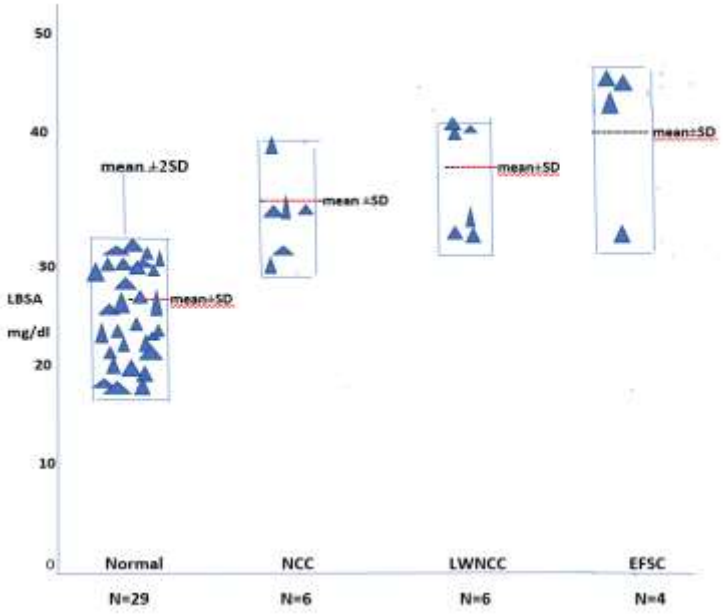

Figure 3. Levels of LBSA in serum from healthy control and cancer patient. NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

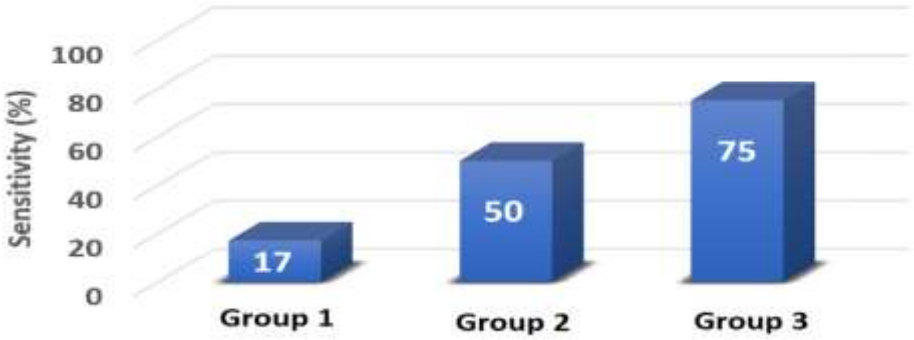

Figure 4. Sensitivity of LBSA in cancer patients. Group (1): Nasal cavity cancer (NCC). Group (2): lateral wall of the nasal cavity cancer (LWNCC). Group (3): Ethmoid and frontal sinuses cancer (EFSC)

The specificity of serum TSA and LPSA are considered in Table 5. Using 87.88 and 33.88 mg/dl as the upper limits of normal for TSA and LPSA respectively.

As can be seen, there were numerous causes of abnormal TSA and LBSA levels and each subgroup had at least one patient with an abnormal serum level of TSA and LBSA

respectively. Over all 25% of these patients had normal TSA and LBSA levels, but the numbers of patients in individual subgroups are too small to define the characteristics of specific malignant.

Table 5. Specificity of the TSA and LBSA test for the patients diagnosed as NCC, LWNCC and EFSC

Clinical condition	No. of cases with normal TSA values	No. of cases with normal LBSA values
NCC (n=6)	2	3
LWNCC (n=6)	1	1
EFSC (n=4)	1	-
Total (n=16)	4	4

NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

A comparison of the overall serum Fucose levels between healthy controls and the various group of cancer patients (Table 6), showed the mean value of serum L-Fucose levels of healthy controls to be 5.49 mg/dl and in various group cancer patients had 17.72 mg/dl, this 32% increase was statistically significant as compared with the healthy control (P<0.001).

The highest level of serum Fucose was found in serum with EFSC, this 37% increase compared with normal controls was statistically significant (P<0.001).

Table 6. Comparisons of serum level of Fucose for the patients diagnosed as NCC, LWNCC and EFSC with healthy control

Clinical condition	Range (mg/dl)	Mean±SD (mg/dl)	*P* value
Control (n=28)	4.21-7.88	5.49±0.93	-
NCC (n=6)	4.96-8.32	6.08±1.80	P<0.001
LWNCC (n=6)	5.11-9.60	6.90±2.55	P<0.001
EFSC (n=4)	8.20-12.30	8.16±3.08	P<0.001
Total (n=16)	4.96-12.30	8.02±3.47	P<0.001

NCC: Nasal cavity cancer, LWNCC: Lateral wall of the nasal cavity cancer, EFSC: Ethmoid and frontal sinuses cancer

Discussion

Modified glycoproteins and glycolipids, which presents major structural component of cell surface glycoconjugates undergoes alteration on neoplastic transformation change in surface enzymes have been associated with malignant transformation of a cell, which may contribute to the aberrant cell-cell interactions, cell-matrix adhesion, cell-cell recognition, antigenicity, and tumor progression. As a result of released glycoconjugates into the circulation through increased turnover, secretion and or shedding from malignant cells leading these glycoproteins and glycolipids elevation in biological fluids [(4)].

In the early tumor progression, the tumor cells have got different surface characteristics and the glycoprotein on the cell membrane would be change on the certain degree and the activity of glycosyltransferases in the cells

would be enhanced [22]. Then, the sialic acid was overexpressed that acted as specific terminal glycan of glycoproteins [23]. Excessive secretion of sialic acid sialylated tumor cells and helped it evade the monitoring and killing of immune system [24,25].

The present study demonstrates that serum TSA and LBSA are higher in cancer patients suffered from nasal and para nasal sinus malignancies as compared to healthy control and the results obtained from the present study was consistent with those of another study that showed increased level of sialic acid form in patients with pancreatic cancer [26], prostate cancer and Bone metastases [27], stomach cancer [3], bladder cancer [4] and oral squamous cell carcinoma [28].

Glycosylation is involved in a variety of biological phenomenon including birth, differentiation, growth, inflammation and play a critical role during malignant transformation [29]. Among different types of oligosaccharides, Fucose is one of the important carbohydrates in oligosaccharide chain. This fucosylation is mainly found in glycoprotein and glycolipids of living beings. Hence, altered fucosylation of glycoproteins is the most representative types of glycan‐related cancer biomarker [30].

The present study revealed significantly higher concentration of L-Fucose in overall among serum cancer patients as compared to healthy controls. Moreover, elevated levels of serum L-Fucose have been in different group of malignancy such as breast cancer [31], leukoplakia and oral cancer patients [32,33], oral squamous cell carcinoma [34] as well as brain tumors [35].

The reason for elevated serum glycoprotein levels in malignancies has not been clearly established, but various views have been put forward by several researchers, wherein they have reported that elevation above the normal level reflects the process of tissue destruction at the site and release of preformed glycoprotein from the tissue or it may be due to local synthesis and release of glycoprotein by the tumor cells or increased glycoprotein levels in diseases are, in whole or in part, associated with tissue proliferation rather than tissue destruction [36] or it may be due to overproduction of glycoprotein or due to polymerization of the ground substance of the connective tissue at the site of tumor invasion with release of solubilized component into the circulation [37].

In conclusions, the combined estimations of serum TSA, LBSA and L-Fucose levels may be used as an additional tool for clinical assessment for cancer detection as well as may be used as a biomarker in the diagnosis of different malignant disease. The limitation of the present study was the small sample patients' size and can be overcome by expanding to a large sample scale for further investigation and can be used as an effective parameter in screening, diagnosis, monitoring of nasal and paranasal sinus malignancies.

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Author contribution

Dr. Mohammed: put the research plan and writing the manuscript. Dr. Mahdi: did the sampling and lab works and Dr Ahmed did the statistical treatments.

Conflict of interest

Authors declare no conflict of interest.

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Premarital Screening Program in Al-Nuaman Teaching Hospital

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Abstract

Background Premarital blood screening helps couples to identify potential health problems and risks for themselves and their offspring, considered a primary preventive approach for couples planning for conception and an important step towards protecting society from spread of diseases especially thalassemia, and allowing people to enjoy life.

Objective To determine the prevalence of minor β-thalassemia, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and venereal disease research laboratory (VDRL) referred to Al-Nuaman Teaching Hospital.

Methods The cross-sectional study as part of premarital screening in Al-Nuaman Teaching Hospital was conducted over six months from May 2017 to the end of October 2017. Total individual of 3027 underwent routine mandatory tests.

Results Regarding HIV and VDRL cases were zero, regarding HBV was no case in August, only one case in May and June, two cases in September and October, and four cases in July, regarding HCV there was no cases in May, June and Aug., two cases in September, and October, and three cases in July, Regarding β-thalassemia minor, one couple in August and another couple in September, those two couples both male and female were thalassemia minor , while the rest either the male or female were thalassemia minor.

Conclusion Premarital screening program is an important project in detecting asymptomatic carriers of hepatitis viral infection and thalassemia. This study showed that incidence of hepatitis B infections 0.33% were more frequent than Hepatitis C infections 0.23%, there was no cases of HIV infection detected so far, and β-thalassemia trait prevalence was 1.92%.

Keywords Premarital, β-thalassemia, virology, screening

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List of abbreviations: EDTA = Ethylenediaminetetraacetic acid, ELISA = Enzyme-linked immunosorbant assay, HBV = Hepatitis B virus, HCV = Hepatitis C virus, HIV = Human immunodeficiency virus, MCH = Mean corpuscular hemoglobin, MCV = Mean corpuscular volume, VDRL = Venereal disease research laboratory

Introduction

Premarital blood screening helps couples to identify potential health problems and risks for themselves and their offspring. A healthy-looking person may have undetected health problems, or be a silent carrier of infectious diseases [(1)]. Premarital screening are important steps for the prevention of genetic blood disorders such as hemoglobinopathy [(2,3)], and considered a primary preventive approach for couples planning for conception and an important step towards protecting society and allowing people to enjoy life [(4)], particularly be important in the prevention of the spread of disease [(5)]. All couples with marriage plans are required to be tested for human immunodeficiency virus

(HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) and to have the appropriate counseling (if required) before completing their marriage plans [6]. Premarital thalassemia screening was first carried out in 1975 by Silvestroni and in Latium, Italy, as part of a school prevention program [7]. Thalassemia is common, incurable, autosomal recessive inheritable haemoglobinopathy that cause significant morbidity and mortality and impose a heavy financial burden on society. A simple blood test before marriage can easily detect carriers of these diseases, to inform couples about their chances of producing affected children and ensure they receive appropriate advice [8]. The goal of complete eradication for β-thalassemia was met in Cyprus, Italy, and Greece [9]. In this program, red cell indices are checked, if mean corpuscular hemoglobin (MCH) <27 pg or mean corpuscular volume (MCV) <80 fl (cut off values) were found in both couples, hemoglobin A2 concentrations will be measured. If it is confirmed as characteristic for minor β-thalassaemia (Hb A2 >3.5), the couples were referred for counseling. In reference hematology books and also in literature, different cut off values have been mentioned for MCH and MCV, which might be due to the difference in the characteristics of the studied populations, such as age and race [10,11].

This research was conducted to determine the prevalence of minor β-thalassemia, HIV, HBV, HCV and Venereal disease research laboratory (VDRL) in individuals underwent routine mandatory premarital tests referred to Al-Nuaman Teaching Hospital. This is because sexual intercourse is an important route of transmission for HBV, HCV and HIV infections. The determination of a carrier status during premarital testing will create awareness between the couples, lead to the protection of the prospective spouse by early vaccination which is imperative [12].

Methods

This cross-sectional study as part of premarital screening in Al-Nuaman Teaching Hospital, was conducted over six months from May 2017 to the end of October 2017, total individual of 3027 (1513 couples and one female her male was outside Iraq) underwent routine mandatory tests and blood samples collected from venous blood was taken into an ethylenediaminetetraacetic acid (EDTA) tube and the complete blood count and red blood cell indices were measured by Abbot automated cell counter on the same day of blood collection. Subjects were considered to have β-thalassemia trait if they had MCV <80 fl, MCH <27 pg and a hemoglobin A2 level >3.5%. A second gel tube blood sample were allowed to clot and centrifuged at 1000 rpm for five minutes. The serum was separated and used for the screening of HIV, HBV and HCV viruses by enzyme-linked immunosorbant assay (ELISA) to detect antibodies in plasma against HCV (indirect ELISA), HIV type 1,2, or subtype 0 (sandwich ELISA), and to detect hepatitis B surface antigen (HBsAg) by (sandwich ELISA). Then for syphilis rapid test cassette one step rapid test.

Couples with safe marriage test results were issued instant compatibility certificates while at-risk couples were asked to attend meetings the counselors explained to the couple members the potential hazards of their proposed marriages and the voluntary nature of their compliance.

Results

This study was conducted to 3027 subjects who were attending the Al-Nuaman Teaching Hospital for premarital screening, age was ranging from 17 years to 47 years of either sex, they were couples males and females with the exception of one couple whose the male was outside Iraq, and the result regarding HIV, VDRL was zero, regarding HBV was no case in August, only one case in May and June, two cases in September and October, and four cases in July, regarding HCV there was no cases in May, June and August, two cases in September, and October and three cases in July (Table 1).

Regarding β-thalassemia minor total percentage was 1.9% as just one couple in August and another couple in Sept. (Table 2).

Table 1. Frequency and percentage of virology, VDRL and Rh screen

Months	No.	HIV No. (%)	VDRL No. (%)	HBV No. No. (%)	HCV No. No. (%)	Rh –ve No No. (%)
May	666	0 (0.0)	0 (0.0)	1 (0.15)	0 (0.0)	39 (5.85)
June	243	0 (0.0)	0 (0.0)	1 (0.41)	0 (0.0)	19 (7.81)
July	760	0 (0.0)	0 (0.0)	4 (0.53)	3 (0.39)	0 (0.0)
August	650	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	29 (4.46)
September	468	0 (0.0)	0 (0.0)	2 (0.43)	2 (0.42)	14 (2.99)
October	240	0 (0.0)	0 (0.0)	2 (0.83)	2 (0.83)	16 (6.66)
Total	3027	0 (0.0)	0 (0.0)	10 (0.33)	7 (0.23)	117 (3.86)

Table 2. Frequency and percentage of β-thalassemia minor

Months	Number of couples per month	Thalassemia minor No.	Couples	%
May	666	5	0	0.75
June	243	6	0	2.47
July	760	14	0	1.84
August	650	20	1	3.08
September	468	9	1	1.92
October	240	4	0	1.67
Total	3027	58	2	1.92

Discussion

HBV is the most common cause of serious liver infection in the world [13]. HBsAg was positive in 0.33% of the individuals entered the study; this result was lower than that of Yassin study [14], which was done in Sulaimani city from November 2008 to February 2009, including all premarital people from age 18 years and above of either sex, shows that the prevalence of HBsAg among premarital people was 0.67%. The result of this study was higher than that of Flichman et al. study [15], which study was done in Argentina from 2004 to 2011 including blood donors which showed that the prevalence of HBsAg decreased from 0.336% to 0.198%.

In this study, the HCV infection was 0.23%, which is higher than that of Aljarbou study [16], which was done in Qassim Region of Saudi Arabia in 2008 shows that the HCV was (0.1%) before getting married, and was equal or slightly lower than Alswaidi and O'Brien study [17], which was done in Saudi Arabia in premarital testing between January and May 2008 shows that 0.33% for HCV and lower than Ismail et al. study [18], which was done in March 2014 and continued up to August 2016 in the Public and Family Health Clinic, Tobruk city, Libya where HCV was (1.2%) infections among individuals who performed pre-employment and premarital medical examination. Current study shows that HIV was 0% similar to Ismail et al. study [18] and lower than Alswaidi and O'Brien study [17] who shows that HIV was 0.03%.

This study shown that thalassemia trait prevalence was 1.92%, which is less than a study conducted in Turkey which found to be 2.6 % by Keskin et al. in 2000 [19]. Among the genetic diseases, the most common disorders are hemoglobinopathies [20]. B-thalassemia is prevalent in Mediterranean countries [21]. Considering that nowadays, the neighbor Mediterranean countries have eradicated the disease almost completely, Turkish Republic of Northern Cyprus (TRNC), Italy and Greece have succeeded in preventing thalassemia infants being born in the last decade, as a result to society screening, pre-marriage carrier detection [22].

In conclusion, premarital screening program is an important project in detecting asymptomatic carriers of hepatitis viral infection and thalassemia. This study showed that incidence of HBV infections was more frequent i.e., 0.33% (10 cases of HBV infections detected out of 3027 individuals) than HCV infections i.e., only 0.23% (7 cases of HCV infection were detected out of 3027 individuals) and there was no case of HIV infection detected so far, and β-thalassemia trait prevalence was 1.92%.

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Conflict of interest

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Assessment of PTEN Deletion or Signal Reduction in Breast Carcinoma Using FISH Technique in A Sample of Iraqi Female Patients

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Abstract

Background PTEN (phosphatase and tensin homolog) gene is located at the 10q23 region that regulates cell proliferation and survival mainly by regulating PI3K (phosphatidylinositol 3-kinase)-AKT (protein kinase B) signaling pathways. PTEN gene is one of the most frequently mutated tumor suppressor genes in human cancer. Loss of PTEN gene can cause overgrowth, proliferation, survival, and metabolism of tumor cells. In breast cancer, loss of PTEN gene is associated with the occurrence of tumor and is significantly correlated with its characteristics.

Objective To assess the frequency of PTEN deletion or signal reduction in breast cancer in a sample of Iraqi female patients using FISH technique and its relation with clinico-pathological parameters (age, tumor size, multicentricity, histopathological types, grade, pathological stage and lymph node status).

Methods This is a retrograde, case control study, which was conducted at the Department of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University for the period between October 2019 to November 2020. It involved 30 patients with breast cancer who underwent mastectomy and 30 patients with benign breast lesions.

Results PTEN gene deletion or signal reduction was observed in 8 (26.67%) out 30 breast cancer patients. There was a significant relation between PTEN gene deletion (or signal reduction) and larger tumor size, higher grade, advanced pathological stage, and metastasis to more than 4 lymph nodes.

Conclusion There was significance difference between cases and control groups regarding PTEN deletion or signal reduction. There was significant correlation between PTEN gene deletion or signal reduction and larger tumor size, higher pathological grade and advanced stage.

Keywords PTEN gene, breast cancer, FISH technique

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List of abbreviations: BAD = BCL2 associated agonist of cell death, BC = Breast cancer, CENP-C = Centromeric protein C, FAK = Focal adhesion kinase, FFPE = Formalin fixed paraffin embedded, FISH = Fluorescent In situ Hybridization, GSK3 = Glycogen synthase kinase 3, H&E = Hematoxylin and Eosin, IDC = Invasive ductal carcinoma, ILC = Invasive lobular carcinoma, IRB = Intuitional Review Board, IRS = Insulin receptor substrate, LOH = Loss of heterozygosity, mTOR = Mammalian or mechanistic target of rapamycin, PI3K = Phosphatidylinositol-3-kinase, PKB or Akt = Protein kinase B, PTEN = Phosphatase and tensin homolog gene, TSC = Tuberous sclerosis

Introduction

The World Health Organization (WHO) reported that the incidence rates of breast cancer (BC) are steadily getting higher (1% to 5% increase every year) in countries of the Eastern Mediterranean Region (including Iraq) [(1)]. In Iraq, the Ministry of Health/ Cancer Registry Council reported that

BC ranks the first among the top ten malignant neoplasms affecting the Iraqi females in 2018; comprising 34.06% (6094 cases) of the total number of female patients with cancer in Iraq [2]. During 2018, 1727 women died from BC making it the second most common cause of mortality after lung cancer [3].

Phosphatase and tensin homolog gene (PTEN) is located at chromosome 10. It is a tumor suppressor gene that regulates vital cell functions such as proliferation, cell motility, genomic stability and survival through controlling the function of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as Akt)/ Mammalian target of rapamycin (mTOR) signaling pathway [4].

The PI3K proteins activate PKB that phosphorylates tuberous sclerosis protein 1 (TSC1) and TSC2 leading to dissociation of TSC1–TSC2 complex (which negatively regulates the activity of the kinase mTOR); Therefore, AKT results in the activation of mTOR complex 1 (mTORC1) and ultimately leading to increased protein and lipid synthesis and decreased autophagy, hence promoting cell growth and proliferation [5,6]. PTEN negatively regulates PI3K signaling and downstream PI3K family members, including AKT, leading to inhibition of growth and survival signals and suppressing tumor formation [7,8].

Thus, PTEN inactivation leads to cells proliferation, promoting cell growth, migration and survival through multiple downstream effectors that enhance the growth of neoplasm [4]. PTEN gene is deleted frequently in various human tumor types, and alterations to PTEN gene were reported to have effect on prognosis in primary and metastatic neoplasms, including BC [9]. The frequency of PTEN deletions or reduced expression in BC varies from 4 % to 63 % in the literature [9,10]. Several studies have provided evidence that PTEN loss is significantly related to aggressive behavior of BC, including larger size of tumor, metastasis to lymph nodes, poor differentiation and advanced pathological stage [9-11].

This study aims to assess the frequency of PTEN deletion or signal reduction using Fluorescent in Situ Hybridization (FISH) technique in BC patients in a sample of Iraqi patients and its relation with the clinico-pathological parameters (including patients' age, tumor size, multicentricity, histopathological types, grade, stage and lymph node involvement.

Methods

This is a case control study, which was conducted at the Department of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University. The study was approved by Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University.

A total of 30 formalin-fixed paraffin embedded (FFPE) blocks of breast tissue specimens from patients who were diagnosed with BC and underwent mastectomy during 2019 and 2020 were collected from Al-Imamein Al-Kadhimein Medical City and from the Oncology Teaching Hospital-Medical City. Thirty FFPE blocks of breast tissues from benign breast lesion were also collected from the same centers and were used as a control group.

Clinical and pathological information were collected from patients' pathology report. From each FFPE tissue blocks, two sections of 5 µm thickness each were obtained. One section was stained with hematoxylin and eosin (H&E) stain for revision of the diagnosis. The other section was placed on positively charged slide for FISH study of PTEN gene using ZytoLight FISH (PTEN/CEN 10 Dual Color) Probe.

Evaluation of PTEN gene had been carried out using Zeiss Axio Imager Z2 fluorescent microscope with 40X objective lens. Three filters had been used RED, DAPI and FITC filter for specimen evaluation. The final result of the slide was processed and produced by MetaSystems Isis software of the fluorescent microscope. The PTEN/CEN 10 dual color probe consists of ZyGreen labeled polynucleotides

which target PTEN gene region on chromosome 10, and ZyOrange labeled polynucleotides, which target the centromeric region of the same chromosome.

Hybridization signals of 50 non-overlapped nuclei were counted manually. Normal cells or cells without a deletion involving the PTEN gene region show, two green and two orange signals. Orange signals represent chromosome 10 centromeres. A cell with a deletion affecting PTEN gene region shows a reduced number of green signals. Presence of fewer PTEN signals than centromere 10 probe signals in >30% of tumor nuclei were considered as heterozygous deletion. Complete absence of PTEN signals in the tumor cells, with presence of centromere 10 signals in >20% of tumor nuclei was considered homozygous deletion [(12)]. Tissue spots lacking any detectable PTEN signals in all (tumor and normal cells) or lack of any normal cells as an internal control for successful hybridization of the FISH probe were excluded from analysis [(9)].

Results

Eight cases of BC out of 30 cases showed PTEN signal reduction or complete deletion, forming 26.67%. Seventy five percent of which (6 cases) showed reduced signal of PTEN gene (only 1 signal out of 2 was deleted) (Figures 1 and 2), while 2 cases demonstrated complete loss of PTEN signal (Figure 3). However, the remaining of the cases (22 cases (73.33%)) showed no deletion or signal reduction of PTEN signal (Figure 4). Regarding control group, none of the patients in the control group showed signal reduction or deletion in PTEN gene expression (Figure 5). There was significance difference between cases and control groups regarding PTEN deletion or signal reduction (P value=0.0024).

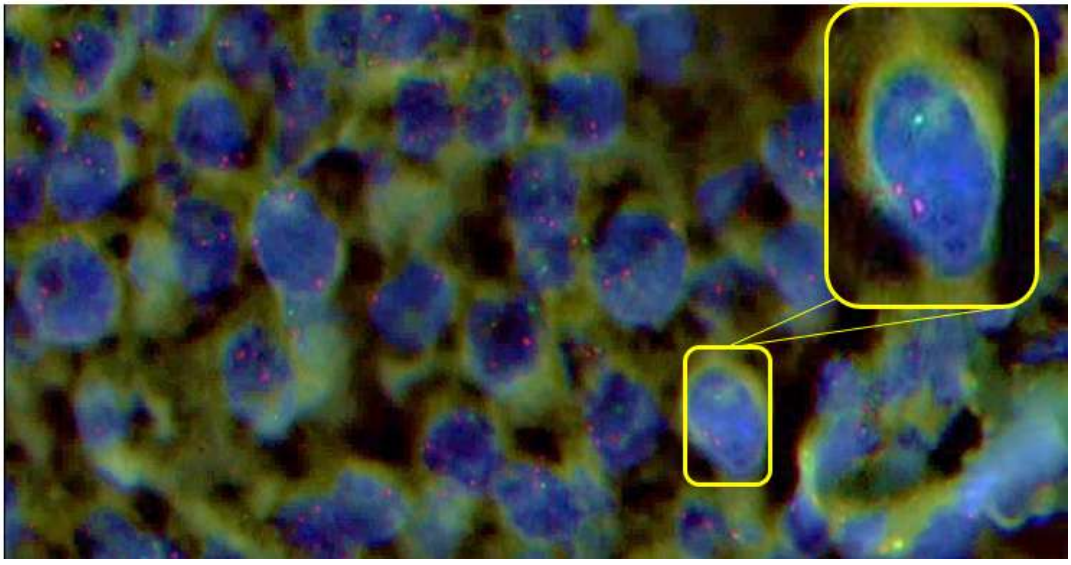

Figure 1. Invasive ductal carcinoma of the breast (grade 3) showing reduced signal of PTEN gene (partial deletion of the gene). Inset on right top corner showing a magnified cell with partial PTEN gene deletion (two orange signals and one green signal). This slide was displayed with 40X magnification.

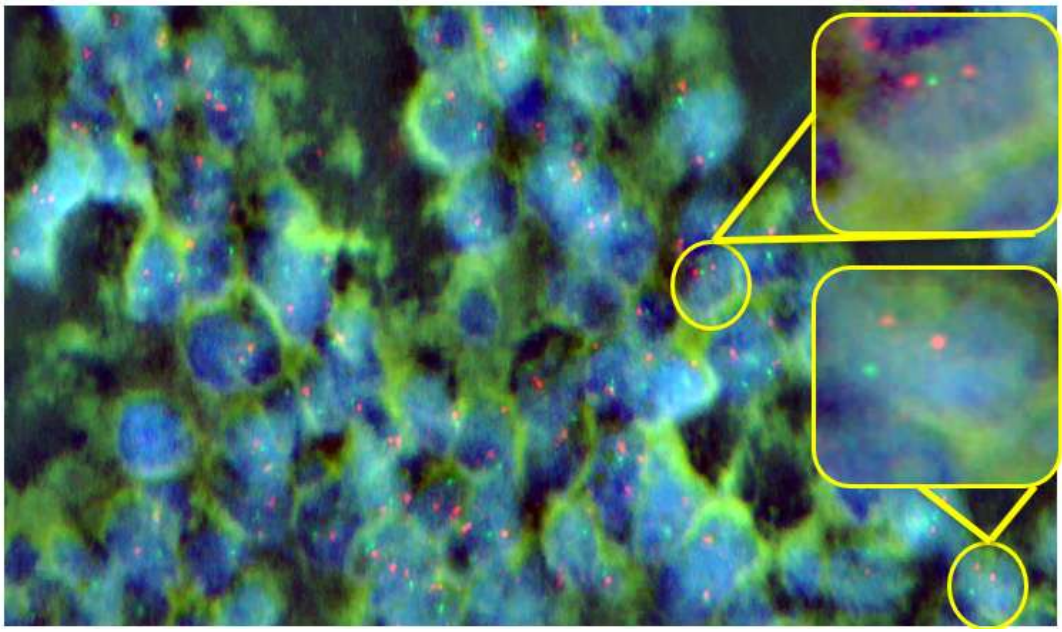

Figure 2. Invasive ductal carcinoma of the breast (grade 3) showing reduced signal of PTEN (partial deletion of the gene). The 2 insets are showing magnified cells with partial PTEN gene deletion (two orange signals and one green signal). This slide was displayed with 40X magnification

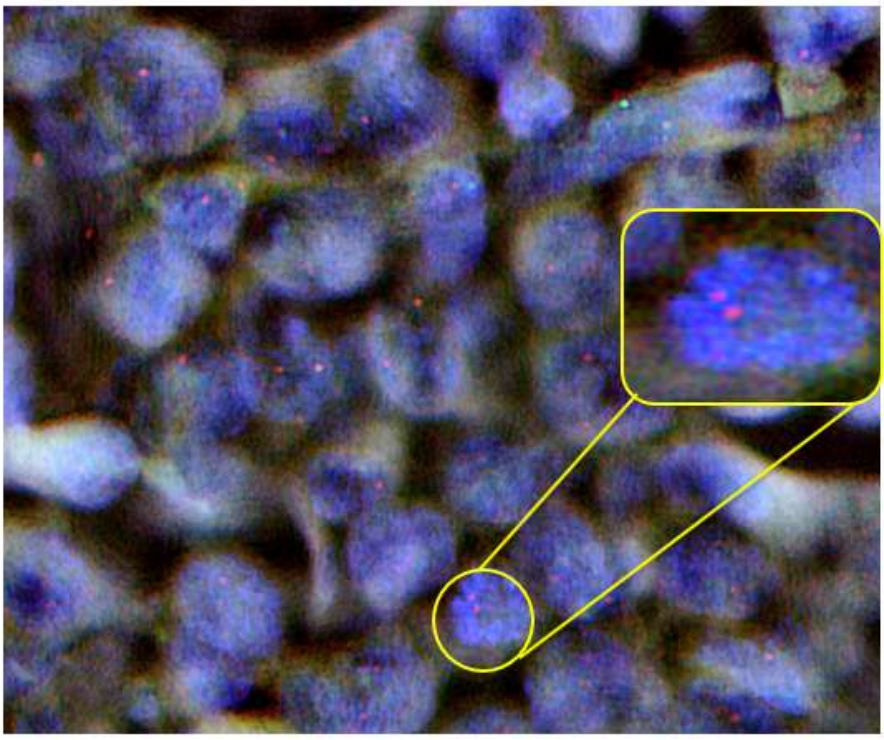

Figure 3. Invasive lobular carcinoma of the breast showing complete deletion of PTEN gene on chromosome 10. The 2 insets are showing magnified cells with two orange signals and loss of both green signals. This slide was displayed with 40X magnification

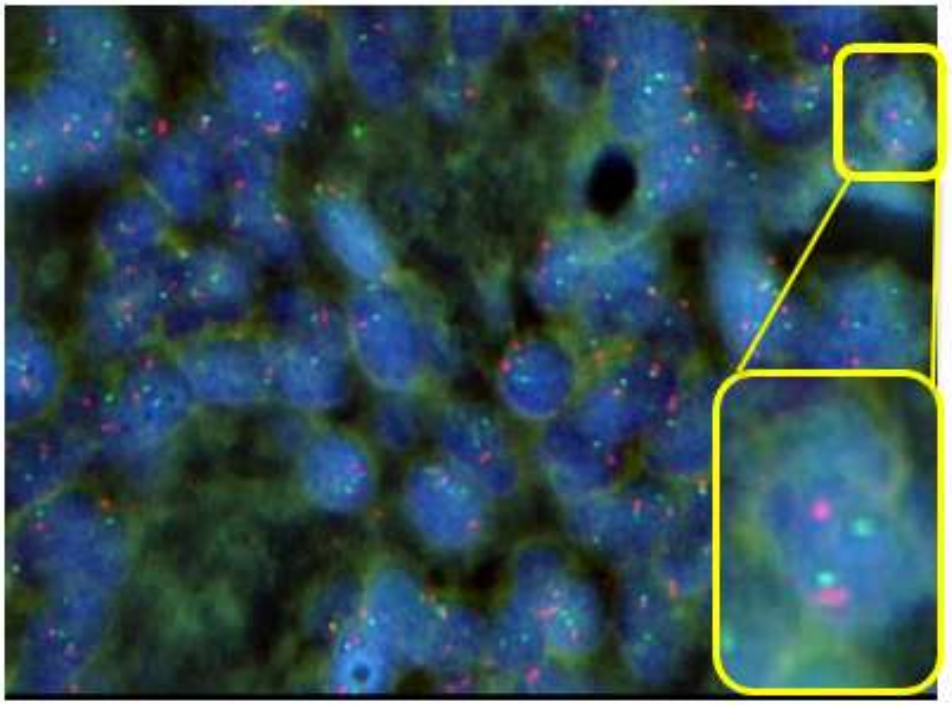

Figure 4. Invasive lobular carcinoma of the breast showing normal signal of PTEN gene. The inset on the right corner is showing a magnified cell with normal PTEN gene signal (two green signals and two orange signals). This slide was displayed with 40X magnification

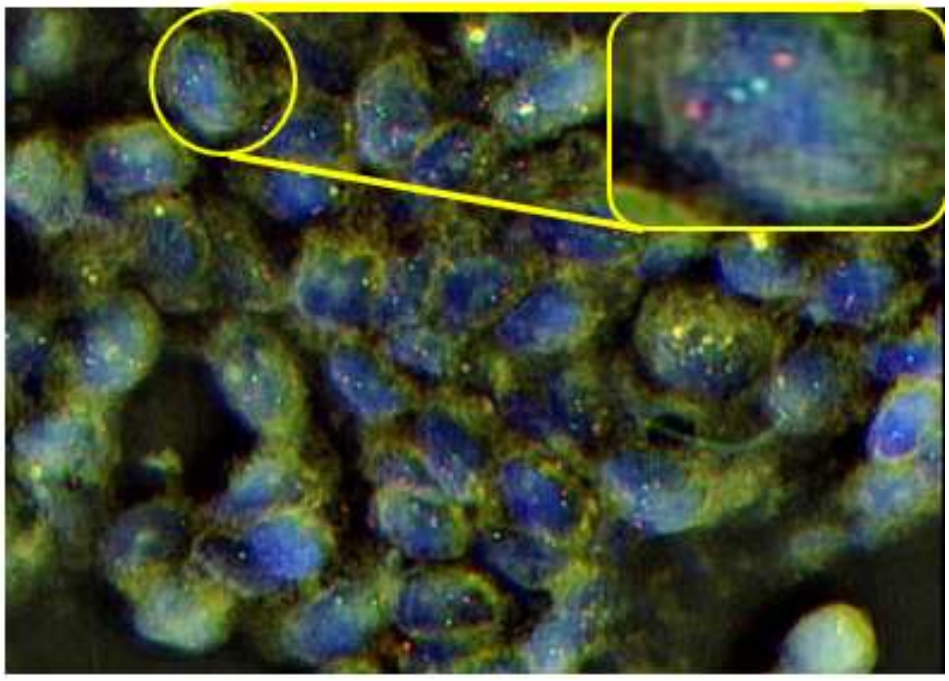

Figure 5. Fibroadenoma of the breast, showing normal signal of PTEN gene. The inset on the right corner is showing a magnified cell with normal PTEN gene signal (two green signals and two orange signals). This slide was displayed with 40X magnification.

Mean age of patients with PTEN deletion was 43.5±11.12 years, while the mean age of BC patients with no deletion in PTEN gene was 55.63±9.53 years. There was no statistically significant difference in mean age between deleted PTEN gene and non-deleted PTEN gene groups (P value=0.7664) (Table 1).

BC patients were categorized into three groups based on the tumor size (group 1: included patients with tumor size <2 cm, group 2: ≥2 cm and <5 cm, and group 3: ≥ 5 cm). Eight patients out of 30 cases (26.67%) were in group 1, group 2 had 16 patients (53.33%) and the remaining 6 cases (20%) of the cases were in group 3. Two out of 16 patients (12.5%) of group 2 displayed reduced signal of PTEN gene. Six out of six patients (100%) in group 3 showed a reduced or deleted signal of PTEN gene. There was a highly statistically significant correlation between reduced or deleted PTEN gene signal and tumor size (P value =0.0001) (Table 1).

Regarding multicentricity, only four out of thirty cases (2 cases were invasive ductal carcinoma (IDC) and the other 2 cases were invasive lobular carcinoma (ILC)); collected reported multicentric breast tumor forming a percentage of 13.33%. Two of them (50%) displayed PTEN gene deletion, the remaining 2 (50%) cases showed no reduction in PTEN gene signals. There was no statistical significance in the relationship between PTEN deletion and multicentricity (P value =0.26) (table 1).

Twenty six out of 30 cases (86.67%) were diagnosed as IDC (Figure 1) and six of them (23.07%) showed PTEN gene deletion (Figure 5 and 6). On the other hand, only 4 out of 30 cases (13.33%) were ILC (Figure 2) and 2 of them (50%) showed reduced or deleted signal in PTEN gene signal (Figure 7). There was no statistical significance in relation between the PTEN reduced or deleted signal with tumor pathological type (P=0.25) (Table 1).

Eighty percent (24 out of 30 cases) were diagnosed as grade II (moderately differentiated) and 4 of them (16.67%) showed PTEN gene reduced or deleted signal. Twenty percent (6 cases) were diagnosed with grade III (poorly differentiated), half of them (4 cases) demonstrated PTEN deletion or signal reduction. No cases were presented with grade I in the case group. There was a statistical significance in the relationship between the PTEN gene reduced or deleted signal with tumor pathological grade (P=0.013) as shown in table 1.

Four cases out of 30 patients with BC were diagnosed with pathological stage I forming 13.33%, all of them showed normal PTEN gene signal. Most of cases were in stage II with 16 cases making 53.33% of the group, however only 2 of them (12.5%) showed deleted PTEN gene or reduced signal. Finally, 10 patients in the cases group presented with stage III (33.33%) and 6 of them (60%) revealed reduced or deleted PTEN gene signal. There was statistically significant difference in relation between the PTEN reduced or deleted signal with tumor pathological grade (P=0.012) (Table 1)

Fourteen patients were recorded with lymph nodes metastasis. From these patients, 6 of them were found to have a reduced signal of PTEN gene. This means that 75% (6 out of 8) of patients with PTEN gene deletion had positive lymph nodes metastasis. However, there was no statistically significant difference between PTEN deletion or signal reduction and lymph node metastasis (P value=0.0606) (Table 1).

All the patients with PTEN gene deletion or signal reduction and lymph node metastasis (6 out of 6 (100%)) were found to have metastasis in 4 or more lymph nodes. On the other hand, there were 8 patients with normal PTEN gene expression who had lymph nodes metastasis; 4 out of 8 (50%) had lymph node metastasis in <4 lymph nodes, and the other 4 (50%) had lymph node metastasis in ≥4 lymph nodes. There was statistically significant difference between PTEN gene deletion and number of lymph nodes invaded (equal or more than 4 lymph nodes), P value=0.0404 (Table 1).

Table 1. Relationship between PTEN gene status and clinico-pathological characteristics of breast cancer patients

Feature		Breast cancer patients with normal PTEN gene	Breast cancer patients with Reduced or deleted PTEN gene	Total	P value
Age (mean ±SD)		55.63±9.53	43.5±11.12		0.76
Tumor size	Group 1	8	0	8	0.0001
	Group 2	14	2	16	
	Group 3	0	6	6	
Multicentric		2	2	4	0.26
Pathological type	IDC	20	6	26	0.25
	ILC	2	2	4	
Grade	Grade I	0	0	0	0.013
	Grade II	20	4	24	
	Grade III	2	4	6	
Pathological stage	Stage I	0	4	4	0.012
	Stage II	14	2	16	
	Stage III	4	6	10	
Lymph nodes invasion	Yes	8	6	14	0.0606
	No	14	2	16	
Number of lymph nodes invaded	<4	4	0	4	0.0404
	≥4	4	6	10	

IDC = Invasive ductal carcinoma, ILC = Invasive lobular carcinoma

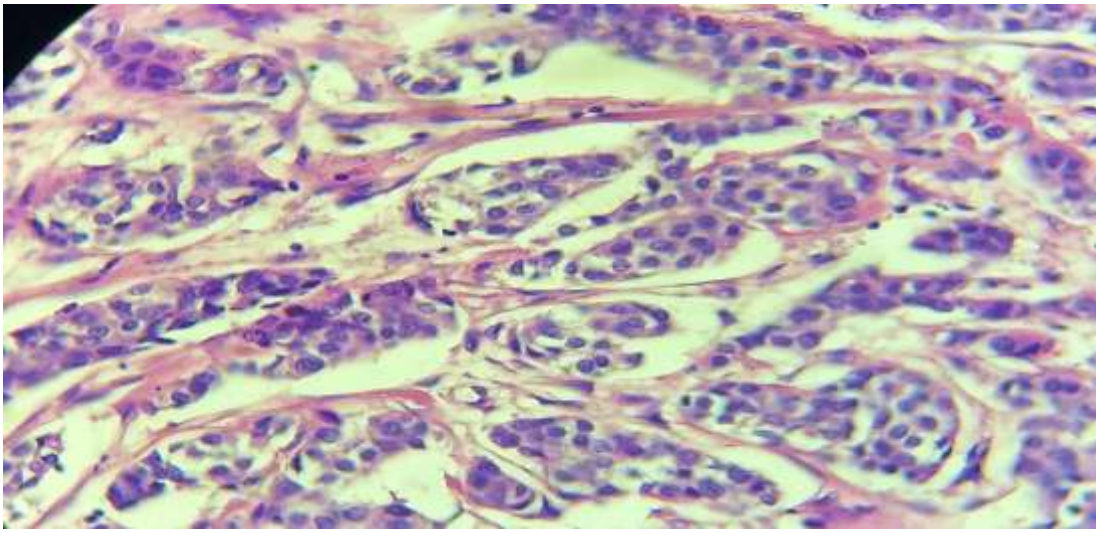

Figure 6. Invasive ductal carcinoma. Section showing malignant mammary cells forming nests. Malignant cells are large pleomorphic with large vesicular nuclei with prominent nucleoli. (H&E, double magnification of (40X) magnification)

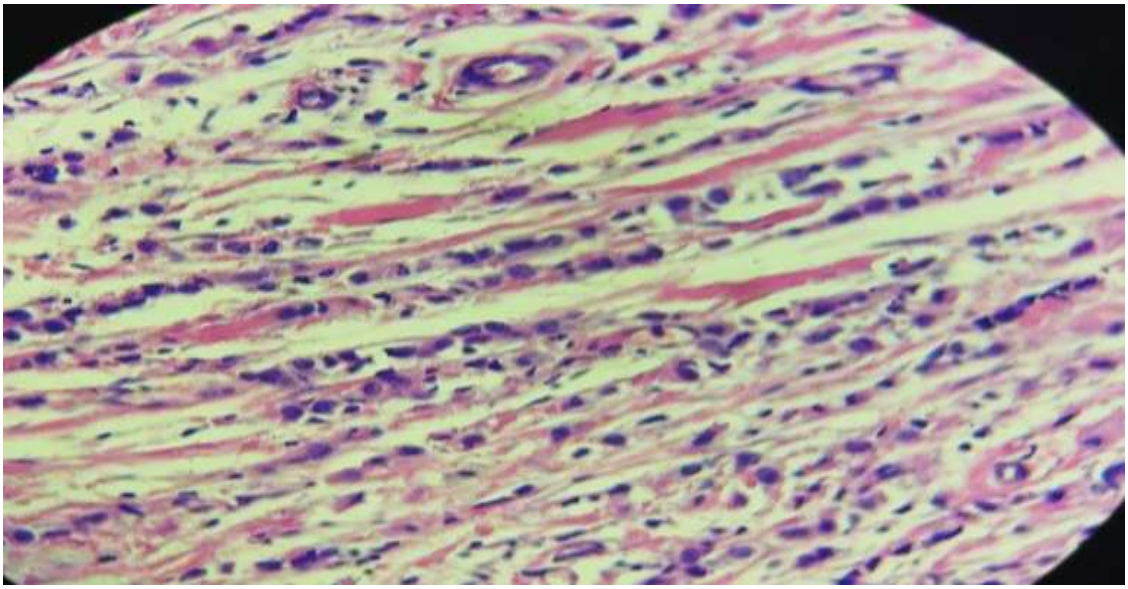

Figure 7. Invasive lobular carcinoma. Section showing breast tissue is invaded by malignant cells forming cords pattern. Malignant cells are arranged in a single line pattern (Indian file). Malignant cells are pleomorphic with different sizes with pleomorphic and hyperchromic nuclei. (H&E, 40X)

Discussion

This study showed a significant PTEN gene deletion or signal reduction in around quarter of the sample patients with BC (26.67% P value=0.0024). The percentage of PTEN gene deletion was varied among other studies; Lebok et al. (2015) 19%, Beg et al. (2015) 6.4%, Al-hamadawi et al. (2015) 58.8% and Bazzichetto et al (2019) 30-40% [9,11,13,14]. This variation in percentage of PTEN gene deletion in BC was mentioned in a meta-analysis which stated that PTEN deletion or reduced expression is present in 4% to 64% of BC patients [10]. These variations in the percentages can be attributed to sample sizes, genetic and racial differences, and techniques used to assess the gene deletion.

Many studies suggested that complete deletion to of PTEN gene or even gene inactivation (deletion to one copy of PTEN gene) can contribute to tumorigenesis or promote the progression of BC [15-18]. In other words, PTEN inactivation leads to high concentrations and activity of phosphatidylinositol 3,4,5-trisphosphate (PIP3) (which is normally present in low concentrations in quiescent cells and increased by stimulation of growth factors). This will lead to high levels of Akt (proto-oncogene) which has antiapoptotic activity by inactivating proapoptotic factor BCL2 associated agonist of cell death (BAD) protein and caspase-9 [15].

The average age of BC patients with PTEN gene deletion or signal reduction was 43.5 years, which is approximately 12 years younger than those with no deletion (55.63 years). This means that PTEN deletion tends to occur in younger BC patients, however there was no statistical significance for this relation. This result goes with the findings of other studies; Kazim et al. in 2019 and Anders et al. in 2008 [19,20].

There was significant relation between PTEN gene deletion or signal reduction and tumor size >2 cm (P value=0.0001). This relation between PTEN gene loss and larger tumor size was established by a previous study done in Iraqi by Al-hamadawi et al. in 2015 [13]. Other studies; Li et al. in 2017, Beg et al. in 2005 and Zhang et al. in 2013 recognized the relation between PTEN deletion and larger tumor size [10,11,21]. When PTEN gene is deleted or inactivated, Akt (which is normally inhibited by PTEN gene) would be active and this promotes

cell cycle progression in cancer and leads to continuous cells division and larger tumor size. This increased tumor size can be attributed to the effect of Akt on glycogen synthase kinase 3 (GSK-3) and mTOR signaling pathway. Akt inhibits GSK-3, which is thought to have a role in promoting cell cycle progression in cancer cells. On the other hand, Akt activates mTOR signaling pathway which is important in ribosomal protein synthesis [22].

Only 2 cases out of 8 (25%) with PTEN deletion showed a multicentric tumor and both of the cases were ILC. There was no statistical significance in relation between PTEN and multicentric tumor (P value=0.26). Unfortunately, there are no data regarding the relation between PTEN gene deletion status and breast tumor multicentricity.

In this study, 75% of patients with deleted PTEN gene were diagnosed with IDC of no special type (NOS) and the remaining 25% were diagnosed with ILC. There was no statistical significance between PTEN deletion and tumor histological type (P value=0.25). Same results were found in a study done by Lopez et al. (2020), in which BC patients with low PTEN expression were 80% IDC, 14.3% ILC and 5.7% for the other types of BC and there was no statistical significance between PTEN deletion status and BC pathological type [23]. Li et al. in 2017 found the same result in which there is no association between PTEN gene loss and BC histological type [10].

The breast tumors with deleted PTEN gene were divided equally between grade II and III (4 cases in each grade). This means that patients with deleted PTEN gene present with higher grade tumors (grade II and III), and there was a statistically significant difference between PTEN gene deletion and higher tumor grade (P value=0.013). This relation was demonstrated in many previous studies Lebok et al. (2015), Li et al. (2017), Al-hamadawi et al. (2015), and Golmohammadi et al. (2016) [9,10,13,24].

It has been demonstrated that PTEN has a role in chromosomal stability and DNA repair and is negatively associated with mitotic index. Loss of PTEN gene function in tumors leads to chromosomal instability, impaired DNA repair and higher mitosis rate which yields to a higher-grade tumor [25]. In addition to that, PTEN gene loss leads to aberrant chromosomal segregation during mitosis which leads to erroneous chromosome inheritance [26].

In the current study, majority of patients with deleted PTEN gene presented with pathological stage III (6 out of 8 (75%)) and the remaining 2 cases (25%) were diagnosed with stage II. This means that patients with deleted PTEN gene tend to present with more advanced tumor stage and there was a statistically significant difference between PTEN gene deletion and higher tumor stage (P value=0.012). This was stated in previous studies, which found PTEN deleted tumors was associated with higher tumor stage; Lebok et al. (2015), Li et al. (2017), Al-hamadawi et al. (2015), Golmohammadi et al. (2016) and Chang et al. (2005) [9,10,13,24,27].

PTEN gene deletion leads to phosphorylation of focal adhesion kinase (FAK) which in turn leads to integrin-mediated cell spread, migration and focal adhesion formation. Another mechanism by which PTEN gene has a role in metastasis is that PTEN gene deletion leads to loss or decrease of E-cadherin, which is crucial for tumor invasion and metastasis [28].

There was no statistical significance between PTEN gene deletion or signal reduction and lymph nodes metastasis (P value=0.6). However, there was statistical significance between PTEN deletion or signal reduction and metastasis to more than 4 lymph nodes (P value=0.4). Many studies showed that PTEN gene deletion was associated with lymph nodes metastasis; Li et al. (2007), Al-hamadawi et al. (2015), Chang et al. (2005), Chung et al. (2004) and Izzo et al. (2021) [10,13,27,29,30]. However, no data were found regarding the relation between PTEN gene deletion status and number of lymph node that BC would metastasize to. Loss of PTEN gene function can weaken intercellular adhesion and promote cancer cell invasion including invasion to lymph nodes [31].

In conclusion, PTEN gene deletion or signal reduction was detected in 26.67% of BC cases. This may reflect the possible role of PTEN gene in the development of BC. There was a significant correlation between PTEN gene

deletion or signal reduction and larger tumor size, high number of lymph nodes metastasis (more than 4 lymph nodes), higher grade and advanced stage. This may be related to the loss of PTEN gene normal function by deletion resulting in impaired control of cell growth, increased cellular proliferation, loss of differentiation and cellular adhesion with enhanced migration and local invasion of BC. However, there is no significant relation between PTEN gene deletion status and age, multicentricity, pathological type and lymph node metastasis (regardless the number of lymph nodes) in patients with BC.

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Author contribution
Dr. Al-Taie bought the kit, stained the slides with H&E stain and FISH probe and wrote the paper. Dr. Qasim supervised the work, examined and confirmed the diagnosis of the slides and reviewed the paper.

Conflict of interest
The authors declare no conflict of interest.

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Anti-Proliferative Activity of *Althaea Officinalis* Extracts on Iraqi Breast Cancer Cell Line AMJ13

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Abstract

Background Breast cancer has been the highest-ranked malignancy among the Iraqi population since 1986. *Althaea officinalis* is a perennial plant and native to Iraq and Asia, Europe and United States of America.

Objective To evaluate the cytotoxicity of *Althaea officinalis* extracts on AMJ13 breast cancer cell line.

Methods The cytotoxic activity of crude, flavonoids, and phytosterols extracts were tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Apoptosis assay was done by Acridine orange–Ethidium bromide dual staining method.

Results The cell line was exposed to serial concentrations of Doxorubicin and extracts from (3.125 to 100 µg/ml) in triplicate of each concentration for 72 hours exposure period. The Inhibitory concentration fifty (IC50) values for Doxorubicin, crude, flavonoids, and phytosterols were as follows: 19.52 µg/ml, 25.18 µg/ml, 39.54 µg/ml, and 47.88 µg/ml, respectively.

Conclusion *Althaea officinalis* extracts have a significant cytotoxic activity and have the ability to cause apoptosis on AMJ13 breast cancer cells. Crude extract have the highest significant cytotoxic activity

Keywords Breast cancer, AMJ13 cell line, *Althaea officinalis*, cytotoxicity assay, apoptosis assay

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List of abbreviations: AMJ13 = Human breast cancer cell line-Iraq, AO/EtBr = Acridine orange–Ethidium bromide, DMSO = Dimethyl Sulphoxide, Dox = Doxorubicin, IC50 = Inhibitory concentration fifty, MCF-7 = Human breast cancer cell line-USA, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, PBS = Phosphate buffer saline, TNF-α = Tumor necrosis factor alpha, VEGFR = Vascular endothelial growth factor receptor

Introduction

Breast cancer happens when cells in the breast begin growing out of proportion and results in malignant tumor that spreads throughout the body [(1)]. It is highly prevalent in females and accounts for second highest number of deaths worldwide [(2)]. Breast cancer has become a major threat to female health in Iraq, where it is the leading cause of death after cardiovascular diseases among women and it has been the highest-ranked malignancy among the Iraqi population in general since 1986 [(3)]. There are three main options available for the treatment of patients with solid tumors: surgery, radiotherapy and chemotherapy. Each treatment can either be applied alone or in combination, depending on the disease [(4)]. Chemotherapy remains the most effective form of treatment once the tumor has spread [(5)]. Although chemotherapeutic drugs and radiation are more powerful maneuvers for treatment of malignancy, they are associated with serious

adverse effects, and tumor resistance against therapy [6,7]. Plants and plant derived products possess the potential to be excellent lead structures and to serve as a basis of promising therapeutic agents for cancer treatment as these are simple, safer eco-friendly, low-cost, fast, and less toxic as compared with conventional treatment methods [8]. Phytochemicals are selective in their functions and acts specifically on tumor cells without affecting normal cells [7]. *Althaea officinalis* (Khatmi) is a perennial plant and native to Iraq and Asia, Europe and United States of America [9]. It is widely used traditionally for the treatment of the irritation of oral, pharyngeal mucosa and associated dry cough, mild gastritis, skin burns and for insect bites [10]. It is also used in catarrh of the mouth and throat, gastrointestinal tract and urinary tract complains, as well as for inflammation, ulcers, abscesses, burns, constipation and diarrhea [11]. The objective of this study was to investigate the cytotoxic activity of *Althaea officinalis* extracts on AMJ13 breast cancer cell line.

Methods

Cytotoxicity assay

To determine the cytotoxic effect of *Althaea officinalis* crude, flavonoids, and phytosterols compared to Doxorubicin cytotoxicity. The MTT cell viability assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was done using 96-well plates. AMJ13 cells were seeded at 1×10^4 cells/well. After 24 hours or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration. Cell viability was measured after 72 hours of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [12]. The absorbency was determined on a microplate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

Cytotoxicity = A-B/A *100

Apoptosis assay

The induced death of (AMJ13) cells was performed using Acridine Orange-Ethidium bromide (AO/EtBr) dual staining method [13]. Briefly, the cells in 96-well plates were treated with Doxorubicin, *Althaea officinalis* crude, flavonoids, and phytosterols and incubated for 16 h. The cells were detached and washed twice using PBS (Phosphate buffer saline), and transferred to a clear 96-well plate. Dual fluorescent dyes (10 µL) were added into the cells at equal volumes. Finally, the cells were visualized under fluorescence microscopy.

Results

Cytotoxic activity of Althaea Officinalis crude, flavonoids, and phytosterols fractions on AMJ13 cell line

The study was done on (AMJ13) cell line. The effect of different concentrations of crude, flavonoids, and phytosterols fractions of *Althaea officinalis* and Doxorubicin from (6.25 to 100 µg/mL) on AMJ13 tumor cell line revealed significant cytotoxic effects on all cell lines where all test substances inhibited cell growth at highest concentrations and reduced at the lower concentrations with the highest growth inhibitory effect presented with Doxorubicin followed by crude, flavonoid, and phytosterols fractions (Table 1). The highest cytotoxic activity of Doxorubicin, crude, flavonoids and phytosterols fractions were achieved at high concentration (100 µg/mL) and the least activity observed at lower concentration (6.25 µg/mL) after 72 h exposure period and the cytotoxic activity increases with concentration as concentration dependent (Figures 1, 2, 3 & 4). Histopathologically, the AMJ13 tumor cells in control group showing continues cell growth and monolayer formation, while the tumor cells treated by

tested agents showed cells detachment and presence of cytoplasmic vacuolation (Figure 5). The IC50 values for Doxorubicin, crude, flavonoids and phytosterols on AMJ13 cell line were calculated by the logarithmic equation. They were equal to 19.52 µg/mL, 25.18 µg/mL, 39.54 µg/mL, 47.88 µg/mL respectively.

Table 1. Cytotoxic activity of serial concentrations of *Althaea officinalis* crude, flavonoids and phytosterols fractions compared to Dox on AMJ13 cell line after 72hrs exposure period, (p<0.05)

Concentration (µg/mL)	Doxorubicin IR% (Mean ±SEM)	Crude IR% (Mean ±SEM)	Flavonoids IR% (Mean ±SEM)	Phytosterols IR% (Mean ±SEM)
0 (Control)	0±0.02	0.0±0.02	0.0±0.02	0±0.02
6.25	22.81±2.79	11.67±1.2	10.33±2.03	9.33±1.45
12.5	33.0±2.082	25.0±3.61	24.0±2.08	24.0±4.58
25	58.67±2.03	51.0±4.36	38.67±1.86	37.33±1.86
50	72.0±2.52	61.0±3.22	50.67±1.2	51.67±2.4
100	81.33±2.03	71.67±3.53	72.0±4.04	71.0±3.22

Each concentration in triplicate and the experiment were repeated twice

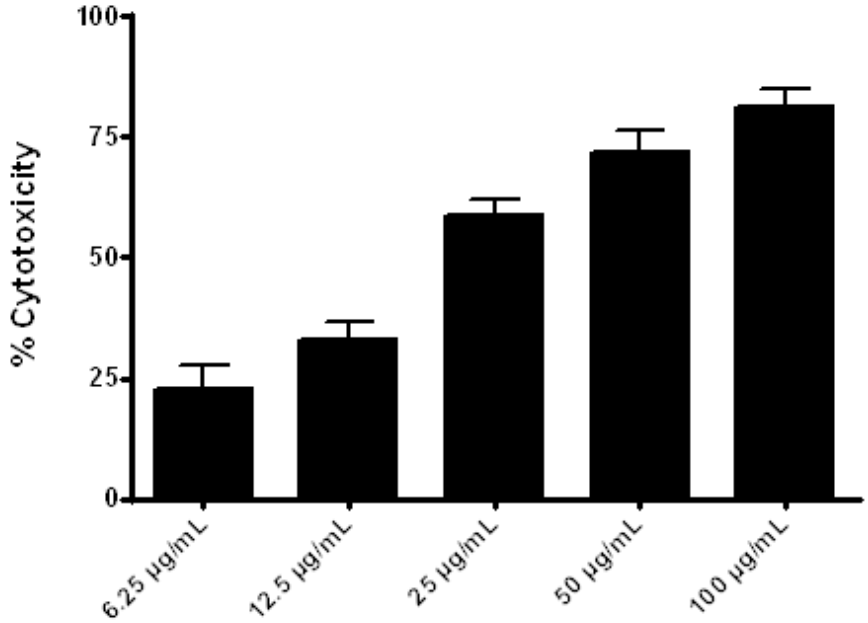

Figure 1. Cytotoxicity effect of Doxorubicin in AMJ13 cells. IC50=19.52 µg/mL

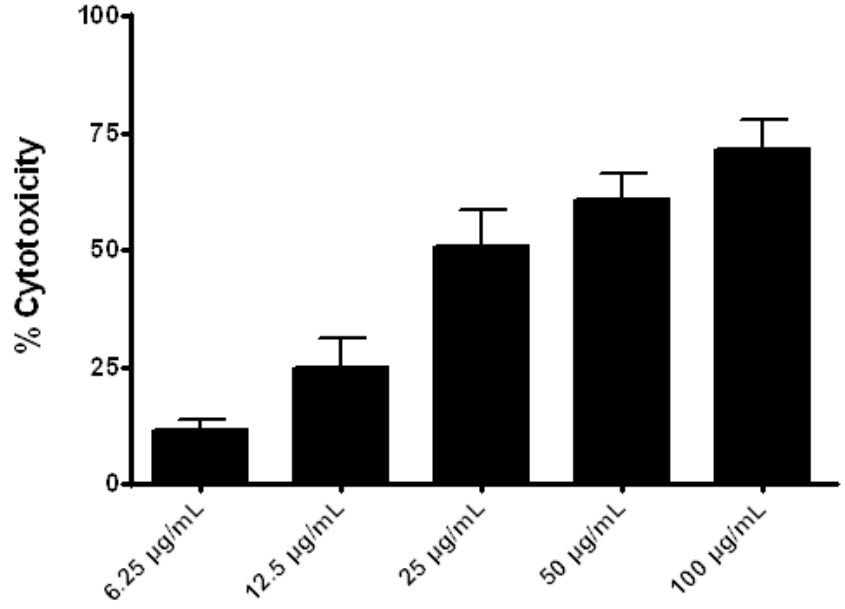

Figure 2. Cytotoxicity effect of crude in AMJ13 cells. IC50=25.18 µg/mL

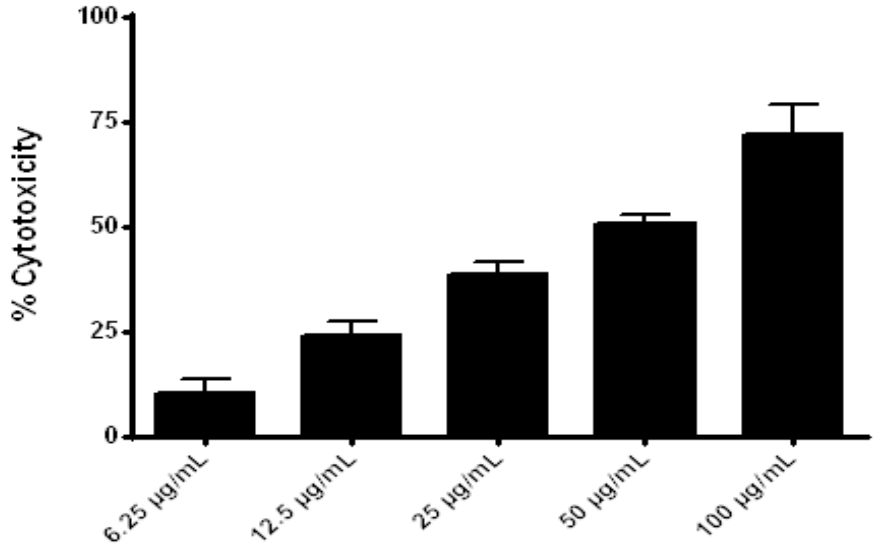

Figure 3. Cytotoxicity effect of flavonoids in AMJ13 cells. IC50=39.54 µg/mL

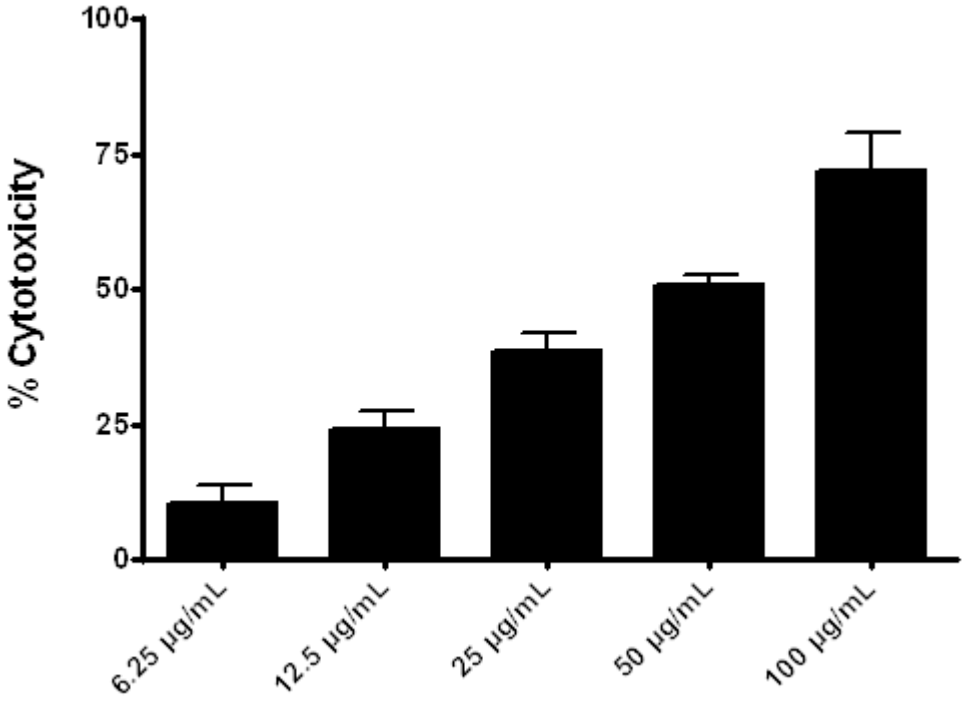

Figure 4. Cytotoxicity effect of phytosterols in AMJ13 cells. IC50=47.88 µg/mL

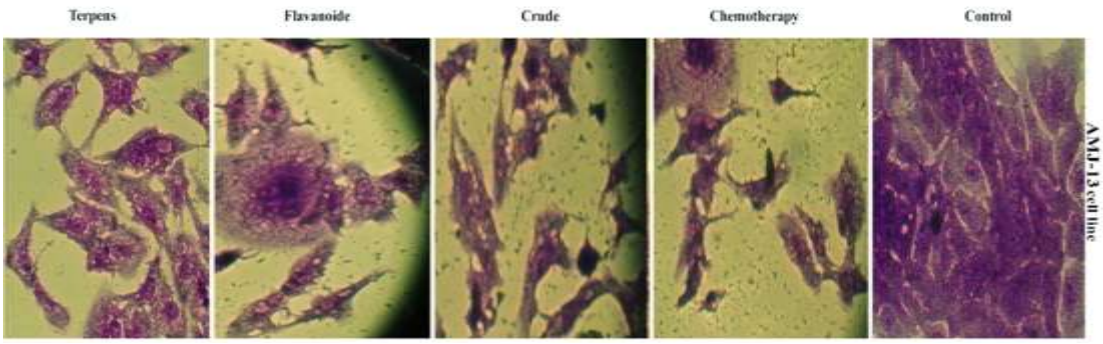

Figure 5. Cytotoxic activity of tested compounds against AMJ13 cells (40X)

Apoptosis assay

In our study, the *Althaea officinalis* extracts induced apoptosis in AMJ13 cells with non-significant effects on normal cells. The most extensive apoptotic effects was shown by crude extract which is comparable to Doxorubicin effects on AMJ13 breast cancer cell line followed by flavonoids fraction and then phytosterols fraction which has the least effect where the viable cells are stained green while the apoptotic cells are stained orange or red (Figure 6).

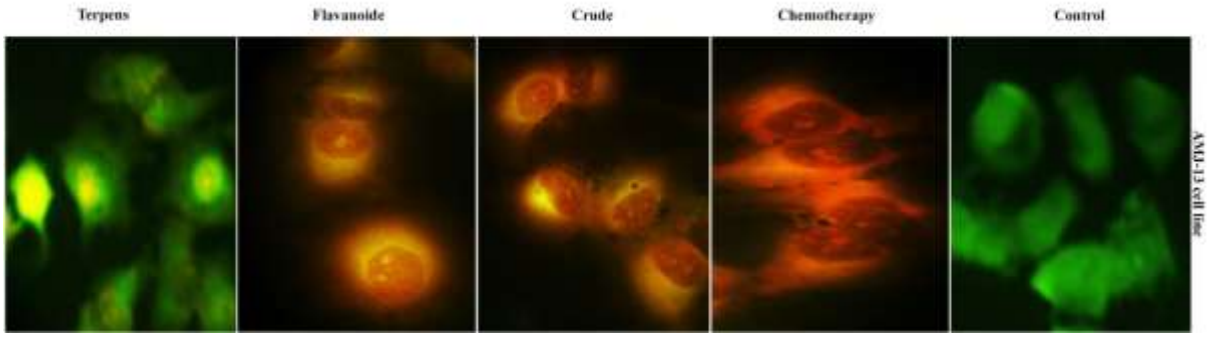

Figure 6. Effect of test substances on AMJ13 cells by apoptosis mechanism using fluorescent microscope

Discussion

The cytotoxic and antiproliferative activity of Doxorubicin and *Althaea officinalis* extracts were tested by the MTT assay, which is a colorimetric assay for evaluating cell viability [(14)]. In this study, in vitro cytotoxicity of Doxorubicin, crude, flavonoids, and phytosterols were evaluated with AMJ13 human breast cancer cell line to determine if these agents have any cytotoxic effect against breast cancer cell line. The results showed that the three extracts of *Althaea officinalis* had significant cytotoxic effect in a concentration dependent manner after 72 h exposure period on AMJ13 breast cancer cell line that was used and could reduce the viability of cancer cell line that were used to a variable extent. Crude extract exert the highest cytotoxicity effect, followed by flavonoids, and phytosterols fractions according to the threshold proposed by Suffness and Pezzuto where they establishes that naturally derived compounds showing a growth inhibitory effect (IC50 ≤ 100 µg/mL) can be considered to be cytotoxic and selected for further studies, whereas the most promising ones are those with an GI50 lower than 30 µg/mL [(15)] where the IC50 of crude, flavonoids, and phytosterols were 25.18 µg/mL, 39.54 µg/mL, 47.88 µg/mL respectively compared to IC50 of Doxorubicin, which is equal to 19.52 µg/mL. The naturally derived compounds and the bioactive constituents could influence the developing and progressing of carcinoma in several manners, for instance, inhibit the development and metastasis of cancer cells, immune-modulation, defensive against carcinogens and improving chemotherapy [(16)]. This study is the novel study all over the world to study the cytotoxic activity of *Althaea officinalis* extracts on AMJ13 cancer cell line. The results agreed with other previous studies that carried out in vitro and confirmed that some flavonoids could inhibit the cell growth of colon, prostate, liver, and breast cancer [(17)]. Previous study showed that scopoletin of *Althaea officinalis* produced dual action on tumoral lymphocytes exhibiting both a cytostatic and a cytotoxic effect on the cell, and also exert apoptosis [(9,18)]. Flavonoids are a class of compounds that have antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties [(19,20)]. The anticancer activity of flavonoids is related to their modulation of signal transduction pathways within cancer cells [(21)]. As a result, flavonoids can inhibit cell proliferation, angiogenesis, and metastasis, while also promoting apoptosis.

Phytosterols are natural products, showing anticancer activity. Campesterol, β-sitosterol, and stigmasterol are the three most common sterols [(22)]. It has been reported that phytosterols have protective effects on various chronic ailments including cardiovascular diseases [(23,24)] and diabetes [(25)]. Moreover, it is suggested that diets rich in phytosterols can reduce the risk of cancer by 20% [(26,27)]. Phytosterols and their oxy-derivatives may offer protection to the human body and inhibit cell proliferation and metastasis [(28)], as well as

the induction of apoptosis [29,30], all of which have been experimentally verified. In addition, phytosterols may also be important in host systems and exert antitumor effects by improving the immune system's identification of cancer, affecting hormone-dependent endocrine tumor growth, and regulating sterol biosynthesis [31,32]. Stigmasterol is antiangiogenic compound that inhibit endothelial cell proliferation, migration, and capillary network formation through the disruption of the TNF-α-VEGFR-2 axis, and it effectively suppress the growth cholangiocarcinoma xenografts by downregulating inflammatory cytokine production, macrophage recruitment and tumor angiogenesis [33]. The antiproliferative activity of stigmasterol also reported in human vascular smooth muscle cells A7R5 [34], hepatoma cells HepG2 [35], and human monocyte cell line U937 [36]. In the most recent study done by Al-Fatlawi at 2019, stigmasterol exerts anti-proliferative activities against the breast (MCF-7) and liver cancer (HepG2) cells. This study also found that stigmasterol helped to regulate apoptotic regulatory genes in cancerous cells where it led to high expression levels of pro-apoptotic genes (bax, p53), and negative expression of antiapoptotic genes (bcl-2). In addition, the stigmasterol treated both cancerous cell lines showed an increase in expression of the gene of caspase-9 and caspase-3. According to the gene expression analysis results, stigmasterol probably activates the apoptosis signaling pathway, and hence genomic DNA fragments were observed through gel electrophoresis. Therefore, this compound may be beneficial therapy without possible side effects on normal cells [36].

The changes in the nuclear morphology of AMJ13 cells after treated with crude, flavonoids, and phytosterols fractions of *Althaea officinalis* and Doxorubicin were studied using AO/EtBr dual staining method. The apoptotic cells were evaluated based on DNA damage. The AO-EtBr dual staining method was employed to stain specific parts of the cell and to determine the distinct apoptotic signs of nuclear alternations. The viable and non-apoptotic cells are stained green while the apoptotic cells are stained orange or red. The results in our study showed the exposure of the cells to the tested compounds caused an increase in membrane disruption and formation of lysosome vacuoles compared to the untreated control cells. The results showed the ability of test fractions to cause cell death due to their ability to penetrate the cell membrane and cause the mRNA expression levels of p53, bax, bcl-2, caspase-3, and caspase-9 (apoptosis is controlled through these pathways) [37]. The most extensive apoptotic effects were shown by crude extract which is comparable to Doxorubicin effects on AMJ13 breast cancer cell line followed by flavonoids fraction and then phytosterols fraction which has the least effect. The results of our study were coincided with preceding studies in that flavonoids and phytosterols have anticancer properties through induction of apoptosis mechanism in cancerous cells [38]. The majority of flavonoids compounds can promote apoptosis, induce cell cycle arrest, and promote cellular differentiation [19]. Alvarez-Sala and his colleagues stated that phytosterols including β-sitosterol produced apoptotic cell death with the induction of DNA fragmentation through the appearance of a sub-G1 cell population [39]. The antitumor activity of stigmasterol might be mediated through the activation of protein phosphatase 2A by ceramide causing apoptosis, as is shown by structurally similar phytosterols [40].

In conclusion, crude, flavonoids, and phytosterols extracts of *Althaea officinalis* exert a significant cytotoxicity and have the ability to cause apoptosis on AMJ13 breast cancer cells. Besides that, crude extract has the highest significant cytotoxic activity on AMJ13 cells.

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Author contribution

Dr Kadhum and Dr Al-shammari: collection, analysis of data, interpretation and discussion

of results. Dr Abd: concept, supervision and revising the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Assessment of Vitamin D Level in a Sample of Iraqi Obese Women

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Abstract

Background High prevalence of both vitamin D (vit. D) deficiency and obesity in world population as reported by many studies drown attention to establish the association and its direction between these two modifiable risk factors and their impact on health status which are still uncertain.

Objective To measure vit. D levels among a sample of obese women, describing some factors that may contribute for vit. D values in this sample and assess the correlation between vit. D levels and body mass index (BMI).

Methods A cross-sectional study was carried out among 100 obese women. Their age ranged between (20-50) years, during the period from 1st February to 1st August 2019. The sample was gathered from laboratory of private medical center at Palestine Street in Baghdad. vit. D was measured by (Ichroma vit D), which is a fluorescence immunoassay.

Results The mean±SD of vit. D values of the participants were found to be (16.05±6.9) ng/ml. Only two women from the sample had sufficient vit. D level of more than 30 ng/ml, (72%) of the participants found to have insufficient vit. D level 10-29.9 ng/ml and the remaining had deficiency in vit. D level <10 ng/ml. A significant association was found between vit. D levels and the age participants and beverages drinking (P≤0.01). Indirect correlation was found between vit. D values of the participants, their BMI values, and waist/ hip ratios and was significant with the last P≤0.012.

Conclusion No association was found between vit. D and BMI, but there is indirect correlation between vit. D and waist/hip ratio. Highest deficiency of vit. D among age group (30-39) years old and among those who were drinking carbonated beverage.

Keywords Obese, women, vitamin D, body mass index

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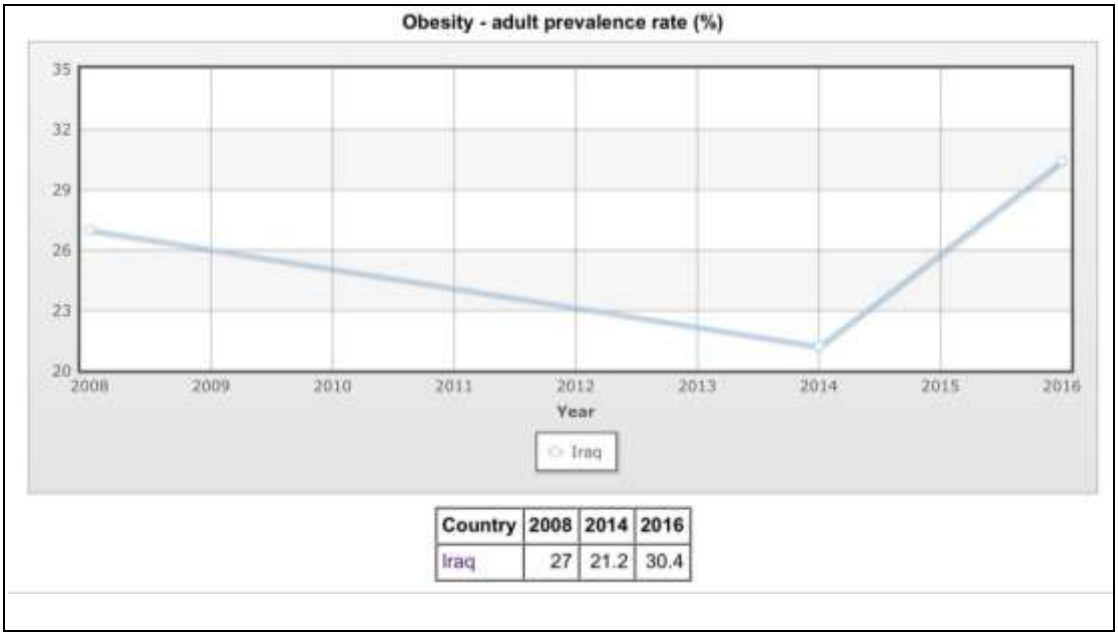
List of abbreviations: BMI = Body mass index, HT = Height, SD = Standard deviation, UV = Ultraviolet light, vit. D = Vitamin D, WHR= Waist hip ratio, WT = Weight

Introduction

Obesity has been known to be a major health problem worldwide [(1)], and considered to be a risk factor for many medical conditions such as cardiovascular disease and type 2 diabetes mellitus [(2)] . As the prevalence of obesity is increasing worldwide [(1)] and in Iraq [(3)], so it has been of great importance to study the possible associated factors with obesity and type of its relation such as vitamin D (vit. D), which is a fat-soluble vitamin synthesized in the body and taken from diet and supplements [(4)]. Vit. D and its metabolites have a wide range of important biological functions in the body, and its linkage with obesity has been a subject of interest by many studies trying to define their relationship as this current study that spotted the light on vit. D level in obese women [(5)] as showed in figure 1.



Country	2008	2014	2016
Iraq	27	21.2	30.4

Figure 1. Prevalence of obesity in Iraq [3]

Definition of Obesity - adult prevalence rate
This entry gives the percent of a country's population considered to be obese. Prevalence rate in Iraq was found to be 27% in (2008), this rate decreased to 21.2% in (2014), but increased to 30.4 % in (2016).

Study objectives
1. To measure vit. D levels in a sample of obese women.
2. To describe factors that may contribute for vit. D values in this sample of obese women.
3. To assess the correlation between vit. D levels and body mass index (BMI) of this sample of obese women.

Methods
A descriptive cross-sectional study was conducted in a laboratory of private medical center at Palestine Street in Baghdad. This center has nutrition and weight healthcare department visited by obese patients.
Data was collected using a convenient sampling technique during the time frame from February 1st till August 1st 2019. Eligible participants include all adult obese women (BMI ≥30), their age range 20 to 50 years old. Women with bone mineralization, malabsorption disorder, parathyroid disorders, genetic disorders of vit. D metabolism, pregnant and lactating women, chronic kidney or liver disease, diabetes mellitus and participants using tonics contain vit. D supplementations were excluded.

Data collection tools
A) Questionnaire
Each participant was interviewed by the researcher to collect information through a questionnaire designed especially for this research after reviewing the literature. It covered some socio-demographic data and some of the risk factors related to vit. D deficiency.

Questionnaire:
1. Serial no. (vit. D).
2. Age
3. Years of education
4. Occupation
5. Address
6. Marital status
7. GPA (gravida, parity, abortion)
   - Marriage duration
   - Years of infertility
8. Wearing Hijab: yes/no
9. Habits

Smoking: yes/no

10. Eating
    - Unhealthy food, fast food
    - Fish such as salmon, tuna
    - Milk and its products.
    - Beverages (cola)
11. Sedentary lifestyle (Physical activity): Yes/No
12. Sun exposure: Yes/No
    - (5-30 min /day) from 10 AM -3 PM
13. Drug history

B) Anthropometric measures

Weight and height were measured using mechanical weight machine with height measuring scale (RGZ-160), also waist and hip circumferences were measured using a holtain flexible metallic tape to determine the waist/hip ratio (WHR).

BMI ranges according to WHO classification [6]:

- ❖Underweight: BMI <18.5 kg/m^2
- ❖Normal weight: BMI 18.5-24.9 kg/m^2
- ❖Overweight: BMI 25-29.9 kg/m^2
- ❖Obese: BMI ≥30 kg/m^2
- ❖Obesity
  - class I 30-34.9 kg/m^2
  - Class II 35-39.9 kg/m^2
  - Class III ≥40 kg/m^2

WHR ranges according to WHO classification [7]:

- ❖Underweight: WHR >0.80 in female, >0.72 in male
- ❖Normal weight: WHR is <0.90 in female, <0.80 in male.
- ❖Overweight: WHR 0.80-0.84 in female, 0.90-0.99 in male.
- ❖Obese: WHR > 0.85 in female, >1.00 in male.

C) Vit. D measurement

This study adopted the quantitative determination of total 25 vit. D level in human serum by fluorescence immunoassay (FIA) using Ichroma vit. D tool.

The cut-off (reference range) of vitamin D [8]:

- ❖<10 ng/ml (<25 nmol/l) - deficiency
- ❖10-30 ng/ml (25-75 nmol/l) - insufficiency
- ❖30-100 ng/ml (75-250 nmol/l) - sufficiency

Ethical consideration

Participant's consent was taken after explaining to them the nature and goals of our study that may help them and the community for better health care, promising the participants to protect their private information but only the necessary information will be used to serve for educational purposes only, for which participants fully understood and agreed.

Statistical analysis

Analysis of data was carried out using SPSS (statistical package for social sciences) version 25. Data were presented as tables and figures, frequency, percentage, mean, standard deviation and range (minimum-maximum values). The significance of association of qualitative data was tested using parson chi-square test (X2-test) with fisher exact test used whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

Results

A total of 100 obese women were recruited for this study. The age of participants ranged from 20-50 years with age group of 30-39 years forming the highest percentage 37%, 73% lived in Risafa side of Baghdad city, 67% were unemployed, 42% of the sample had finished college education and 75% of the sample were currently married.

The mean±SD of the BMI of the participants was found to be (34.83±3.99). More than half (60%) of the participants were of class 1 obesity (BMI = 30-34.9 Kg/m^2), 29% of class 2 obesity (BMI = 35-39.9 Kg/m^2) and the remaining were of class 3 (BMI ≥40 Kg/m^2). While less than half (47%) had high WHR ≥0.85. The mean±SD of the WHR of the participants was found to be (0.8±0.17) as showed in figures 2 and 3.

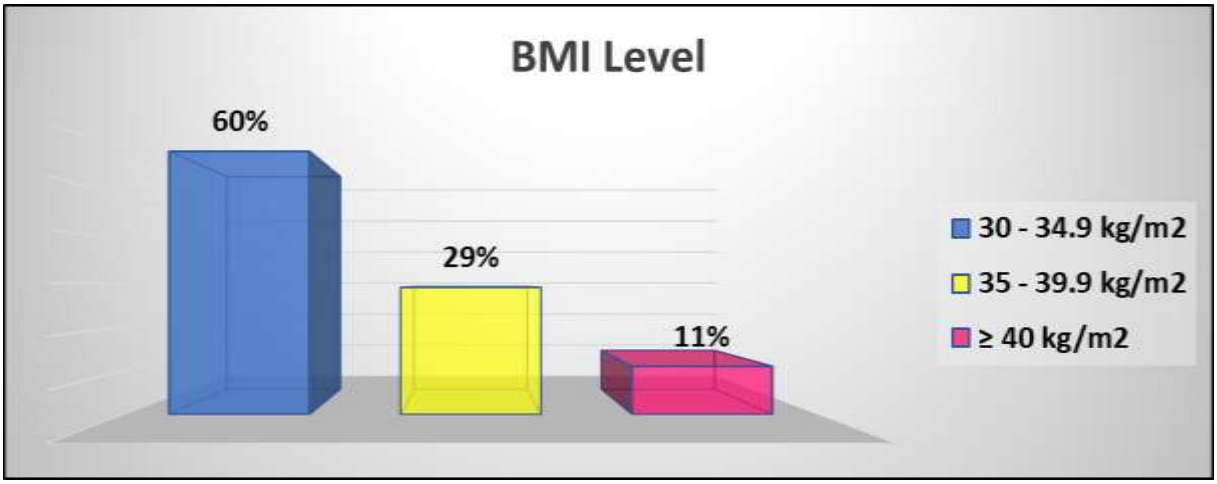


Figure 2. Distribution of study participants by body mass index (BMI)

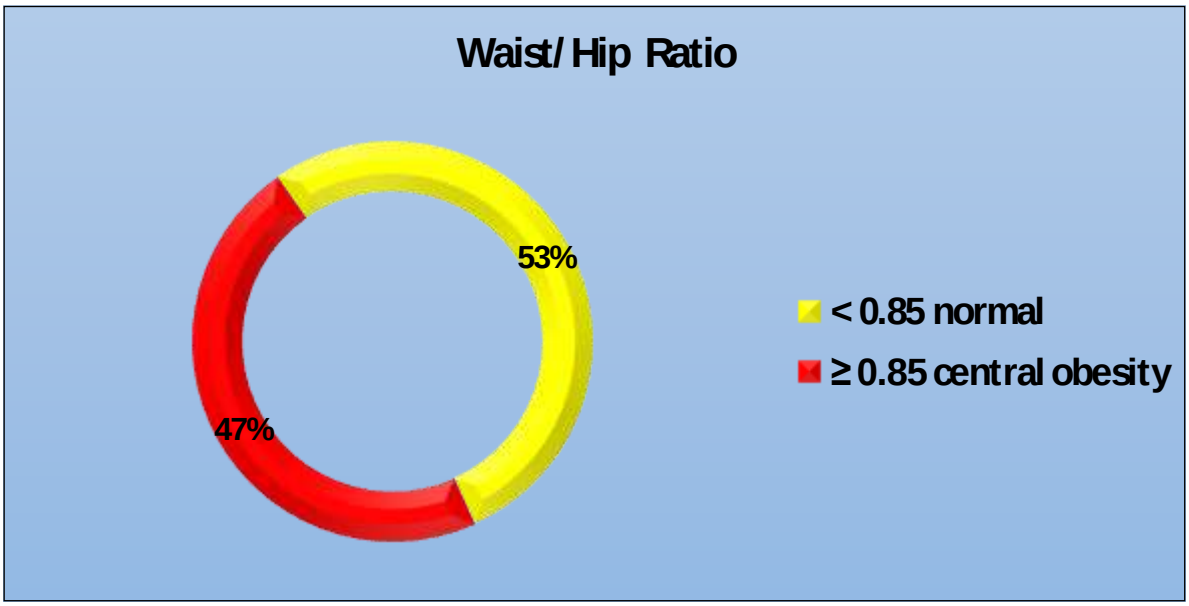


Figure 3. Distribution of study participants by waist/hip ratio (WHR)

The mean±SD of vit. D values of the participants was found to be (16.05±6.9) ng/ml; and only two women from the sample had sufficient vit. D level of more than 30 ng/ml who were excluded from the association tests for its validity (Figure 4).

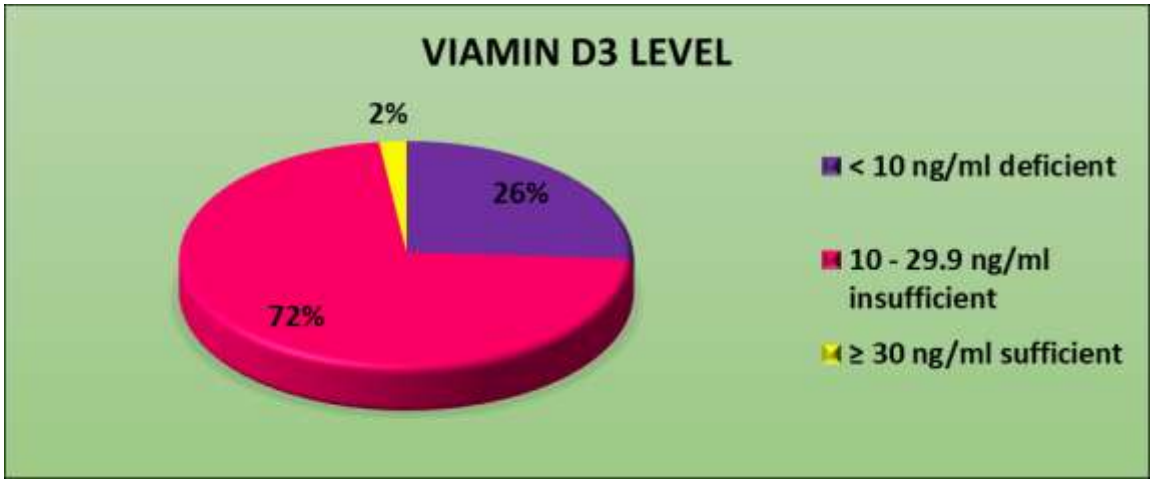


Figure 4. Distribution of study participants by vitamin D level

Although no significant association was found between vit. D levels and the other socio-demographic characteristics, but vit. D was more deficient among unemployed (31.3%), those with primary education (35.7%), and unmarried women (33.3%) (Table 1).

Table 1. Distribution of vitamin D level of study participants according to socio-demographic characteristics

Variable		Vitamin D Level <10 n= 26 (%)	Vitamin D Level 10-29.9 n= 72 (%)	Total (%) n= 98	P value
Age (Year)	20-29	5 (18.5)	22 (81.5)	27 (27.6)	**0.013**
	30-39	16 (43.2)	21 (56.8)	37 (37.8)	
	≥40	5 (14.7)	29 (85.3)	34 (34.7)	
Address	Risafa	19 (26.8)	52 (73.2)	71 (72.4)	0.981
	Karkh	7 (25.9)	20 (74.1)	27 (27.6)	
Occupation	Unemployed	21 (31.3)	46 (68.7)	67 (68.4)	0.308
	Student	1 (20.0)	4 (80.0)	5 (5.1)	
	Employee	4 (15.4)	22 (84.6)	26 (26.5)	
Years of education	Primary or less	10 (35.7)	18 (64.3)	28 (28.6)	0.670
	Middle	4 (22.2)	14 (77.8)	18 (18.4)	
	Secondary	3 (25.0)	9 (75.0)	12 (12.2)	
	College or higher	9 (22.5)	31 (77.5)	40 (40.8)	
Marital status	Currently married	18 (24.3)	56 (75.7)	74 (75.5)	0.429
	Unmarried	8 (33.3)	16 (66.7)	24 (24.5)	

Chi-square test (x2-test)

A significant association was found between vit. D levels and beverages drinking with highest deficiency being in participants who reported drinking beverages 17 (40.5%) (P≤ 0.01). Although no significant association was found between vit. D levels and other lifestyle

factors, but vit. D was more deficient among those wearing hijab 23 (28%), those without sun exposure 17 (32.7%) compared to those reported exposed to the sunlight P=0.142. Those reported eating fast food 15(33.3%), not eating fish 7(29.2%), not drinking milk 17 (34.0%), and had sedentary lifestyle 23 (28.4%) were deficient with vit. D more but without a significant association. out of the 100 participants 82% were wearing hijab, 47% reported sufficient sun exposure, 14% were smokers, 47% of the sample reported eating fast food, (75%) eating fish, 50% drinking milk, and 42% drinking beverage (on daily basis). Also, 83% of the sample reported having sedentary life style (Table 2).

Table 2. Distribution of vitamin D level of study participants according to their lifestyle

Variable		Vitamin D Level <10 n= 26 (%)	Vitamin D Level 10-29.9 n= 72 (%)	Total (%) n= 98	P value
Wearing hijab	Yes	23 (28.0)	59 (72.0)	82 (83.7)	0.548
	No	3 (18.8)	13 (81.2)	16 (16.3)	
Sun exposure	Yes	9 (19.6)	37 (80.4)	46 (46.9)	0.142
	No	17 (32.7)	35 (67.3)	52 (53.1)	
Smoking	Yes	3 (21.4)	11(78.6)	14 (14.3)	0.754
	No	23 (27.4)	61 (72.6)	84 (85.7)	
Fast food	Yes	15 (33.3)	30 (66.7)	45 (45.9)	0.160
	No	11 (20.8)	42 (79.2)	53 (54.1)	
Eating fish	Yes	19 (25.7)	55 (74.3)	74 (75.5)	0.763
	No	7 (29.2)	17 (70.8)	24 (24.5)	
Drinking milk	Yes	9 (18.8)	39 (81.2)	48 (49.0)	0.087
	No	17 (34.0)	33 (66.0)	50 (51.0)	
Drinking beverages	Yes	17 (40.5)	25 (59.5)	42 (42.9)	**0.007**
	No	9 (16.1)	47 (83.9)	56 (57.1)	
Sedentary lifestyle	Yes	23 (28.4)	58 (71.6)	81 (82.7)	0.547
	No	3 (17.6)	14 (82.4)	17 (17.3)	
Drug history	Yes	3 (23.1)	10 (76.9)	13 (13.3)	0.930
	No	23 (27.1)	62 (72.9)	85 (86.7)	

Fisher's Exact Test

Indirect significant (P=0.012) correlation was found between vit. D values of the participants and WHR as 51% of them had WHR <0.85, while non-significant indirect correlation was found with their BMI values (Figures 5 and 6).

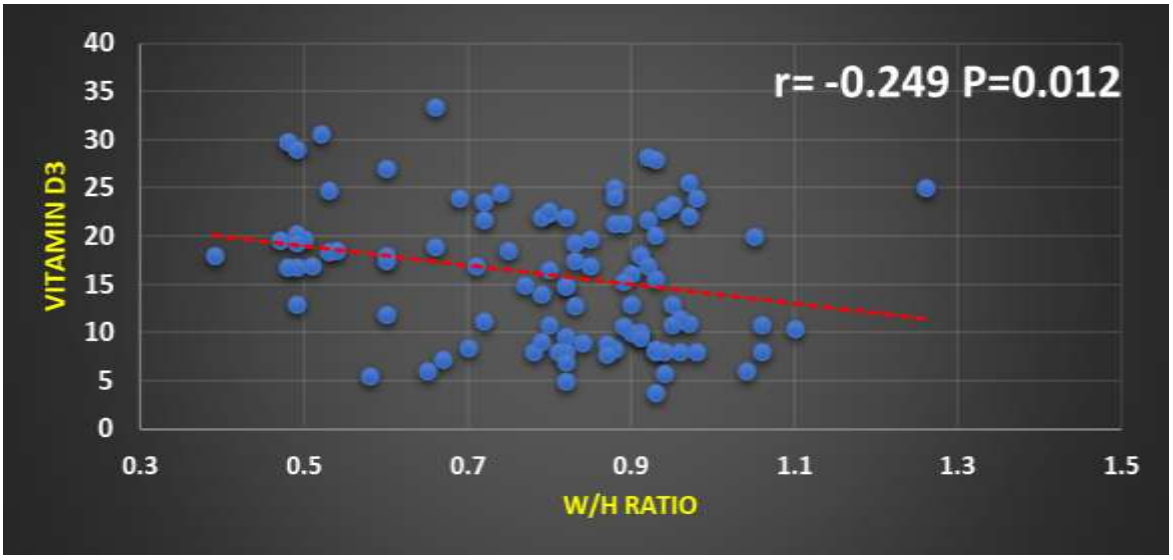


Figure 5. The correlation between vitamin D and waist/hip ratio

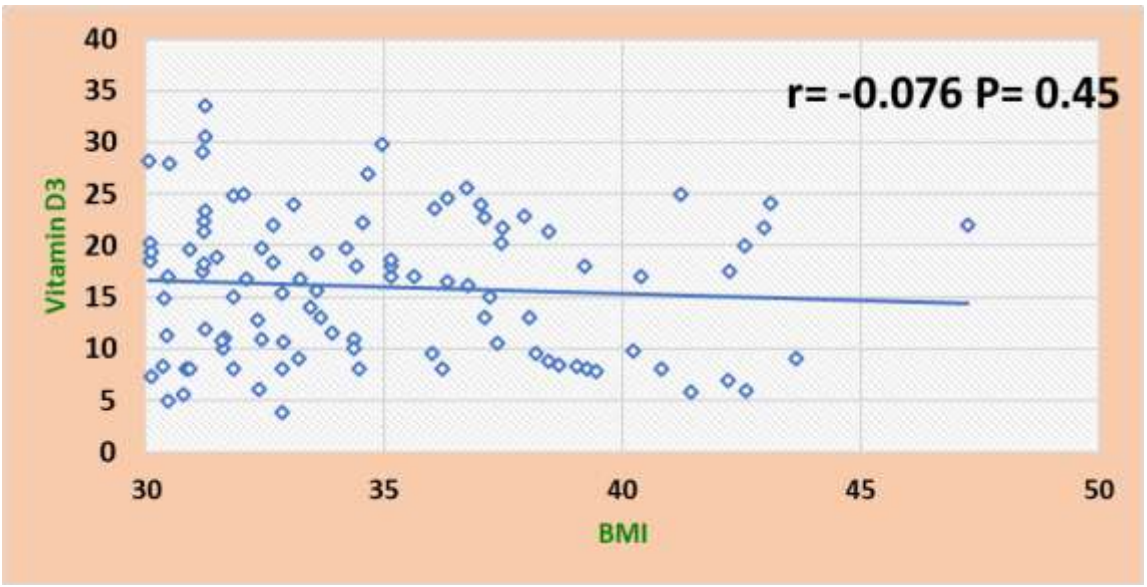


Figure 6. The correlation between vitamin D and body mass index

Discussion

Vit. D and its metabolites are known to play directly or indirectly an important role in many body functions through a wide range of mechanisms and interactions. Because of this, vit. D drew the attention of many researchers trying to determine the type and direction of the relation between vit. D and its possible affecting factors especially its diagnosis and treatment are relatively achievable. This cross-sectional descriptive study spotted the light on the relation between vit. D and BMI with other effecting factors, the most accurate way to measure vit. D using total 25-hydroxy level in human serum by blood test, fluorescence immunoassay (FIA). The advantages of this method include higher sensitivity detection of the analyte, simplified reagents and simpler assay designs [9]. The study sample consisted of 100 obese healthy females, most of them were aged between (30-39) years, finished college, married, multigravida and unemployed. Clearly

vit. D deficiency is highly prevalent among this study subjects, 98% of them are below serum vit. D (29.9 ng/ml), and 26 % of study subjects were below serum vit. D level (10 ng/ml) with mean serum vit. D level (16.05±6.9 ng/ml). These results agreed with a recent study conducted in Basrah during 2019 [10] on 57 healthy subjects, in a study conducted in Karbala during 2016 [11] on 60 healthy women, and in study was conducted in Qatar in 2017 [12] conducted on 102342 subjects. These results of high vit. D level deficiency among different study samples is not surprising since these were mentioned in many previous studies [10-11] locally, regionally [12] and worldwide [13], which necessitate for further workup to reveal vit. D relation to its determinant factors especially the modifiable factors, and this was the focus of the current study. This study found a significant association between serum vit. D level and participants age at P value of (0.013) with highest deficiency among (30-39) years old, these results are in consistent with studies conducted in Kufa city 2018 [14] and in Duhok study [15]. On the other hand, Basrah [10], Qatar [12] and Oman [13] studies found serum vit. D level increased with increasing age. These variations in the studies results may be attributed to the sample size, subjects' age range, methods of assay use and other factors intersecting with each other's. In the current study, no significant correlation between serum vit. D and BMI was found, this is in accordance with results of previous studies in Kufa [14], Babil [16] and Oman [13], in contrast to other studies that found a significant inverse correlation in Baghdad [17], Qatar [12] and Spain [18]. Positive correlation was found between vit. D and BMI in studies from Basrah [10] and Iran [19]. On other hand, in this study, central obesity seems to play an important role in serum vit. D level as shown by the significant correlation between serum vit. D level and WHR, as with the increase of WHR, there was decrease in serum vit. D level. This may be explained by the fact that adipose tissue as it is a storage site for vit. D, sequester more vit. D as it is increased, limiting its serum availability. These results were consistent with previous studies conducted in Oman [13], Spain [18] and Boston [20], in contrast to other studies in Babil [16] and Iran [21], which found no association with WHR. Ultra violet light (UV) provided by sunlight is essential for endogenous vit. D synthesis produced by skin; outdoor sun exposure and physical activity important to increase vit. D concentration. In this study, results showed that vit. D deficiency was more among those not exposed to sunlight, and reporting sedentary lifestyle despite non-significant association, but was similar to previous studies that found a positive linear association between physical activity and sun exposure with vit. D level in Basra [10], Karbala [22], Qatar [12], and France [23]. Apparently, sun exposure and hijab wearing clothes were adherently related, vit. D level was lower in those wearing hijab, clearly for the same reason of low sun exposure. This finding was also reported in previous studies from Basra [10], Oman [13], and Iraq [24]. The later study from Iraq in 2016 also found that hand and face in hijab wearing participants exposed to sunlight is not enough for sufficient vit. D synthesis [24]. This current study found no association between smoking and vit. D level, in contrast to Basra study [10] that found an increase of vit. D level in smoking participants that was attributed to the outdoor activity of this group. In the current study, no association was found between dietary intake and vit. D, this agreed with study conducted in Jordan 2016 [24] while In korea 2017 [25] found that a positive association between dietary intake and vit. D concentration. In this current study, it was found that vit. D was significantly more deficient among carbonated beverages consumers (P≤0.001), this agreed with study in Canada 2014 [26] whom found that women with higher intake beverages had lower concentration of vit. D. carbonated beverages contain a higher concentration of fructose, which derived from corn syrup and used as sweetener. Fructose may have an effect on vit. D metabolism.

In conclusion, no association between vitamin D and body mass index was found. There is indirect correlation between vit. D and central obesity (WHR) was found. Highest deficiency of vit. D were among age group (30-39) years old

and among those who drinking carbonated beverages.

Authors recommend that multi-level educational strategies should be implemented to prevent and intervent in the modifiable risk factors for vit. D deficiency in the community as for adequate sun exposure and avoiding carbonated beverage. Also, enhancement of routine vit. D checking among risky groups in the community. In addition to promote people for achieving optimal weight through increasing physical activity and adopting healthy life style and foods.

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Author contribution

Dr. Abdulkader: collection of data, statistical analysis, interpretation and writing of manuscript. Dr. Al-Saffar: the research plan and study design, final revision of the manuscript.

Conflict of interest

The author declares no conflict of interest.

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Radiological and Clinical Results of Pemberton Osteotomy for Delayed Presentation of Iraqi DDH Patients

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Abstract

Background Pelvic osteotomy is a major procedure required for lately presented with developmental dysplasia of hip (DDH) patients; one of the needed osteotomies of the pelvis in such patients is Pemberton osteotomy (P.O.).

Objective To evaluate the clinical and radiological results of the P.O. for delayed presentation of Iraqi DDH patients.

Methods The study was conducted at Al-Imamein Al-Kadhimein Medical City from 2014-2020. We did the osteotomy for 32 hips of 25 patients (no one of them have neuromuscular disease). Of them 28 hips were Tönnis stage IV, 4 stage III, and 2 stage II.

Results The clinical status of all patients has improved according to modified McKay criteria, 26 of them have excellent score, 4 have good score, 2 with fair results, but no one has bad results. Radiologically, we score Severin's classification as 62.5% with excellent, 28.1% with good, 9.4% with fair stage, and zero for bad stage. The average acetabular index corrected from 41.34° into 16.17°. The center edge angle of wiberg reached 32.34°. The VCA of Lequesne became 29.66°. And the ACM angle became 43.33°. Avascular necrosis was reported as 1 hip was class I and 1 hip as class II according to Kalamchi McOwen classification.

Conclusion Although Salter R.B. when published his article about his osteotomy discussed the P.O. as one option for pelvic osteotomies meanwhile Pemberton P.A. still not publishing his article until after 4 years even it was practiced well before that time! We found that P.O. is very useful and dependable for clinical improvement and for correcting the radiological parameters to normal values yet it is simple, safe, practical, and effective osteotomy.

Keywords Pemberton osteotomy, delayed DDH, Tonnis classification, Severin's classification, modified McKay classification, Kalamchi McOwen classification

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List of abbreviations: ACM = Angle of acetabular depth, AI = Acetabular index, AVN = Avascular necrosis, CEA = Center edge angle, DDH = Developmental dysplasia of hip, VCA = Vertical center anterior acetabulum angle

Introduction

Management of Developmental dysplasia of hip (DDH) patients impose great challenge to the pediatric orthopedic surgeon at any age group,

but it is more challenging at the walking age and older [1,2]. At this age group, the open reduction for dislocated hips is mandatory and it could be so for subluxated ones immediately or after it becoming dislocated completely. After the age of 1.5 years, the growth and development of dysplastic hip becoming slow and may not get deeper and well covering unless pelvic osteotomy is done [3,4]. Many surgeons design and others modify a number of osteotomies; Pemberton osteotomy (P.O.) remains one of the older types, yet it still provides very good outcomes [5]. Unlike Salter who believed that the deficit in the anterior and lateral hip coverage is caused by excessive acetabular anteversion and hence its osteotomy re-orient the acetabulum into more retroverted position, Pemberton believe that the acetabulum is not retroverted but it is undergrown anteriorly and laterally and need not to re-orient but to augment the anterior and lateral coverage by re shaping the upper part of the acetabulum [6].

This study was made to evaluate the improvement achieved for DDH patients by pericapsular P.O. radiologically and clinically.

Methods

Thirty-two hips (25 patients) were treated at Al- Al-Imamein Al-Kadhimein Medical City from 2014-2020; of them 12 have bilateral DDH and 8 were unilateral with 3 males and 22 females. There ages ranges from 56 months (4 8/12 years) to 77 months (6 5/12 years) with average of 62 months (5 2/12 years). Of them 2 hips were grade II, 4 hips grade III, and 26 were grade IV according to Tonnis classification [7].

All of patients have had preoperative pelvic anteroposterior (AP) radiograph and validated for tilting and rotation for classification and measuring acetabular index (AI) [8,9]. The mean AI preoperatively was 41.34 (ranging from 30-46). All were prepared for general anesthesia with preparation of 1 pint of blood. All have had adductor muscles myotomy except 3 hips. All have had open reduction and P.O. at the same session. Twenty-seven (27) patients have had femoral shortening at the time of open reduction, and 10 have had derotation with the shortening. The rotation was judged clinically intraoperatively. In the first 6 cases the C-arm fluoroscope was used for performing the P.O.

All patients were put in one and half hip spica for 5-6 weeks after that a Petrie cast under general anesthesia was used for another 5-6 weeks, after that they were freely moving at home for another 2 weeks before starting official physiotherapy.

Postoperatively all patients received parenteral antibiotics for 5 days then oral antibiotic for another 5 days with regular oral analgesia for 5 days and parenteral analgesia on need. Stitches of the adductor myotomy were removed after 2 weeks and the stitches of the remaining wounds were removed with the removal of the hip spica under general anesthesia.

Postoperatively AP X-ray view was taken at the day of surgery, 6 weeks, after removal of Petrie cast (10-12 weeks), and at 16 weeks post operatively unless it is needed for some times to excludes fractures during physiotherapy. Then every 2 months for the first year and then at 1-year interval. A false lateral profile view was taken at the last interview. The average follow-up was 36.66 months (range from 8-68 months).

Results

All the 32 hips immediately post operatively achieved radiological reduction and the osteotomy seems maintaining its position (i.e., the bone graft not slipped away). The osteotomy is healed by the 2nd radiological examination (i.e., at the 6th week). Post operatively, the hips were 20 hips class IA, 9 hips class IB and 3 class II of modified Severin's criteria [10]. The average AI at the 1st X- ray examination was 19.23° (range from 11°-23°), and at the last interview was 16.17° (range from 7°-21°). The angle of acetabular depth (ACM), the center edge angle (CEA) of Wiberg, and vertical center anterior acetabulum angle (VCA) was recorded at the last interview [11-13]. The average ACM was 43.33° (range from 40°-52°). The average CEA of Wiberg was 32.34° (range from 25°-37°). The average VCA was 29.66° (range from 22°-34°). According to

modified McKay criteria [14], 26 achieved excellent results, 4 had good results, and 2 had fair results. No one had bad results (Figures 1 & 2).

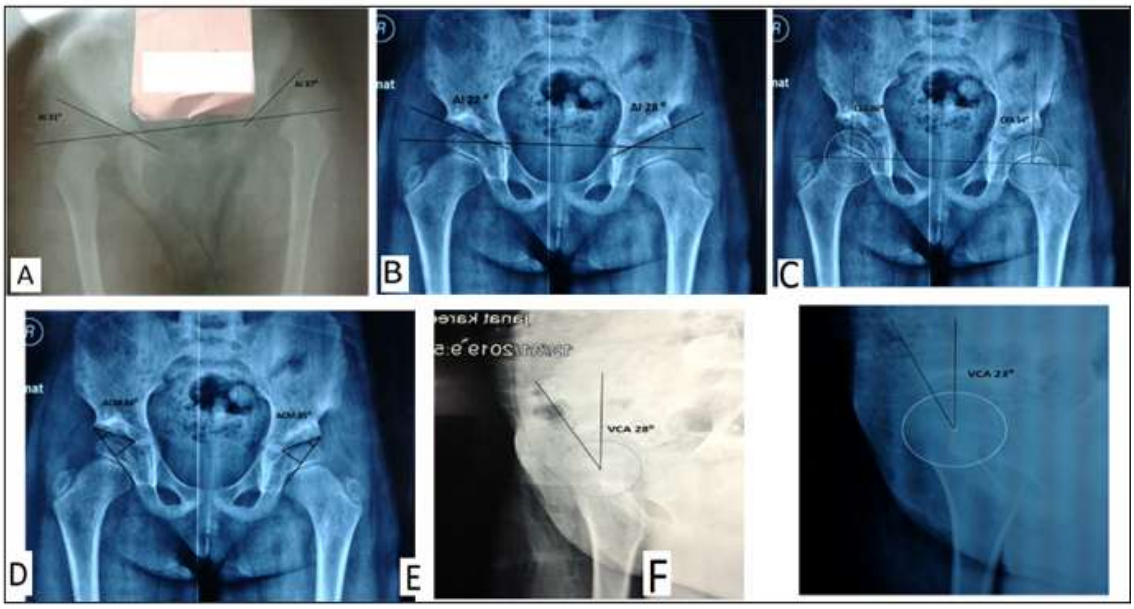

Figure 1. Patient of 4 3/12-year age at presentation with bilateral DDH. The left side had open reduction plus Salter osteotomy and the right side had open reduction plus Pemberton osteotomy. A: preoperatively the AI of right side is 31° and of left side is 37°. B: after 29 months post Pemberton osteotomy of the right side the AI is 22°; and on the left side, 36 months post Salter osteotomy, the AI is 28°. C: the right CEA is 26° and the left CEA is 14°. D: the right ACM is 36° and the left ACM is 35°. E: the right VCA is 28°. F: left VCA is 23°

Complications

No patient has infection of wound. Two patients have supracondylar femoral buckling greenstick fracture in one of their femurs during physiotherapy; one was treated by hip spica for 2 weeks and the second discovered after healing. Two hips in 2 patients had avascular necrosis (AVN); one was grade I and the other was grade II according to Kalamchi and McOwen classification [15].

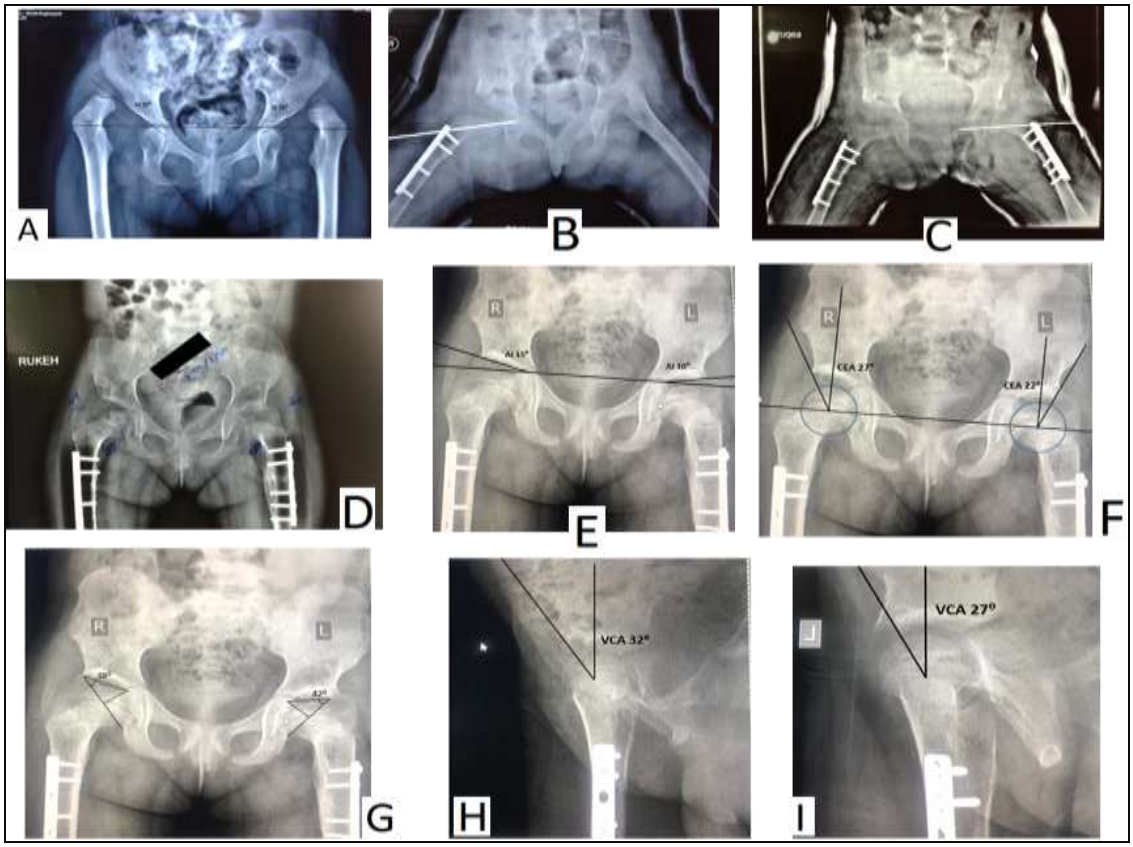

Figure 2. Patient of 4 8/12-year age at presentation with sever bilateral grade IV DDH with a well-defined false acetabulae. A: The AI of right and left hips are 39° and 39° respectively at the presentation. B: immediately post operatively of right hip open reduction + femoral shortening and derotation at the subtrochanteric region + Pemberton osteotomy. The reduction was held by K- wire for 6 weeks. C: the same was done for the left side. D: the patient 1 year after surgeries with concentric reduction, well developed acetabulae, and complete healing of the femoral osteotomies. E: 3 years after surgeries, the AP view of the hips have AI of right and left hips are 15° and 10° respectively. F: CEA right hip is 27° and 22° of the left hip. G: the ACM angle of the right hip is 38° and 42° of left one. H: the right VCA is 32°. I: the left VCA is 27°

Discussion

Pelvic osteotomy is the key for successful treatment of DDH patients older than 1.5 years [16,17]. In our experience, Salter osteotomy seems the most commonly used osteotomy in such patients [6]. Yet still other osteotomies like Pemberton, Shelf, and even Dega osteotomy are in use by some pediatric orthopedic surgeons [5]. Although Salter introduced his osteotomy (at 1961) 4 years after Pemberton osteotomy (at 1957), it gains more popularity at our pediatric orthopedic surgery society. Pemberton osteotomy needs relatively fewer soft tissues dissection around the sciatic notch thus, decreasing blood lose and avoiding violation of the structures passing through it, especially if the C-arm device is used to determine the limits of posterior limb of cutting in the outer and inner ilium tables. Also, the omitting of using Gigli saw (which is used in Salter osteotomy) will reduce the time and complications of saw jamming during cutting the innominate bone. In Pemberton osteotomy, we can direct the coverage whether anteriorly or laterally according to patient's acetabulum deficit. This versatility of Pemberton osteotomy in directing the coverage, also present in the magnitude in

reducing AI angle, which may reach to zero or even much which can't be done in Salter osteotomy without jeopardizing posterior hip stability. One of the advantages of Pemberton osteotomy is the stability of it of holding the bone graft without the need of fixation and so omitting the need for another surgery for hardware removal under anesthesia. In Pemberton osteotomy, we are augment the anterior +/- lateral acetabular deficiency thus, increasing the acetabulum retroversion and improving the acetabulum encompassing around femoral head without affecting posterior stability. Although some texts entail a relatively large acetabulum and small femoral head as a prerequisite for Pemberton osteotomy [(18)], however, Pemberton himself didn't says this as a primary requirement for his pericapsular osteotomy, even more he did it in patient who have hip subluxation without performing open reduction i.e., a primary pelvic osteotomy in hip dysplasia as a sole surgical procedure. Although the Pemberton osteotomy will reduce the circumference of acetabulum margin, actually it increases the acetabulum volume by increasing its depth and by this it makes the hip even more stable.

Many authors evaluate the Pemberton pericapsular osteotomy and compare the results with other osteotomies regarding the clinical and some radiological results especially on anteroposterior views to evaluate the ability of the osteotomy in restoring the normal architecture and appearance of the acetabulum in DDH patients in coronal view [(19-24)]. Kessler et al. have a mean postoperative AI of 18°, versus the preoperative mean of 33° (with 15 gain) [(25)]. Mehmet et al. have 22.42 gain in AI [(26)]. Badrinath et al. found 14.2 gain in AI [(19)]. Gordon et al. in their study estimated the CEA as 28° [(27)]. Aydin et al. found a preoperative mean AI of 40.53 and final AI of 12.04°, (gain of 28.49) and a mean CEA of 35.5° [(28)].

To our knowledge, only recently Wang et al. study, the anterior coverage of the osteotomy radiologically in some details where he has VCA of 43° [(29)], (normal value of VCA is more than 25°). The table 1 shows the results of some studies that evaluate the results of Pemberton osteotomy.

It is clear that there are some differences in the methods in evaluating DDH patients preoperatively and postoperatively (like preoperative classification and the postoperative results). Also, because of the great differences in the results of the same issue like AVN, we can see clearly the wide range of differences in the results of these studies or articles [(30,31)]. And because of the wide range of parameters in patients with DDH (like age at surgery, preoperative methods of treatment such as traction or splintage, gender, uni or bilaterally hips, and the need of other procedures such as femoral surgeries, all of these can make the results very variable for these studies and articles. So, we need to unify the patients in very similar criteria and make studies for groups of patients with very similar associated pre and postoperative conditions.

The results in the current study in regard to clinical finding seems to be close to other studies that evaluate Pemberton osteotomy elsewhere. The measured parameters on the AP and false profile of hip in the current study reached the patient with the operated hips to the normal values in the normal population [(32,33)].

The good radiological parameters approaching the normal figures with good clinical patient performance plus the relative surgical simplicity of Pemberton osteotomy and its many advantages, we are encouraging pediatric orthopedic surgeons to practice it in their work and popularize it. At the same time, it needs much more extensive and longer follow up studies to delineate its validity and its scope in treating DDH patients at different age groups.

Table 1. Summary of some studies with their results compared with our current one

		Balioğlu et al (26)	Badrinath et al (19)	Aydin et al (28)	Ertürk et al (23)	Current study
Duration		2001 - 2006	2006 -2015		2001 – 2009	2014 – 2020
Number		14	38	91	50	32
Tonnis classification	I	-	-	-	-	0 (0.0%)
	II	5 pt	-	24.2 %	-	2 (6.2%)
	III	-	-	41.8 %	12 pt	4 (12.5%)
	IV	9 pt	-	34.1 %	38 pt	28 (81.2%)
Severin's classification	ex	78.57% Ia	92 %	86.8 %	-	20 (62.5%)
	good	7.14% Ib	5 %	7.7 %	-	9 (28.1%)
	fair	7.14% II	3 %	5.5 %	-	3 (9.4%)
	bad	7.14% III	0 %	0 %	-	0 (0.0%)
AI	Pre	41.92	24.3	40.53	40.21	41.34
	post	19.5	10.1	12.04	20.11	16.17
	Gain	22.42	14.2	28.49	20.1	25.17
CEA		24.24	33.9	35.5	31.01	32.34
VCA		-	-	-	-	29.66
ACM		-	-	-	-	43.33
McKay staging	v.g	92.85%	63 %	89 %	-	26 (81.3%)
	good	7.14%	24 %	2.2 %	-	4 (12.5%)
	fair	-	0 %	8.8 %	-	2 (6.2%)
	poor	-	5 %	0 %	-	0 (0.0%)
Kalamchi-McEwen classification	I	85.71%	5 %	9.9 %	-	1 (3.0%)
	II	14.28%	0 %	7.7 %	-	1 (3.0%)
	III	-	3 %	1.1 %	-	0 (0.0%)
	IV	-	0 %	0 %	-	0 (0.0%)

ACM = Angle of acetabular depth, AI = Acetabular index, CEA = Center edge angle, VCA = Vertical center anterior acetabulum angle, ex = excellent, v.g = very good

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Author contribution
The patients were operated on and followed for their outcome by Dr. Chhaily, Dr. Joda, and Dr. Hafith. Research conduction, statistical analysis done by Dr. Chhaily, Dr. Abbood, and Dr. Joda.

Conflict of interest
There was no conflict of interest.

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Assessment of Plasma Level of CTHRC-I in Patients with Rheumatoid Arthritis

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Abstract

Background Rheumatoid arthritis (RA) is a chronic progressive, autoimmune disease that affects about 1.5% of the community. New markers are needed for early diagnosis of RA as seronegativity in early RA remains a major limitation of both anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF).

Objective To measure the plasma levels of the collagen triple helix, repeat containing-1 (CTHRC-1) protein in RA patients.

Methods 103 RA patients (56 new diagnostic without treatment group and 47 patients on treatment were included in this study according to American College of Rheumatology (ACR) criteria, in addition to 25 subjects as healthy control group. CTHRC-1 level was measured by using Immunoassay System in plasma samples.

Results The mean and SD of CTHRC1 was (49.10±6.51 ng/ml) in RA patients was significantly higher than its mean in healthy controls (6.20±2.81 ng/ml), (p value =0.002). The distribution of CTHRC1 was insignificantly associated with patient treated or not (P value 1.000, 0.273) respectively, or were the patients had positive or negative RF (P value 0.118, 1.000) respectively.

Conclusion Using the Immunoassay System for CTHRC-1 quantification, CTHRC1 could be used as a plasma marker that can aid in the diagnosis of RA although it has no association with seropositivity with RF or treatment.

Keywords Collagen triple helix repeat containing-1, rheumatoid arthritis, serum rheumatoid factor, anti-CCP

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List of abbreviations: Anti-CCP = Anti cyclic citrullinated peptide, CRP = C-reactive protein, CTHRC1 = Collagen triple helix repeat containing 1 protein, ESR = Erythrocyte sedimentation rate, RA = Rheumatoid arthritis, RF = Rheumatoid factor

Introduction

The collagen triple helix repeat containing 1 protein (CTHRC1) is a veiled modulator of signaling, which is a key controller of joint remodeling, it promotes cell proliferation and immigration [(1)]. CTHRC1 that showed expression in murine investigational arthritis is increased in the synovium. These monoclonal antibodies have very little cross-reactivity with the skeletal isoforms [(2)]. CTHRC1 very sensitive and specific indicator of myocardial damage [(3)]; their assays have been beset by some analytical problems [(4)]. Others suggest that this CTHRC1 is not affected by rheumatoid factor (RF) [(1)].

This study aimed to evaluate the benefit of plasma CTHRC1 as a marker that help in diagnosis of rheumatoid arthritis disease (RA) disease and could be a marker for difference between seropositive and seronegative RA, and their correlation with other markers.

Methods

Data were collected during the period from March 2020 to September 2020. This study included (103) RA patients of less than 1 year duration that were classified to new diagnostic without treatment group (56 patients) and early treated group (47 patients), in addition to (25 healthy control group). Their ages were > 18 years. All recruited patients were seen in Rheumatology Outpatients Clinic in Teaching Baghdad Hospital in Baghdad. The diagnosis of RA was according to the American College of Rheumatology criteria for RA [(5)]. Venous blood specimens (5 ml) were collected from each subject; 3 ml of each sample for serum. Serum CTHRC1 was detected by using the Immunoassay System (VEDALAB Inc, 28091 cat. number, France). The RF IgG concentrations in both healthy controls and patients were measured on the Immunoassay System (Chorus Inc, 86038 cat. number, Italy), (Chorus Anti-CCP device, 86094 cat. number, Italy) of Anti-CCP kit and (AGAPPE, 52009002 cat. number, Switzerland) of CRP kit.

Statistical analysis

Statistical analysis was performed using the SPSS 10 statistical pack up. Mean and standard deviation (SD) were used to express variables. Unpaired t-test was used to compare the difference in mean between two continuous variables, differences were considered statistically significant at $P<0.05$. For comparing more than two groups, the one-way ANOVA method was used to determine if there is statistical significance across the groups or not. Receiver-operating characteristic (ROC) curves were used to evaluate the diagnostic utility of marker as estimated by the area under the curve (AUC). [(6)].

Results

Demographical and serological data of study groups

Table (1) shows demographical characteristics of patients and controls. There was no significant difference in mean age between patients and controls (42.22±11.23, 36.4±11.15 years) respectively; p value =0.126). While there was significant difference in the sex distribution, although majority of patients and controls were females but males were more in patients than in control (p value =0.030). In patients, 29.1% have positive family history of RA which significant different from controls were none of them have (p value =0.040).

Table 1. Demographical and serological data of study groups

Parameters		Patients (No. = 103)	Control (No. = 25)	P value
Age (Yrs)	Mean±SD	42.22± 11.23	36.40±11.15	0.126
Sex No. (%)	Female	77 (74.8)	22 (88.0)	0.030
	Male	26 (25.2)	3 (12.0)	
Disease Duration (Months) No. (%)	(1-6)	46 (44.7)	---	0.003
	(7-12)	57 (55.3)		
Family History No.	No	73 (70.9)	25 (100)	0.04
	Yes	30 (29.1)	0 (0.0)	
	Negative	36 (35.0)	25 (100)	

Serological data were illustrated in table 2 were the difference between patients and controls were highly significant as majority of RA patients had elevated ESR (79.62%), positive CRP (86.4%), positive Anti-CCP (91.3%), and (65.0%) of them had positive RF while only 8% of controls has elevated ESR, and none of them had positive CRP, Anti-CCP or RF (p value 0.001, <0.001, 0.001 and 0.001) respectively.

Table 2. Serological data of study groups

Parameters		Patients (No. = 103)	Control (No. = 25)	P value
ESR	Elevated	82 (79.62)	2 (8.0)	0.001
No. (%)	Normal	21 (20.40)	23 (92.0)	
CRP	Positive	89 (86.4)	0 (0.0)	<0.001
No. (%)	Negative	14 (13.6)	25 (100)	
Anti-CCP	Positive	94 (91.3)	0 (0.0)	0.001
No. (%)	Negative	09 (08.7)	25 (100)	
RF	Positive	67 (65.0)	0 (0.0)	0.001
No. (%)	Negative	36 (35.0)	25 (100)	

ESR: Normal value for female =0-20 mm/h, Normal value for male = 0-9 mm/h, CRP: Positive value (more than 6 mg/l) in serum

The mean and SD of CTHRC1 was (49.10±6.51 ng/ml) in RA patients and (6.20±2.81 ng/ml) in healthy control group, the difference was significant (p value =0.002) (Table 3).

Table 3. Comparison of Collagen triple helix repeat containing 1 protein (CTHRC1) between patients and controls

Parameter		Patients (No. = 103)	Control (No. = 25)	P value
CTHRC1 (ng/ml)	Mean±SD	49.10±6.51	6.20±2.81	0.002

Distribution of CTHRC1 marker according treatment and seropositivity of RF

Table (4) shows association of "CTHRC1" marker with treatment of patients and their seropositivity for RF patients. It showed that there is no significant association of CTHRC1 distribution whether the patients were treated or not (P value 1.000, 0.273) respectively, or were the patients had positive or negative RF (P value 0.118, 1.000) respectively.

Table 4. CTHRC1 marker distribution according to RF Patients (treated and Untreated (positive and negative) with comparisons significant

RF Groups	CTHRC1	No. & %	Treatment		Total	P value
			Untreated Patients	Treated Patients		
RF ±ve	Positive	No.	2 (33.3)	8 (80.0)	10 (62.5)	0.118 NS
	Negative	No.	4 (66.7)	2 (20.0)	6 (37.5)	
	Total	No.	6 (100)	10 (100)	16 (100)	
RF-ve	Positive	No.	3 (50.0)	0 (0.0)	3 (42.9)	1.000 NS
	Negative	No.	3 (50.0)	1 (100)	4 (57.1)	
	Total	No.	6 (100)	1 (100)	7 (100)	
P value			1.000 NS	0.273 NS		

NS: Non-significant at P>0.05

Discussion

There is an unmet need for specific and easy-to-measure biomarkers to diagnose RA patients and distinguish patients with high disease activity who are at increased hazard of developing erosive, joint destructive disease.

The present study revealed that prevalence of RA was more in female patients than male patients similar to result in 2014 showed (75.25% vs 24.75%, respectively) [(7)]. Few studies have shown opposite result more frequent in men than in women (72% versus 55%, respectively) [(8)].

In present study, the percentage of patients had 7-12 months of disease duration more than the 1-6 months of patients' groups, while study reported the patients fulfilling the American College of Rheumatology (ACR) criteria at presentation, 53% with disease duration of ≤3 months, compared with 94% of patients who presented with disease duration of >12 weeks, therefore, the strongest predictor of persistent disease was a disease duration of >3 months [(9)]. Although family history of RA is an old concept [(10)], present data appeared patients had family history less than those with no family history of RA. Somers and his colleagues reported a high rate of RA in female offspring with a maternal history of RA [(11)].

Early diagnosis of RA is important for preventing joint damage via treatment. For patients having typical symptoms, the disease could be easily diagnosed, often in the first year of disease onset. For many patients with atypical symptoms, it could take more time to diagnose. Consequently, specific and sensitive serological tests are required for diagnosis. Furthermore, ESR and CRP with high positive results, although they are nonspecific for the diagnosis of RA, but they are important assisting markers for the diagnosis of RA. Simultaneous finding of RF, Anti-CCP, CRP and ESR is helpful for the confirmed diagnosis of RA [(12)]. Patients that had positive RF and Anti-CCP more than with negative test results, recently the new criteria for diagnosis of RA have been introduced in addition to Anti-CCP, together with RF [(13)]. Thus, these parameters could be used as specific serologic markers for RA. It is showed that Anti-CCP have the power to predict the development of RA in patients with early arthritis, and the possibility of future onset of RA in certain high-risk populations [(14)].

The most important finding of this study was that concentrations of high sensitivity, CTHRC1 were higher in patients with RA compared to controls. The difference remained statistically significant after adjustment for demographic characteristics (P=0.002). Other results reported that patients with seropositive RA do not have falsely elevated CTHRC1 levels [(1)].

Current study found that neither seropositivity for RF nor treatment of patients have significant effect on CTHRC1. This finding agree with a study that reported no significant

association between CTHRC1 level and inflammatory markers, acute phase reactants. This suggests that active inflammation may not be the primary driver of CTHRC1 elevation in RA [15].

In conclusion, CTHRC1 may be plasma marker that can aid in the diagnosis of RA.

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Author contribution

Dr. Alwan: collection of data, interpretation and writing of manuscript. Abdul-Ridha and Ali: final revision of the manuscript.

Conflict of interest

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Comparative Study between Obstructive and Non-Obstructive Renal Anomalies Among a Group of Iraqi Children

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Abstract

Background Congenital anomalies of the kidney and the urinary tract (CAKUTs) are relatively common birth defects and account for 40-50% of the etiology of chronic kidney disease (CKD) in children worldwide.

Objective To study the types of renal anomalies (RA) and to compare between obstructive and non-obstructive RA in relation to demographic data and complications.

Methods A descriptive study conducted in Al Imamein Kadhimein Medical City spanning the period from the 1st of February 2020, to the 30th of July 2020. Data collected were: type of renal anomaly, age of diagnosis, family history of RA, consanguinity, clinical presentation and associated complications.

Results In this study, 160 patients were included. Males were more affected than females 1.54:1. Most of the patients, 78 (48.8%), diagnosed were ≤5 age group. Most of the patients, 124 (77.5%), had negative family history, vesicoureteral reflux (VUR) was the commonest anomaly detected in 67 patients (41.9%), followed by renal agenesis in 24 patients (15.0%). Urinary symptoms were most common presentation in 93 patients (58.1%). A higher complication was urinary tract infection (UTI) (62.5%). Comparison between obstructive and non-obstructive RA, reveled age of diagnosis and hydronephrosis has significant difference. While family history, UTI, failure to thrive, and chronic kidney disease stages was not significant.

Conclusion The commonest renal anomaly was VUR, the most prominent complication was UTI. Gender, age of diagnosis and hydronephrosis has significant difference between obstructive and non-obstructive RA.

Keywords Renal, anomalies, children

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List of abbreviations: CAKUT = Congenital anomalies of the kidney and the urinary tract, CKD = Chronic kidney disease, ESRD = End-stage renal disease, FTT = failure to thrive, GFR = Glomerular filtration rate, HN = Hydronephrosis, MCDK = Multicystic dysplastic kidney, PUJ = Ureteropelvic junction obstruction, PUV = Posterior urethral valve, RA = Renal anomalies, UTI = Urinary tract infection, VUR = Vesicoureteral reflux

Introduction

Congenital anomalies of the kidney and the urinary tract (CAKUTs) are relatively common birth defects observed in 3-6 per 1,000 live births and account for 40-50% of the etiology of chronic kidney disease (CKD) in children worldwide [1-2].

Many different developmental abnormalities are classified as CAKUT, including underdevelopment or absence of a kidney (renal hypodysplasia or agenesis), a kidney formed of fluid-filled sacs called cysts {multicystic dysplastic kidney (MCDK)}, swelling

of the kidney resulting from the inability of urine to drain from the kidney into the bladder {hydronephrosis (HN)}, an extra ureter leading to the kidney (duplex kidney or duplicated collecting system), a blockage in a ureter where it joins the kidney {ureteropelvic junction obstruction (PUJ)}, an abnormally wide ureter (megaureter), backflow of urine from the bladder into the ureter {vesicoureteral reflux (VUR)), and an abnormal membrane in the urethra that blocks the flow of urine out of the bladder {posterior urethral valve (PUV)} [3].

The genes most commonly associated with isolated CAKUT are PAX2, which is also associated with renal coloboma syndrome. [4,5].

Inheritance of CAKUT is complex and not completely understood. About 10-20% of cases are thought to occur in families. When inherited, CAKUT most commonly follows an autosomal dominant pattern, less commonly follows an autosomal recessive pattern, while in many cases, the inheritance pattern is unknown or the condition is not inherited [6,7].

It is important to diagnose these anomalies early and initiate therapy to minimize renal damage, prevent or delay the onset of end stage renal disease (ESRD), and provide supportive care to avoid complications of ESRD [8].

This research aimed to study the types of renal anomalies (RA) in relation to clinical presentations, age of diagnosis, family history and complications and to study the difference between obstructive and non-obstructive types of RA according to demographic data and complications.

Methods

This descriptive study included 160 children with RA who were recruited at Pediatric Nephrology Clinics of Al Imamein Al-Kadhimein Medical City, Central Child Teaching Hospital, Child Wellfare Teaching Hospital and Karbala Pediatric Teaching Hospital spanning the period from the 1st of February 2020 to the 30th of July 2020.

A direct interview with the patients themselves or their parents, also review of patient's files; collecting the following data: type of renal anomaly, age of diagnosis, family history of renal anomalies, consanguinity, clinical presentation and associated complications.

Height and weight measurements for all patients was done. The following investigations: renal function test (blood urea, serum creatinine), urinalysis and urine culture, in addition to prenatal ultrasound.

The patients were categorized into two main groups:

Group 1: Obstructive anomalies including PUV, PUJ, VUJ, ureterocele, duplex system, megaureter.

Group 2: Non-obstructive anomalies including VUR, renal agenesis, MCDK, polycystic kidney diseases, renal hypoplasia, ectopic kidney, horse-shoe kidney, ectopic ureter.

Of the 160 studied patients with RA, 23 patients of them had combined renal anomalies; 14 patients out of these 23 had combined obstructive and non-obstructive, accordingly these 14 patients were excluded from comparison of obstructive and non-obstructive to avoid fallacies.

Urinary tract infection (UTI) was considered positive when patient had symptoms and findings on urinalysis, confirmed by a urine culture [9,10]. The diagnosis of failure to thrive (FTT) is considered if a child's weight is below the 5th percentile [11].

Reference values for normal blood urea and serum creatinine according to age was applied. [12]. Stages of CKD, was categorized according to estimated glomerular filtration rate (GFR) based on serum creatinine using Schwartz formula [13].

The study has been conducted in accordance to the terms of the code of ethics in research of Ministry of Health in Iraq and The Iraqi board of Medical Specialty Ethics Committee.

Statistical analysis

The data analyzed by the statistical package for social sciences (SPSS-version 23) and Microsoft

office Excel programs (2013) and Graph Pad Prism (6). Most of data were presented as frequency and percentage except for age of presentation and age of diagnosis which expressed as mean±standard deviation. Fisher exact test and Yates' chi square test was used to compare between frequencies, while unpaired t-test was used to compare between means. P value <0.05 was considered statistically significant and highly significant if <0.001.

Results

Age at time of presentation was ranged between 1 day to 16 years, and the mean was 47.07±49.89 months, while the age at time of diagnosis was ranged between prenatal diagnosis to 13 years old with a mean 31.36± 37.44 months. The most frequent age group at time of presentation was 1-5 years age group including 84 patients (52.5%). The most frequent age group at time of diagnosis also was 1-5 years including 78 patients (48.8%), followed by below one year of 48 patients (30.0%), including 13 patients diagnosed prenatally. There were 97 males (60.6%) and 63 females (39.4%) with male:female ratio 1.54:1. Most of the patients had negative family history 124 (77.5%), and most of their parents were not consanguineous (58.1%) (Table 1).

Table 1. Distribution of 160 Patients according to demographic data

Parameter		No.	%
Age at presentation (yr)	<1	41	25.6
	1-5	84	52.5
	5-10	27	16.9
	>10	8	5.0
Age at diagnosis (yr)	Prenatal Dx	13	8.1
	<1	48	30.0
	1-5	78	48.8
	5-10	19	11.9
	>10	2	1.2
Sex	Male	97	60.6
	Female	63	39.4
	M:F ratio = 1.54:1		
Family history	Positive	36	22.5
	Negative	124	77.5
Consanguinity	Positive	67	41.9
	Negative	93	58.1

Urinary symptoms were most common presented in 93 patients (58.1%), followed by fever detected in 77 patients (48.1%). UTI was the most frequent complication accounted for (62.5%), followed by renal impairment (34.4%) (Table 2).

Among the study group, 183 renal anomalies were detected: 23 patients have combined renal anomalies. VUR was the most frequent seen in 67 patients (41.9%), 15 patients with VUR were associated with neurogenic bladder. Second rank was for renal agenesis in 24 patients (15.0%) (Table 3).

Table 4 shows that from total of 160 patients, 23 of them had combined RA, 14 patients out these 23 had combined obstructive and non-

obstructive RA, these 14 patients were excluded from comparison to avoid fallacies. The other 9 patients were involved, because they have either combined obstructive or combined non-obstructive RA, so regarded as same category. Accordingly, we have 146 patients for comparison. Regarding gender; most of those having obstructive RA were males 29/33 patients (87.9%), while only 60/113 patients (53.1%) of non-obstructive RA were males, this difference is highly significant P-value 0.0002. Regarding the age of diagnosis: most of the patients that had obstructive RA 23/33 (69.7%) diagnosed at or below 1 year, while most of the patients 66/113 (58.4%) of non-obstructive RA diagnosed at 1-5 age group, this difference had statistical highly significant P-value 0.0001. Seven patients of non-obstructive RA 7/113 (6.2%), and 5 patients out of 33 (15.2%) of obstructive RA diagnosed prenatally. Most of the patients with non-obstructive and obstructive RA had negative family history, (77.9%, 66.7%) respectively, P-value was not significant 0.6186.

Table 2. Distribution of 160 patients according to clinical presentation and associated complications

Clinical presentation	No.	%
Urinary symptoms	93	58.1
Fever	77	48.1
Abdominal symptoms	41	25.6
Acute kidney injury	15	11.9
Prenatal	13	9.4
Accidentally diagnosed	11	6.9
Improper weight or height gain	10	6.3
Complications	**No.**	**%**
Urinary tract infection	100	62.5
Renal impairment	55	34.4
Failure to thrive	38	23.8
Stones	15	9.4

*Patients may have more than one clinical presentation and more than one complication

Comparison between obstructive and non-obstructive RA in relation to complications as shown in table (5). UTI was seen in 74 patients (65.5%) non-obstructive RA and 20 patients (60.6%) with obstructive RA, p-value was statistically insignificant. Failure to thrive had insignificant difference between the 2 groups. Twenty-six (78.8%) of the obstructive patients' group had HN with only 35 patients (30.9%) of non-obstructive RA had HN, P-value was highly significant <0.0001.

CKD stage 1 was the commonest in 30 patients (20.6%), stage 1,2,3 mainly non-obstructive (22.1%), (4.4%) respectively, while stage 4 mainly obstructive (6.7%), the p-values was insignificant for CKD 0.6473.

Regarding ESRD: nearly equal figures of 8 patients (7.1%) non-obstructive RA, and 3 patients (9.1%) obstructive RA, P-value was not significant 0.7119.

Table 3. Patients' distribution according to types of renal anomalies

Anomaly	No.	%
Vesicoureteral reflux **	67	41.88
Renal agenesis (single kidney)	24	15.00
Ureteropelvic junction obstruction	20	12.50
Posterior urethral valve	12	7.50
Multicystic dysplastic kidney	11	6.88
Polycystic kidney	10	6.25
Renal Hypoplasia	8	5.00
Ectopic kidney	7	4.38
Uretocele	6	3.75
Vesicourethral junction obstruction	6	3.75
Duplex system	6	3.75
Others***	6	3.75

Number is 183, includes 23 patients combined anomalies. ** 15 patients with VUR were associated with Neurogenic bladder. *** Others includes ectopic ureter, Horse-shoe kidney, Megaureter

Table 4. Comparison between obstructive and non-obstructive Renal anomalies in relation to demographic data

Data		Non-Obstructive No.113 No. (%)	Obstructive No.33 No. (%)	Total 146 No. (%)	P value
Gender	Male	60 (53.1)	29 (87.9)	89 (61.0)	0.0002
	Female	53 (46.9)	4 (12.1)	57 (39.0)	
Age of diagnosis	<1	32 (28.3)	23 (69.7)	55 (37.7)	0.0001
	1-5	66 (58.4)	6 (18.2)	72 (49.3)	
	5-10	13 (11.5)	4 (12.1)	17 (11.6)	
	≥10	2 (1.8)	0 (0.0)	2 (1.4)	
Family history	Positive	29 (25.7)	5 (15.2)	34 (23.4)	0.6186
	Negative	88 (77.9)	22 (66.7)	110 (76.7)	

Discussion

In this descriptive study of CAKUT, male predominance (60.6%) was evident with an approximate male/female ratio 1.54:1. A population-based case-control study in Taiwan showed that males had a 1.83-fold greater risk of CAKUT than females as show in Tain *et al.* study [14], Katsoufis *et al*. study show 1.15:1 [15], which are nearly similar to our study. while in Turkish study by Bulum *et al.* in 2013 [16] showed a female predominance, M/F ratio 0:84, which is disagreed with current study, this was due to selection of patients of CAKUT in relative families only.

Authors noticed a considerable delay between the age of presentation (mean 47.07±49.89 months) and that of diagnosis (a mean 31.36±37.44 months), about 1.5 year, which occurred due to fact that the study was done in tertiary centers and patients had seen by different medical centers before referral. During comparison with Iraqi study by Hasoon in 2009 [17] we noticed there is nearly similar gap between age of presentation and age of diagnosis (about 1 year).

Table 5. Comparison between obstructive and non-obstructive Renal anomalies in relation to complications

		Non-Obstructive No.113 No. (%)	Obstructive No.33 No. (%)	Total 146 No. (%)	P value
UTI		74 (65.5)	20 (60.6)	94 (64.4)	0.6806
Failure to thrive		30 (26.5)	4 (12.1)	34 (23.3)	0.1033
Hydronephrosis		35 (30.9)	26 (78.8)	61 (41.8)	< 0.0001
Chronic kidney disease stages	I	25 (22.1)	5 (15.1)	30 (20.6)	0.6473
	II-III	5 (4.4)	1 (3.1)	6 (4.1)	
	IV	6 (5.3)	2 (6.7)	8 (5.5)	
End-stage renal disease		8 (7.1)	3 (9.1)	11 (7.6)	0.7119

Prenatal diagnosis was done for only (8.1%) of the patients, while in Gomez Huertas *et al*. study, fetal anomalies encountered approximately 30-50% of all malformations [18], this difference reflects our limited delay experience in prenatal diagnosis.

Positive family history found in 36 patients (22.5%), in Weber study, family history is identified in 10- 50% of affected children [7].

Positive consanguinity was seen in 67 patients (41.9%), which is nearly similar to Egyptian study 49.5% by Soliman et al. [19]. This reflects the high rate of consanguineous marriage in Arab population.

Regarding clinical presentation(s) of the study patients, urinary symptoms were the commonest (58.1%), followed by fever (48.1%), In Aksu et al. study, a significant number of children had low eGFR or hypertension at presentation [20].

UTI was the commonest complication (62.5%), this is similar to Capone study [3].

Different CAKUT have been identified, of which VUR was the commonest abnormality (41.9%), followed by renal agenesis (15.0%), then PUJ (12.5%). Soliman et al. [19] reported PUV (36.4%) as the commonest followed by primary VUR (19.6%) and PUJ followed by VUR in (18.7%). Aksu et al. [20] reported PUJO in (62.7%). This might be related to inclusion of patients from urology department in these studies.

During comparison of non-obstructive and obstructive RA in relation to demographic data, we notice that obstructive RA has male predominance, which is similar to Egyptian study by Soliman et al. 2015 (80.3%) [19]. Regarding age of diagnosis, most of the obstructive RA patients (69.7%) diagnosed at or below 1 year, while most of the patients of non-obstructive RA diagnosed at 1-5 age group (58.4%), this had statistical highly significant P-value 0.0001. Common obstructive RA like PUV presented early in life that led to earlier diagnosis [21,22]. Family history was of no significance statistically. A familial clustering of VUR has been described with a prevalence of 27-51% in siblings of patients with VUR and a 66% rate of VUR in children whose parents had reflux [23,24].

There was no statistically significant difference in the UTI, FTT and ESRD. This was similar to Egyptian study by Soliman et al. in 2015 [19]. But there was significant difference in HN between obstructive and non-obstructive RA, as (78.8%) of obstructive RA had HN, while only (30.9%) of non-obstructive RA had HN similar to study by Rasouly and Lu [25].

For the stages of CKD: compared to Egyptian study; obstructive group patients had advanced CKD stages (I: 45.5%, II–III: 22.7%, IV: 31.8%) in comparison to the non-obstructive group (I: 62.9%, II–III: 31.4%, IV: 5.7%) (P = 0.021). This difference might be due to high percent of PUVs in Egyptian study [19].

In conclusion, the commonest renal anomaly was VUR, followed by Renal agenesis, then PUJ. Most common presentation was urinary

symptoms. The most prominent complication was UTI. Gender, age of diagnosis and HN has significant difference between obstructive and non-obstructive RA.

Improving antenatal diagnosis, collaborative management between the obstetrician, the pediatric nephrologist and the urologist in order to provide optimal care for children with renal anomalies.

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Author contribution

Dr Ali: Concept of the article, collection of patients, data analysis, final supervision. Dr Kadhim: Data analysis, writing the parts of the article. Dr. Al-Obaidy: Concept of the article, collection of patients.

Conflict of interest

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Post-Cholecystectomy Pain Assessment Using Visual Analogue Scale and Numerical Rating Scale

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Abstract

Background Pain defines as unpleasant sensory and emotional experience associated with actual or potential tissue damage. Postoperative pain should be assessed regularly and documented carefully as it is a significant part of postoperative care. Pain scales are useful for assessment and monitoring the effectiveness of treatment. The 100 mm visual analogue scale (VAS) and the numerical rating scale (NRS) are the most commonly used one.

Objective To compare between VAS and NRS for assessing post-operative pain in patients undergoing elective cholecystectomy.

Methods A cross-sectional study was done in Al-Imamein Al-Kadhimein Medical City from the period of 1st of October 2019 to the 1st of March 2020. Fifty patients were enrolled in this study. All underwent elective cholecystectomy. Patient consents were obtained. Pain assessment was done when patients fully recovered and asking for pain relief and then one hour after giving analgesia.

Results Of the 50 patients, females were 42 (84%). Laparoscopic cholecystectomy has been done for 45 (90%); 33 (66%) patients received parenteral opioid and paracetamol, the remaining 17 (34%) received only paracetamol. There were no significant correlations between most of the suggested risk factors and pain perception apart from type of medications used for pain control. There is strong correlation between NRS and VAS before and after analgesia (P< 0.01), while no observed significant effect or relation between other demographic and surgical parameters on pain score rating.

Conclusion This study validated that both the NRS and the VAS are two comparable acute pain scores mostly used in practice. NRS, which is easy, less pain inducing and more user friendly in the post-operative period has a strong linear association with VAS, thus can be substituted for VAS in assessment of postoperative pain.

Keywords Pain, cholecystectomy, Visual analogue scale, numerical rating scale

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List of abbreviations: NRS = Numerical rating scale, r = Correlation coefficient, Tx = Treatment, VAS = Visual analogue scale

Introduction

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [1].

Pain should be assessed regularly and documented carefully as it is a significant part of postoperative care according to the American Pain Society Guidelines [2].

Pain scales are useful for assessment of the severity of this noxious feeling and to monitoring response and effectiveness of analgesia, of these, Visual Analogue Scale (VAS) and Numerical Rating Scale (NRS) are the two

scores widely used for acute pain assessment. Although VAS is recognized as most appropriate one, it is relatively complex and uncomfortable compared to NRS especially in the early postoperative period as the patient have to move and to put a mark on the VAS sheet, while they only have to say a number in a few second in case of NRS [3].

Visual analogue scale (VAS)

It is a horizontal line (usually 100 mm long) anchored on either end by the terms "no pain" or "worst pain imaginable". The patients are asked to make a mark on the line that represent how much pain they have and the score is obtained by measuring from the low end of the scale to patient's mark, a change of 10 for the 100 mm pain VAS would be the minimal clinically importance difference, and the VAS of 33 or less signifies acceptable pain control after surgery. The main benefit of VAS is that the score appears to have the qualities of ratio data and may be treated as such statistically. The VAS also has a large number of response categories, which mean that it is considered to be more sensitive to change in pain intensity than measures with limited number of responses, it is an analog scale formatted without numbers [4] (Figure 1).

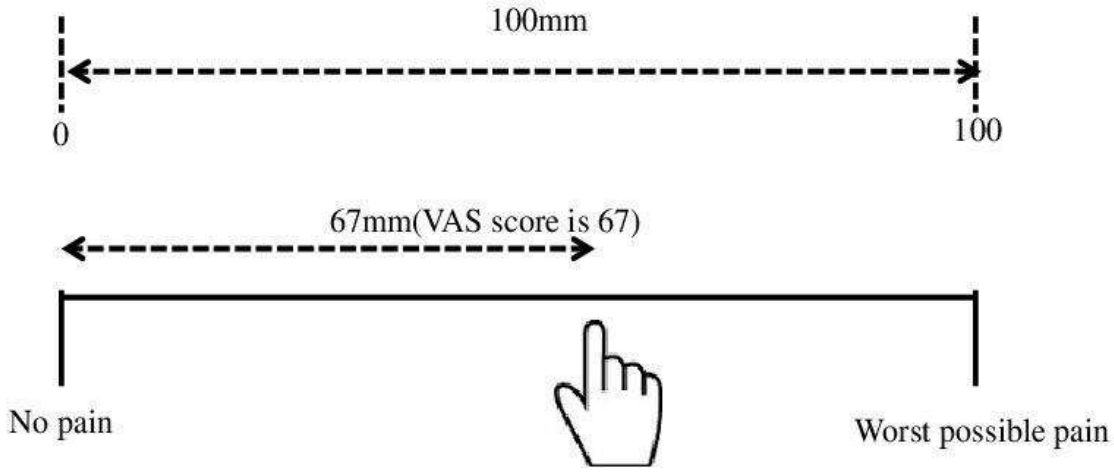


Figure 1. Visual analogue scale model

Numerical rating scale (NRS)

In a NRS, patients are asked to circle or to say vocally the number between 0 and 10, 0 and 20 or 0 and 100 that fits best to their pain intensity. Zero usually represents 'no pain at all' whereas the upper limit represents 'the worst pain ever possible'. In contrast to the VAS, only the numbers themselves are valuable answers, meaning that there are only 11 possible answers in a 0-10, 21 in a 0-20 and 101 in a 0-100 point NRS. It thus allows only a less-subtle distinction of pain levels compared to VAS, where there are theoretically unlimited numbers of possible answers [5]. NRS have shown high correlations with other pain-assessment tools in several studies [6]. As it is easily possible to administer NRS verbally, it can be used in telephone interviews. On the other hand, results cannot necessarily be treated as ratio data as in VAS. A change on the NRS of 20% between two time-points of an assessment is regarded as being clinically significant [7] (Figure 2).

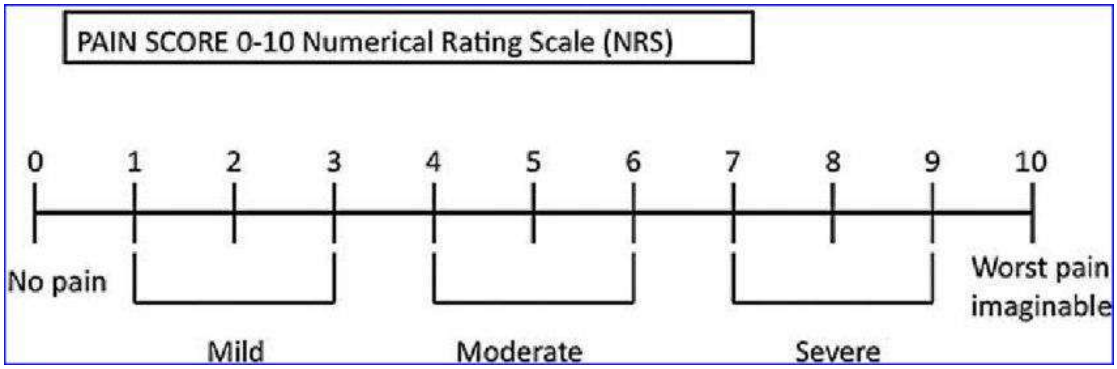


Figure 2. Numerical rating scale

This study aimed to assess postoperative pain for patients underwent cholecystectomy using VAS and NRS and the relation to age, sex, educational status, duration of surgery, use of drains, surgical procedure, type of analgesia and to compare and correlate between the two scales in assessing postoperative pain.

Methods

A cross-sectional study at the surgical ward of Al-Imamein Al-kadhimein Medical City from the period of 1st of October 2019 to 1st of March 2020. Fifty cases were enrolled in this study. All of them submitted to cholecystectomy. All the patients were consented verbally.

Patients were excluded if they had poor language comprehension, drug and alcohol dependence, psychiatric disorder and or concurrent serious medical disorder impairing the completion of questionnaire. Patients interviewed for pain assessment at two separate occasion using VAS and NRS within 24 hours from surgery, the first was after recovery from general anesthesia and patients start asking for pain relief, then one hour after instillation of the analgesia (Acetaminophine Paracetamol vial 1 g and /or Opioid Pethidine 100 mg) intravenously. Data was analyzed using statistical package for social sciences (SPSS) version 21

Results

The mean age and duration of surgery were (42.78±12.96) year, (1.1±0.5) hour respectively. The mean score for NRS before and after treatment were 8.46, 5.66, while for VAS before and after treatment were 8.28, 5.12 subsequently, as shown in table (1).

Table 1. Means of parametric data

Parameter	Mean	SD	Range
Age (yr)	42.78	12.96	22-71
Duration of operation (hr)	1.1	0.5	1-2
NRS before Rx	8.46	1.56	5-10
NRS After Rx	5.66	2.17	2-10
VAS Before Rx	8.28	1.62	4-10
VAS After Rxx	5.12	2.26	2-10

Majority of the study population were females 42 (84%). About 80% of patients have academic achievement of primary or secondary school (36%, 21%) respectively. Ninety percent of patient had their operation finished with laparoscopic approach. Drains have been left in nearly 90% of cases. Around two third of patient have their pain being controlled by combined usage of Paracetamol and Opioid 66%, table (2).

Table 2. Frequencies of non-parametric data

Parameter		Frequency	Percentage
Gender	Females	42	84.0
	Males	8	16.0
Educational status	None	1	2.0
	Primary	18	36.0
	Secondary	21	42.0
	College	10	20.0
Type of surgery	Laparoscope	45	90.0
	Open	5	10.0
Drainage	Yes	44	88.0
	No	6	12.0
Post-operative drug	Opioid+Paracetamol	30	60.0
	Paracetamol	15	30.0
	Not achieved pain relief	5	10.0

There was no significant correlation between the two scores in relation to the age and duration of surgery before and after analgesia as shown in table (3).

Table 3. Correlation between NRS and VAS with age and duration of surgery

Pain scale		NRS		VAS	
		Before Rx	After Rx	Before Rx	After Rx
Age (yr)	r	0.021	0.122	0.067	0.032
	p	0.883	0.321	0.643	0.621
Duration (hr)	r	0.018	0.101	0.112	0.054
	p	0.129	0.142	0.438	0.461

The relation of pain score rating and sex have been assessed, both sex have comparable pre and post analgesia results as shown in table (4). The effect of educational level on pain assessment was as follows, those with college level of education have similar score of 7.8 for (NRS and VAS) while those who have primary educational level have (NRS 8.78) and (VAS 8.67) as shown in in table (5).

The mean of pain score was comparable in relation to the type of cholecystectomy, NRS (8.44, 8.6), and VAS (8.36, 8.6) before analgesia for laparoscopic and open surgery respectively. Also for post analgesia scores, as shown in table (6) below.

Patients in whom drain were used have higher mean pain scores (NRS 8.39) and (VAS 8.18) in comparison without drain (NRS 7.4) and (VAS 7.0), but it was statistically insignificant as shown in table (7).

Table 4. Effect of sex on pain assessment score

		Females N=42 Mean±SD	Males N=8 Mean±SD	P value
NRS	Before Rx	8.45±1.48	8.5±2.0	0.950
	After Rx	5.11±1.2	5.43±1.6	0.360
VAS	Before Rx	8.38±1.61	8.75±1.67	0.348
	After Rx	5.2±1.6	5.31±1.2	0.251

Table 5. Effect of educational status on pain assessment score

	Primary N=18 Mean±SD	Secondary N=21 Mean±SD	College N=10 Mean±SD	P value
NRS before Rx	8.78±1.59	8.43±1.47	7.8±1.62	0.286
VAS before Rx	8.67±1.37	8.19±1.54	7.8±2.2	0.388

Table 6. Effect of type of surgery on pain assessment score

		Laparoscope N=45 Mean±SD	Open N=5 Mean±SD	P value
NRS	Before Rx	8.44±1.5	8.6±2.19	0.884
	After Rx	5.31±1.6	5.9±1.9	0.679
VAS	Before Rx	8.36±1.5	8.6±1.67	0.382
	After Rx	5.18±1.9	5.7±1.3	0.563

Table 7. Effect of drain on pain assessment score

	Drain N=44 Mean±SD	No drain N=6 Mean±SD	P value
NRS before Rx	8.39±1.6	7.4±1.1	0.261
VAS before Rx	8.18±1.6	7.0±1.67	0.300

Both pain scores respond comparably to analgesia, those patients received opioid and Paracetamol 1 g vial report lower mean score (NRS 5.15, VAS 4.61) than those received Paracetamol only as shown in table (8). Significant reduction in pain scores after analgesia were observed in both NRS and VAS (p value <0.001) as shown below (Figures 3 and 4) subsequently.

Table 8. Effect of type of analgesia on pain assessment score

	Opioid ± paracetamol N=33 Mean±SD	Paracetamol N=17 Mean±SD	P value
NRS after Rx	5.15±2.12	6.65±1.97	<0.01
VAS after Rx	4.61±2.09	6.12±2.29	<0.01

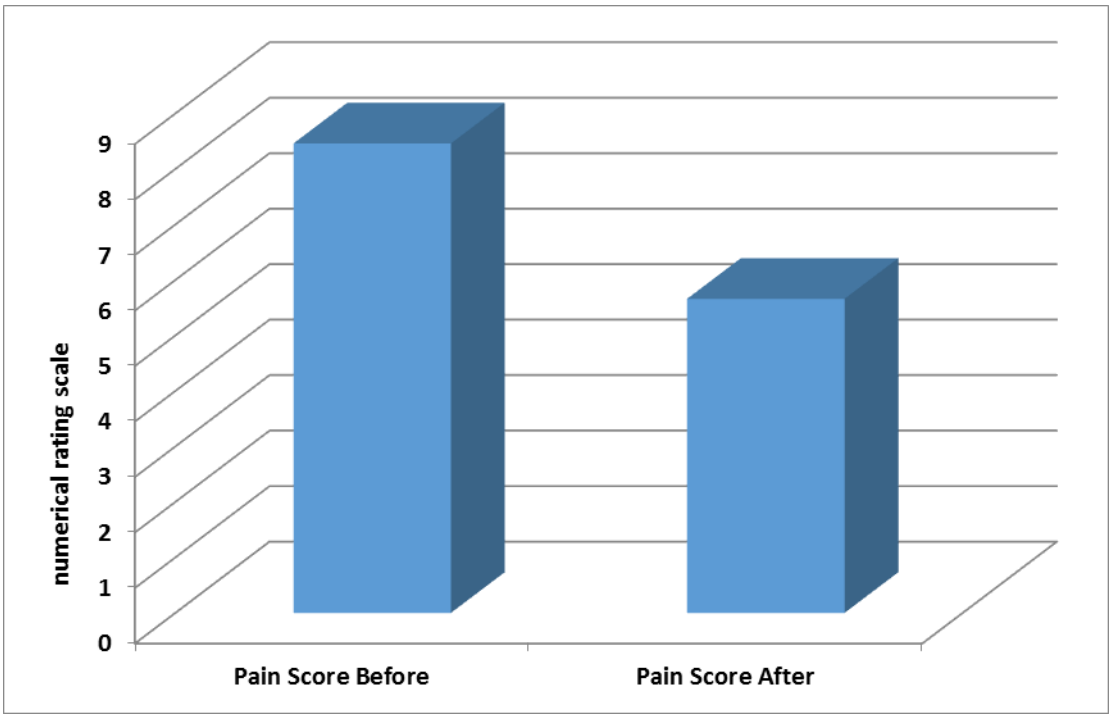


Figure 3. Comparison of NRS before and after treatment by paired test

The scatter plot drawn for the findings shows linear association between values for the two scores before analgesia. It also shows similar distribution throughout the length of the line representing the linear association, r= 0.581, p value <0.001, accordingly there is a strong correlation between NRS and VAS (Figure 5).
Also post analgesia scattered plot test signifies a strong correlation between the NRS and VAS after treatment (r = 0.821), (p value <0.001) (Figure 6).

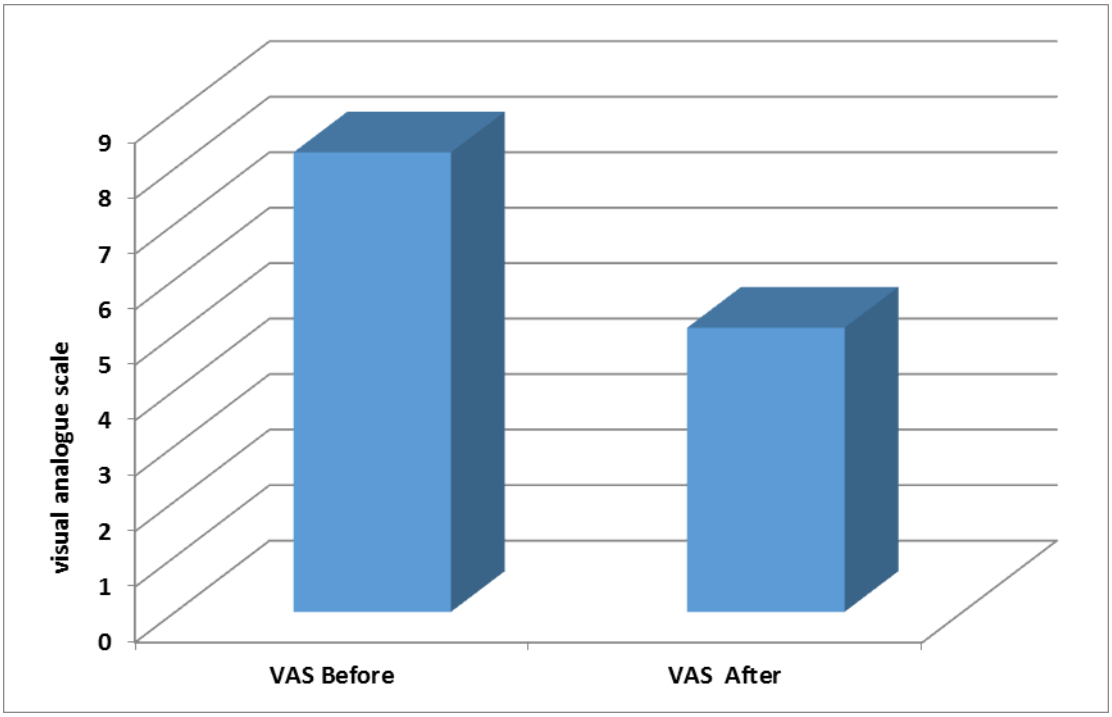
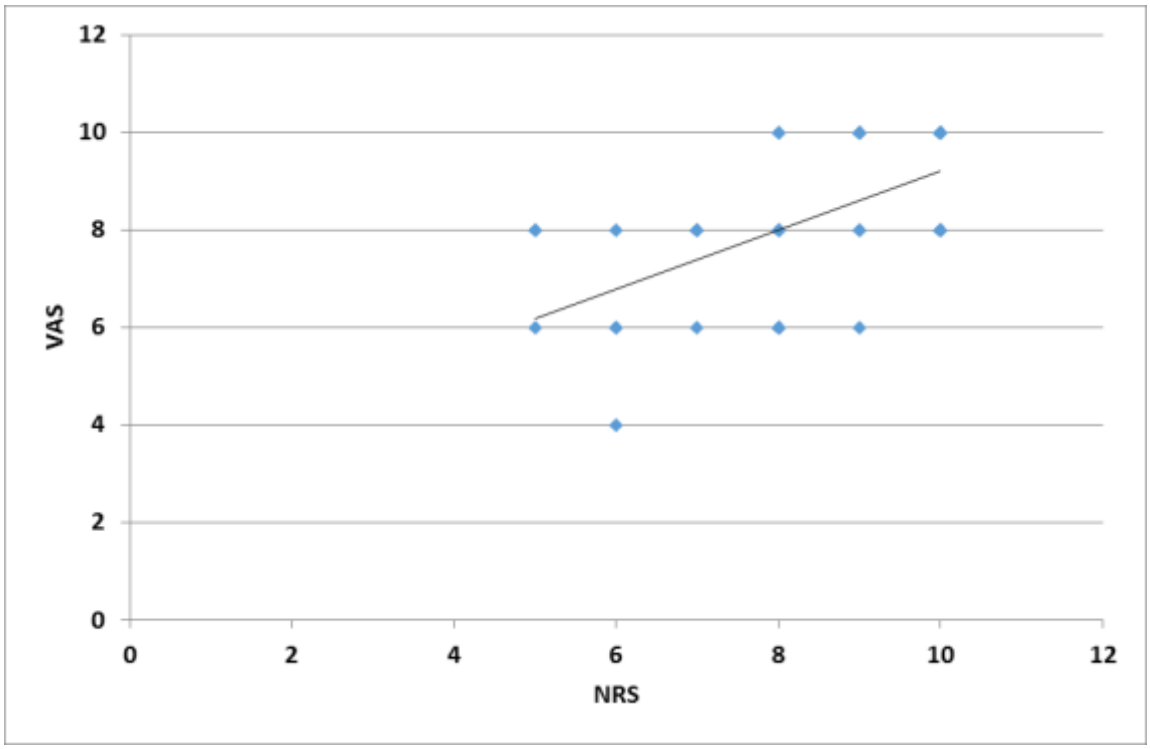
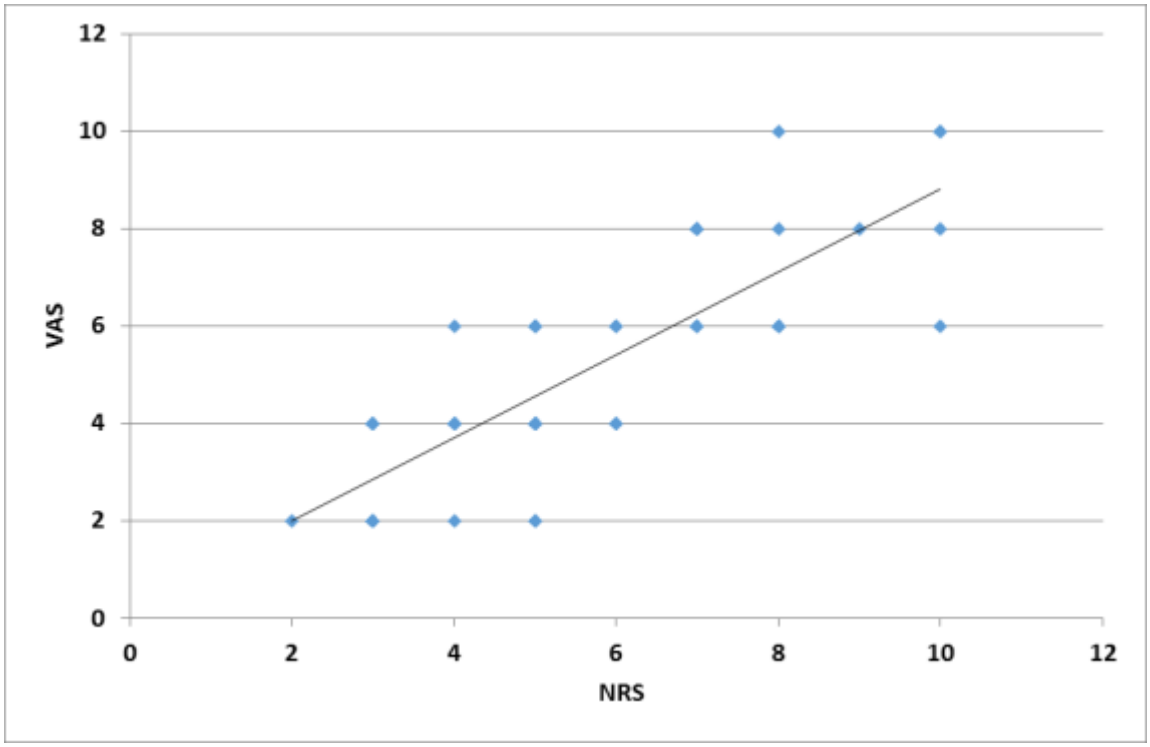


Figure 4. Comparison of VAS before and after treatment by paired test



r= 0.581, p value <0.001

Figure 5. Correlation between numerical rating scale and visual analogue score before analgesia



r= 0.821, p value <0.001

Figure 6. Correlation between numerical rating scale and visual analogue score after analgesia

Discussion

Optimal care of surgical patients mandates effective postoperative control of pain, it is an essential human right [5]. Acetaminophen (Paracetamol) is commonly used alone or in combination with opioids in the management of moderate to severe pain [8].

Laparoscopic Cholecystectomy (LC) has become a gold standard surgical treatment of symptomatic gallstones, although LC has proven to have certain advantages and a common day case procedure, it is not of pain-free procedure [9]. Some patients after LC still experience moderate or even severe pain and may require opioid treatment. Although pain decreases with time but it is severe enough to interfere with daily activities in a substantial number of patients [10].

In our series, the mean age of patients is 42.78 years, which is comparable to that of Khalaf et al., at Al-Basra General Hospital south of Iraq 43.5 years [11].

The mean duration of operation in this study was 1.1 hour. A multivariate analysis done by Lowndes et al., they found a mean operative time of 1.16 hour [12]. Neither the age nor the operative times have significant effect on pain score assessment by (NRS or VAS). In comparison with other studies shows the same results which state that there was no significant difference between age, duration of surgery and pain rating [13,14].

Response to analgesia

Female patients constitute majority of the study group, which is equivalent to other studies [11,15], due to hormonal effect, still we didn't identify significant effect of gender on pain scoring, although Nguyen and Nguyen assessed post-cholecystectomy pain and they found that Females were more likely experience postoperative pain than males in Vietnam [16], this is attributed to environmental and physical differences between two communities. Dabbagh and Ure et al., found that female patients suffer more postoperative pain than male patients, they consider sex as a

significant predictor of postoperative pain [17, 18].

In regard to the effect of patients' education on pain perception, a higher level of education was associated with lower pain score in comparison with primary educational states, although statistically the differences were insignificant. Fadaizadeh et al., found that educational status has no significant contribution to pain score [19].

In this series, most of the procedures completed laparoscopically, although pain perception and need for opioid was less for laparoscopic group, still no significant differences between open and laparoscopic approach in relation to pain scores. Hendolin et al., compared between laparoscopic and open cholecystectomy patients in regard to analgesic doses frequency and timing, they found that both group experience similar pain as measured by VAS in the recovery room, but the first analgesic dose was earlier in open group and need for opioid was more than laparoscopic group ($p<0.01$) [20].

Although insignificant, pain score was higher in those with postoperative abdominal drain especially at drain site. Routine drain use is still debatable, it is thought that drain will reduce pain related to CO_2 accumulation or prevent biloma and or hematoma. According to the Cochrane Database Systemic review randomized clinical studies by Gurusamy et al., they show no benefit of a drain [21]. Nagpal. et al., found no significant advantage of using drain after laparoscopic cholecystectomy, therefore, its routine use cannot be recommended as a means to reduce pain/ nausea/ vomiting as there is higher incidence of postoperative pain and longer duration of hospital stay with its use [22]. We prefer avoidance of drain as a routine procedure unless indicated in complicated or difficult cases

Our study showed that there is positive significant association between type of analgesia and postoperative pain score, patients who received opioid and Paracetamol have lower pain score than patient who received Paracetamol only. Alimian et al., showed that although Paracetamol (1-4 g in 24 hours) is not enough for postoperative pain relief, especially in first postoperative six hours, and patients needed some doses of Meperidine (Pethidine), after eight hours the adequacy of analgesia was similar in two groups [23]. Moffat et al., and Cataldo et al., stated that Paracetamol produce 31-37% decrease in the morphine demand during the first 24 hours after surgery [24,25].

Of the many pain scales used to assess pain worldwide, VAS, NRS and Verbal Rating Scale (VRS) are the three widely used pain scales to assess acute pain. Although they have a comparable range of accuracy, from these three scales VAS is recognized as most appropriate to assess acute pain, but is relatively complex [3]. In this series we found a strong comparable linear correlation between the two scales (VAS and NRS) for pain assessment before and after commencing analgesia as shown in figure 3 and 4 respectively. A similar result demonstrated by Gajasinghe et al., in their study, they found that the linear regression model and Pearson's correlation statistics of the VAS and NRS show stronger linear relationship between them [3]. Hjermstad et al., in their review articles show that NRS or VAS all work quite well and the most important choice is not the type of scale per se, but the conditions related to its use such as methods of administration, time frames, information related to the use of scales, interpretation of cut-offs and clinical significance, and the use of appropriate outcome measures and statistics in clinical trials, still better compliance was reported for the NRS relative to the other scales in 15 studies, whereas 16 studies did not provide any such information, lower compliance on the VAS was found in nine studies, associated with higher age, degree of trauma, or other impairments [5].

In conclusion, NRS and VAS are two comparable acute pain scores mostly used in practice. NRS which is easy, less pain inducing and more user friendly in the post-operative period has a strong linear association with VAS, thus can be substituted for VAS in assessment of postoperative pain.

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Conflict of interest
Author declares no conflict of interest.

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Detection of Chromosome 13q14 Deletion, Chromosome 11q22 Deletion and Trisomy 12 in Chronic Lymphocytic Leukemia Patients

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Abstract

Background Chronic lymphocytic leukemia (CLL) is a malignancy of mature B cells. The genetic factors have been found to play a role in the pathogenesis of the disease. Deletions of the long arm of chromosome 13, specifically involving band 13q14 (del(13q14)) constitute the single most frequently observed cytogenetic aberration in CLL, occurring in ~55% of all cases, followed by structural abnormalities of ch 12(trisomy 12), del 11q22.3-q23.1, del 6q21-q23, del 17p13.1, and 14q.

Objective To detect frequencies of chromosome 13q14 deletion, chromosome 11q22 deletion and trisomy 12 in in CLL patients using FISH technique, and to investigate the relation between those chromosomal abnormalities and clinical features and hematological parameters.

Methods This cross-sectional study was conducted on fifty newly diagnosed patients with CLL. Three ml of fresh blood were taken from each patient at admission and then transfer it to FISH unit for blood preparation. Slide preparation was done by using dual color fusion gene probe. The slides were read in the second day by fluorescent microscope.

Results Deleted chromosome 13 was found in 24 out of 50 patients, (48%). Trisomy chromosome 12 was found in 15 out of 50 patients, (30%). Deleted chromosome 11 was found in 12 out of 50 patients, (24%).

Conclusion In a sample of Iraqi adult with CLL, the most frequent chromosomal abnormality was deletion of chromosome 13 followed by trisomy of chromosome 12 and chromosome 11 deletion.

Keywords Chronic lymphocytic leukemia, FISH, deleted chromosome 13, trisomy chromosome 12

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List of abbreviations: Ab = Antibody, Ag = Antigens, ALC = Absolute lymphocyte count, BM = Bone marrow, CBC = Complete blood count, CD = Cluster of differentiation, CLL = Chronic lymphocytic leukemia, DAPI = Anti fade.4.6.diamidino2.phynlindole, DAT= Direct antiglobulin test, Del = deletion, FISH = Fluorescence in situ hybridization, Hb = Hemoglobin, IQR = Inter quartile range, IWCLL = International workshop group on CLL, LAP = Lymphadenopathy, LPD = Lymphoproliferative disorder, M CLL = Mutated chronic lymphocytic leukemia, MCL = Mantle cell lymphoma, miR-15a = MicroRNA-15a, miR-16-1 = MicroRNA-16-1, , miRNA= MicroRNA, mRNA = Messenger RNA, NHL = Non Hodgkin lymphoma, PAS = Periodic acid Schiff, PBF = Peripheral blood film, PLT = Platelet, SSC = Sodium saline citrate, TRI = Trisomy, WBC = White blood cells

Introduction

Chronic lymphocytic leukemia (CLL) is a malignancy of mature B cells characterized by blood and marrow lymphocytosis. Varying degrees of lymphadenopathy, splenomegaly, and blood cytopenia develop as the neoplasm progresses. CLL is the most prevalent adult leukemia in Western societies [(1)].

It is characterized by a marked degree of clinical heterogeneity, ranging from patients that harbor a highly stable disease with a nearly normal life expectancy to patients with a rapidly progressive disease [2].

In Iraq, leukemia was the third most common cancer accounting for (6.48 %) of all cancers; CLL represents the 7th most common type of leukemia and constitutes (4.91%) in males, (4.29%) in females of all leukemia according to Iraqi cancer registry 2015 [3].

The incidence of chromosomal abnormalities has geographic variations and is variable across the world [4].

Genetic alterations in CLL can include chromosomal alterations, mutations, alterations in the expression of miRNAs and epigenetic modifications. Molecular cytogenetic remains an integral component of the clinical management of CLL, stratifying patients into risk groups associated with poor (11q or 17p deletion), intermediate (trisomy 12 or normal karyotype) and good prognosis (13q deletion as sole aberration), with important implications relating to commencement or escalation of therapy [5].

In the peripheral blood, the morphology of these cells looks like mature lymphocytes [6]. In CLL, lymphocytes typically show B-surface antigens, as shown by CD19, CD20dim, CD21 and CD23 [7].

Based on immunophenotypic characteristics and giving one point to each one of the following: CD5+, CD23+, FMC7 weak, SmIg (κ/λ staining) weak and CD79b weak. Matutes and Catovsky showed that in patients with a score of 4-5, the diagnosis is virtually always CLL, while in those cases with a score <3, the diagnosis of CLL is extremely unlikely [8].

Chromosome 13 deletion seems to protect against the transformation of CLL into diffuse large B-cell lymphoma, as the absence of del(13 q14) characterizes patient subgroups at high risk of developing Richter syndrome [9-12], whereas those with combined tri12 and NOTCH1 mutation fair worse as cells from patients with tri12 and concurrent NOTCH1 mutations may be more resistant to apoptosis leading to a less favorable course [13,14]. Deletion ch 11 q leads to loss ATM gene (which encodes a protein involved in DNA repair). Mechanistically, automated teller machine (ATM) is able to signal DNA damage and to mediate p53 activation which, as a major player assuring genome stability, can ultimately trigger apoptosis or oncogene-induced senescence if the damage is not repaired. Therefore, damaged cells are not adequately removed and ATM deficiency causes genomic instability [13,15].

The aim of current study is to detect frequencies of chromosome 13q14 deletion, chromosome 11q22 deletion and trisomy 12 in CLL patients using FISH technique, and to investigate the relation between those chromosomal abnormalities and clinical features and hematological parameters.

Methods

Selection of patients

A total of 50 (32 males and 18 females) newly diagnosed patients with CLL who were attending the Hematology Outpatient Clinic at the Medical City were included in this study during the period from February 25, 2018 until December 15, 2019.

Data collection

Full personal and demographic information was obtained, including date of birth, sex, residence, date at diagnosis, and duration of illness. Complete clinical data were obtained regarding the presenting complaint(s), including pallor, abdominal distention, any history of recurrent infections, chronic diseases. Immunophenotyping result was obtained from flowcytometry department and according to it we do scoring to the patients depending on Mutuate Scoring System. Immunophenotypic scoring system is used for the diagnosis of CLL and to differentiate CLL from other B cell malignancies [2]. Scores in CLL are usually >3, in other B-cell malignancies the scores are usually ≤3 [8].

Table 1. The scoring system for diagnosis of chronic lymphocytic leukemia [16]

Marker	Points	
	1	0
CD5	Positive	Negative
CD23	Positive	Negative
FMC7	Negative	Positive
smIg	Low	Medium/High
CD22/CD79b	Low / Negative	Medium/High

SmIg= surface membrane immunoglobulin

All patients were thoroughly examined for pallor, lymphadenopathy, hepatomegaly and/or splenomegaly. A staging system is a standardized way for the cancer care team to epitomize information about how far a cancer has spread, as Rai system in the United States [17].

Table 2. Rai and Modified Rai staging system [4]

Risk Group *	Stage **	Description
Low	0	Lymphocytosis only
Intermediate	I	Lymphocytosis plus enlarged nodes
	II	Lymphocytosis plus enlarged liver or spleen with or without enlarged nodes
High	III	Lymphocytosis plus anemia (Hb less than 11) with or without enlarged nodes, liver, or spleen
	IV	Lymphocytosis plus thrombocytopenia (platelet count less than 100 x 10^9/L) with or without anemia, enlarged nodes, liver or spleen

*Modified Rai system (1987). **Rai system

A detailed explanation of the aim of the study was provided to the patients. Participation was strictly voluntary. A written consent was obtained from each patient. The study strictly obeyed the instructions of the Declaration of Helsinki for Human Rights and Institutional Review Board in College of Medicine, Al-Nahrain University.

Laboratory methods

For each patient, 3 ml of ethylenediaminetetraacetic acid blood samples was aspirated under strict aseptic techniques. The laboratory workup included the following:

1. Complete blood count: It was done using an automated (Cell-DYN, RUBY Abbott Diagnostic, USA) at Teaching Laboratories of Medical City. The diagnosis was made by flowcytometry.
2. Reticulocyte count: It was counted manually after staining with new methylene blue stain using the standard methods to exclude the presence of hemolytic anemia for the purpose of clinical staging of the disease.
3. Direct agglutination test was done by a spin tube technique to exclude autoimmune hemolytic anemia prior to clinical staging.

4. Blood film was prepared and stained with Leishman's stain using the standard procedures.
5. Fluorescent in situ hybridization technique (FISH) include: blood preparation, slide preparation, slide reading.
6. About 750 microliters of blood added by pasture pipet to 5 ml of peripheral blood sample in centrifugate tube mixing well by pasture pipette. Then the solution stored at 4 °C in refrigerator for 1 hour, after that the solution centrifugated at room temperature for 10 min at 1000 rpm and discard the supernatant by sucking it off carefully with pasture pipet (1 ml of supernatant was left in the tube to avoid loss of material).

For hypotonic treatment, the pellet was resuspended in 5ml of KCL (37 °C) and incubated at 37 °C for 20 min. Then 1 ml of caryos (fixative material) slowly added to tube, mix carefully and leave it in refrigerator 4 °C or 30 min.

Solution centrifugated in (10 min and 1000 rpm), discard the supernatant, five ml of caryos added to solution and stored in refrigerator at (4 °C) overnight. In second day start with washing to remove all RBC so centrifugate the solution, discard the supernatant and added 5 ml of caryos. Then repeated washing three time, then five ml of caryos added to solution and stored in refrigerator (4 °C) until slide preparation start. The specimen was studied in rundown order by meta system fluorescents microscope. For purposes of this paper, orange signals are referred to as O, green signals are referred to G.

For each specimen at least 100 interphase nuclei from different areas of the same slide were scored. Results were considered clonal when the percentage of cells with any given chromosome abnormality exceeded the established cut-off value. Following internal validation of probes, the cut-off values were established at 3% for trisomy 12, 6% for chromosome 11, 8% for chromosome 13. The cut-off values for both gains and losses were determined by statistical evaluation of FISH results from control people [11].

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion). The level of significance was considered at P-value of equal or less than 0.05.

Results

This descriptive, cross-sectional study included 50 patients with CLL (32 males and 18 females). Their ages ranged from 45-76 years, with a mean age of 62.02±8.66 years and median age of interquartile range (IQR) 64 years (14) (Table 3).

They were 34 (68%) patients with lymphadenopathy, 5 (10%) patients with hepatomegaly, 9 (18%) patients with splenomegaly, 16 (32%) with hepatosplenomegaly and 40 (80 %) patients with pallor. According to modified Rai staging they were 20 (40 %) in intermediate risk group, 30 (60 %) patients in high-risk group. According to immunological score, patients were distributed as 7 (14%) patients in score 3 and 43 (86 %) in score 4 (Table 4).

The Hb levels of the studied patients ranged from 4.4-16.3 g/dl, with a mean of 10.97±2.45, whereas the absolute lymphocyte count (ALC) ranged from 5.5-161, with a mean of 19.02±31.6, prolymphocyte% ranged from 1-8 with a mean of 1.64±1.47 (Table 5).

Table 3. Distribution of chronic lymphocytic leukemia patients according to age and gender

Characteristic		Results
Age (years)	45-49, *n* (%)	6 (12.0%)
	50-59, *n* (%)	11 (22.0%)
	60-69, *n* (%)	22 (44.0%)
	70-76, *n* (%)	11 (22.0%)
	Mean ±SD	62.02±8.66
	Median (IQR)	64 (14)
	Range	45-76
Gender	Male, *n* (%)	32(64%)
	Female, *n* (%)	18(36%)
	Male:Female	1.8:1"

n: number of cases; SD: standard deviation

Table 4. Clinical characteristics, score, staging of chronic lymphocytic leukemia patients

Characteristic	Result
LAP, *n* (%)	34 (68 %)
Organomegaly	
Hepatomegaly, *n* (%)	5 (10%)
Splenomegaly, *n* (%)	9 (18%)
Hepatosplenomegaly, *n* (%)	16 (32%)
Pallor, *n* (%)	40 (80%)
Score	
Score 3, *n* (%)	7 (14%)
Score 4, *n* (%)	43 (86%)
Stage	
II, *n* (%)	20 (40%)
III, *n* (%)	25 (50%)
IV, *n* (%)	5 (10%)

Table 5. Hematological parameters of CLL patients

Characteristic		Result
Hb g/dL	Mean±SD	10.97 ±2.45
	Median (IQR)	10.8 (3.6)
	Range	4.4 -16.3
Platelet count 10^3/µl	Mean±SD	155.65 ±76.55
	Median (IQR)	129 (93.25)
	Range	25.3 -324
WBC count 10^3/µl	Mean ±SD	34.59 ±36.36
	Median (IQR)	24 (11.8)
	Range	7 -196
Absolute lymphocyte count	Mean ±SD	19.02 ±31.60
	Median (IQR)	7.25 (10.88)
	Range	5.5 -161
Prolymphocyte%	Mean ±SD	1.64 ±1.47
	Median (IQR)	1 (1)
	Range	1 -8

n: number of cases; SD: standard deviation; IQR: inter-quartile range

Out of the 50 studied patients, 2 (4%) had non-chromosomal aberrations and 48 (96%) had chromosomal aberrations. Chromosome 11 deletion was seen in 12/50 (24%) of patients and the percent of deleted cells has ranged from 7-19% with a mean of 15.64±4.11%. (Figures 1 and 2). Chromosome 12 trisomy was seen in 15/50 (30%) of patients and the percent of trisomy 12 cells has ranged from 15-17% with a mean of 16.21±0.58% (Figures 3 and 4). Chromosome 13 deletion was seen in 24/50 (48%) of patients and the percent of deleted cells has ranged from 8-32% with a mean 15.71±5.57 (Figures 5 and 6) (Table 6).

Three diploid cytogenetic cases are observed in three patients, two patients had chromosome 11 deletion and trisomy 12 and one patient had chromosome 11 deletion and chromosome 13 deletion and the characteristics of those diploid cases is showed in table 6.

Table 6. Chromosomal abnormalities in patients with chronic lymphocytic leukemia

Abnormality		Result
Chromosome 11 deletion	*n* (%)	12 (24%)
	Mean of deleted cells ±SD	15.64±4.11
	Range deleted cells	7-19
Chromosome 12 trisomy	*n* (%)	15 (30%)
	Mean of trisomy cells ±SD	16.21±0.58
	Rangeof trisomy cells	15-17
Chromosome 13 deletion	*n* (%)	24 (48%)
	Mean deleted cells ±SD	15.71±5.57
	Range deleted cells	8-32
Diploid cases, n (%)		3 (6%)
Ch 11 del & ch13 del n (%)		1 (2%)
% of deleted cells/% of deleted cells		**7%/9%**
Ch 11 del & +12 n (%)		1 (4%)
% of deleted cells/% of trisomy cells		**9%/10%**
Ch 11 del &+12 n (%)		1 (4%)
% of deleted cells/% of trisomy cells		**7%/6%**

n: number of cases; SD: standard deviation; ch: chromosome; +: trisomy; del: deletion

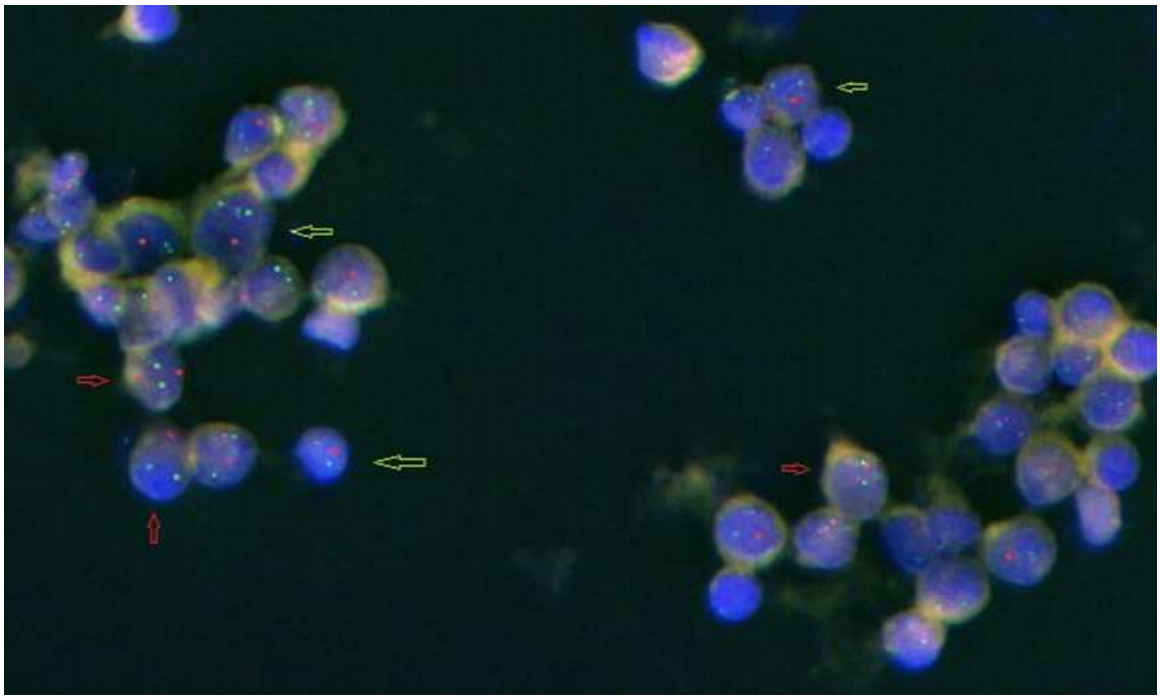

Figure 1. FISH analysis using XL ATM/11cen probe detect deletions in 11q22.3 show leukemic cells with deletions, one orange signal and two green bright fluorescence signal spots (green arrow). Red arrow represents normal FISH pattern with two orange signals and two green signals, on power 40X.

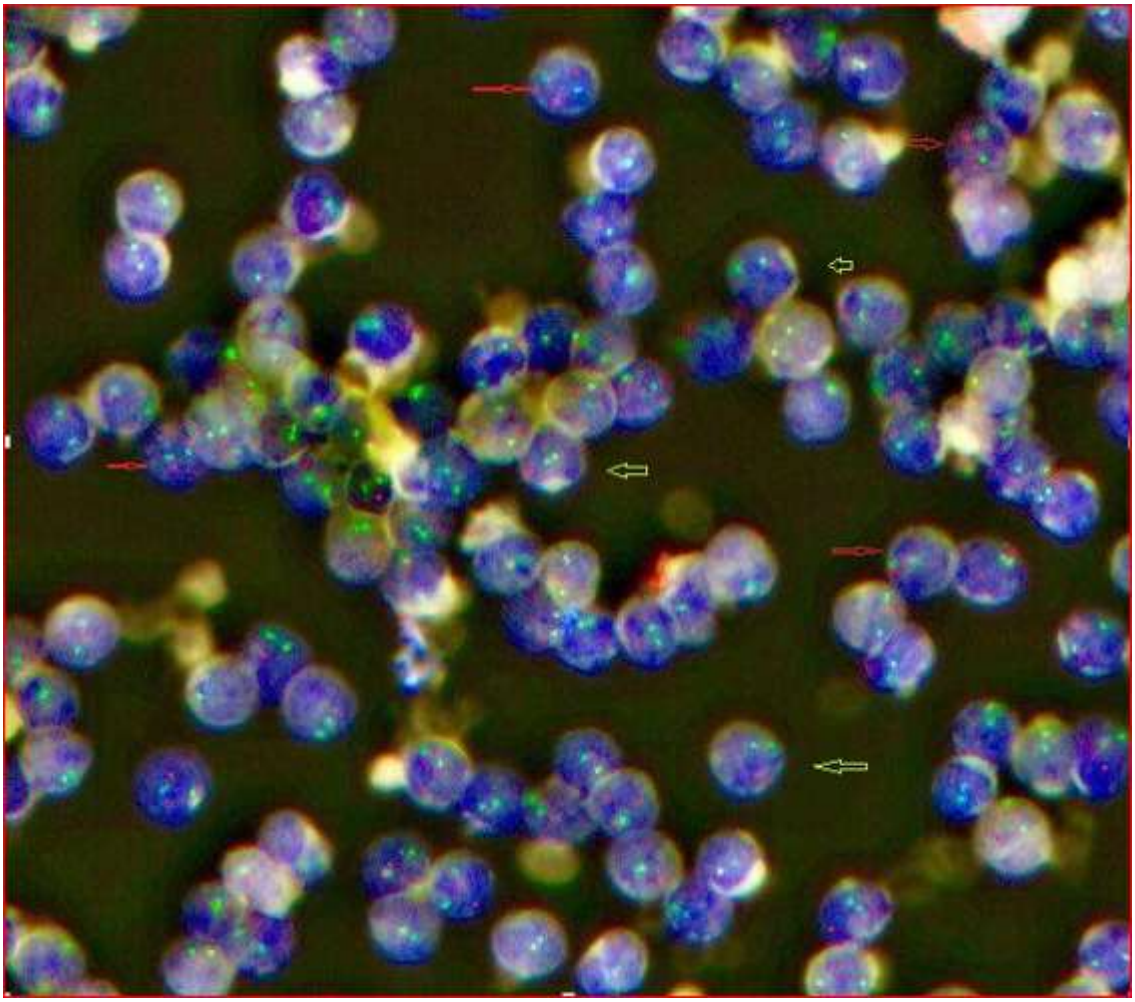

Figure 2. FISH analysis using XL ATM/11cen probe detect deletions in 11q22.3 show leukemic cells with deletions, one orange signal and two green bright fluorescence signal spots (green arrow|). Red arrow represents normal FISH pattern with two orange signal spots and two green signal spots, on power 40X.

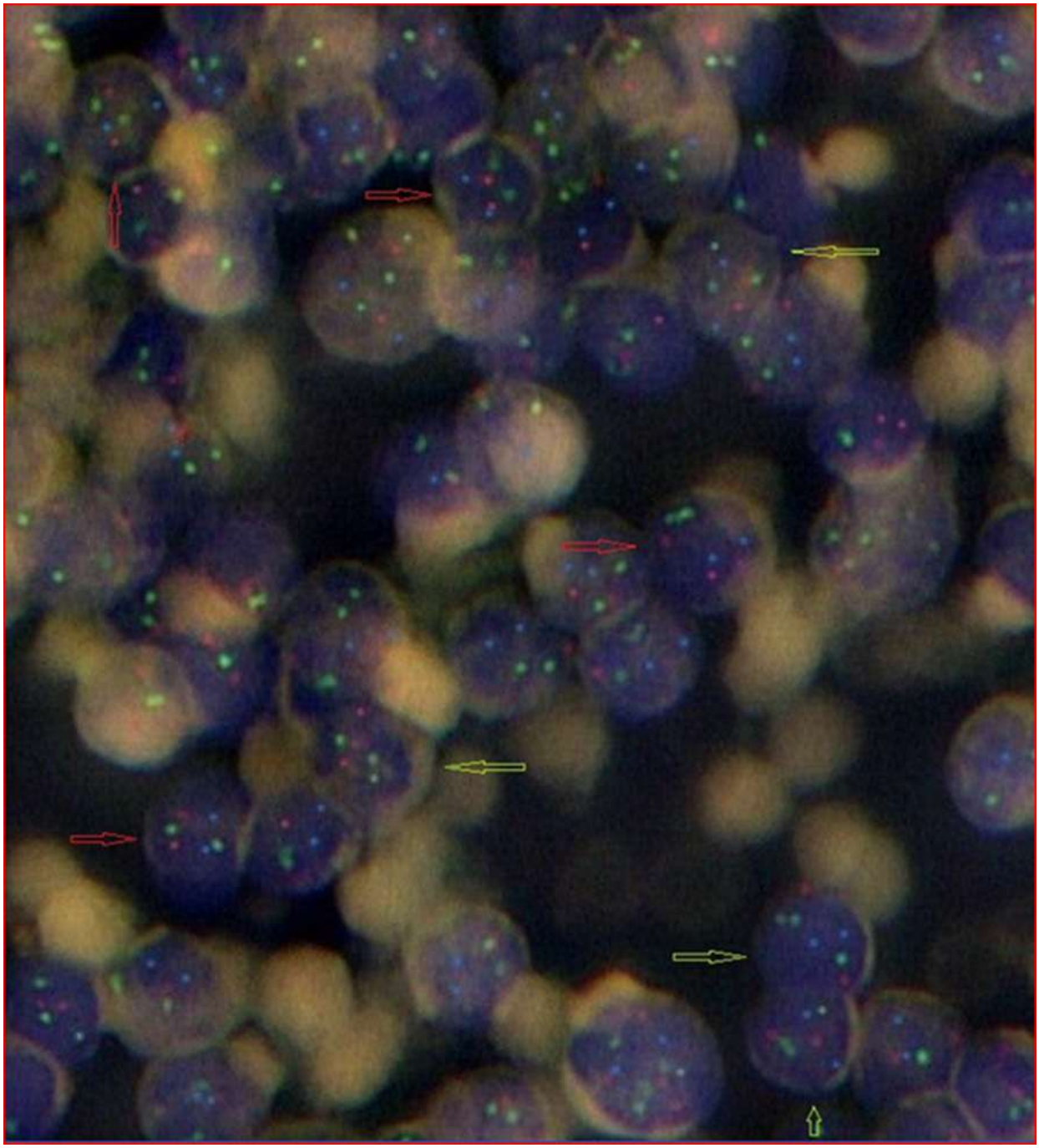

Figure 3. FISH analysis using XL DLEU/LAMP/12cen LSI probe show leukemic cells with trisomy 12, yellow arrows 3 green signals for chromosome 12 and two orange signal, two blue signals for chromosome 13 on power 60 x. Normal FISH pattern with red arrows for chromosome 12

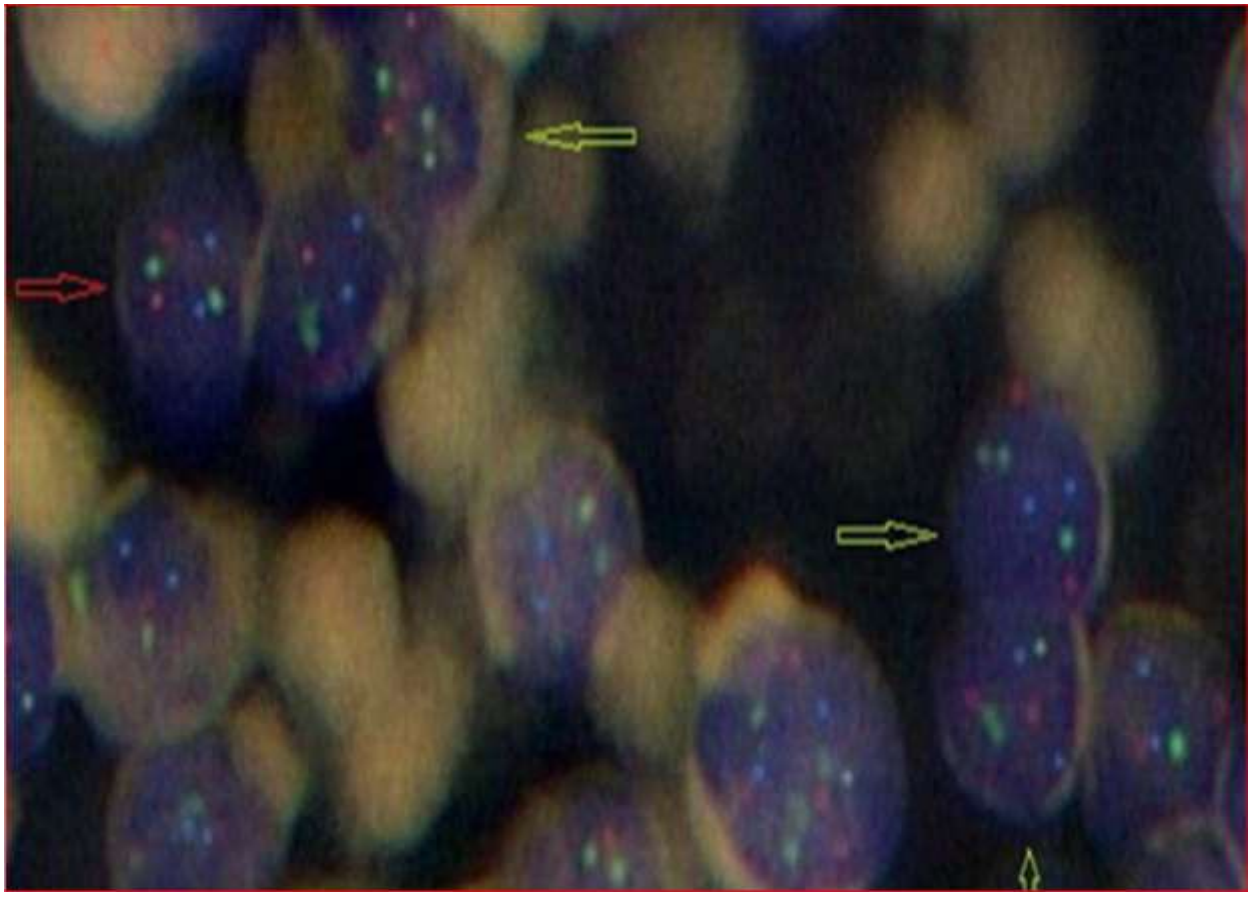

Figure 4. FISH analysis using XL DLEU/LAMP/12cen LSI probe show leukemic cells with trisomy 12, green arrows 3 green signals for chromosome 12 and two orange signal, two blue signals for chromosome 13 on power 60 x. Normal FISH pattern with 2 green signals, two orange signals, two blue signals red arrows

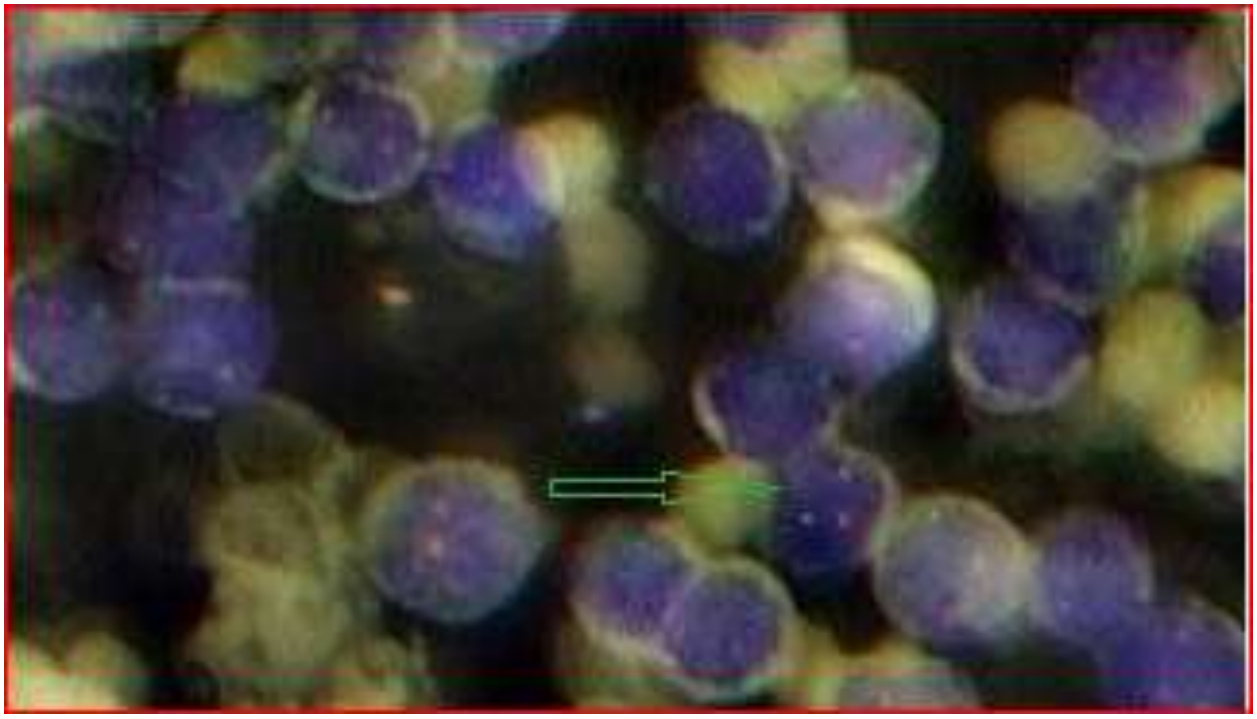

Figure 5. FISH analysis using XL DLEU/LAMP probe detect deletions on chromosome 13q show leukemic cell with monoallelic deletion of chromosome 13 as detected by FISH, the orange (spectrum: orange)-labeled probe detects the minimal deleted region, the green (spectrum: green)-labeled probe detects a terminal DNA segment of the long arm of chromosome 13 to facilitate its identification. Chromosomes are counterstained by DAPI, on power 60x

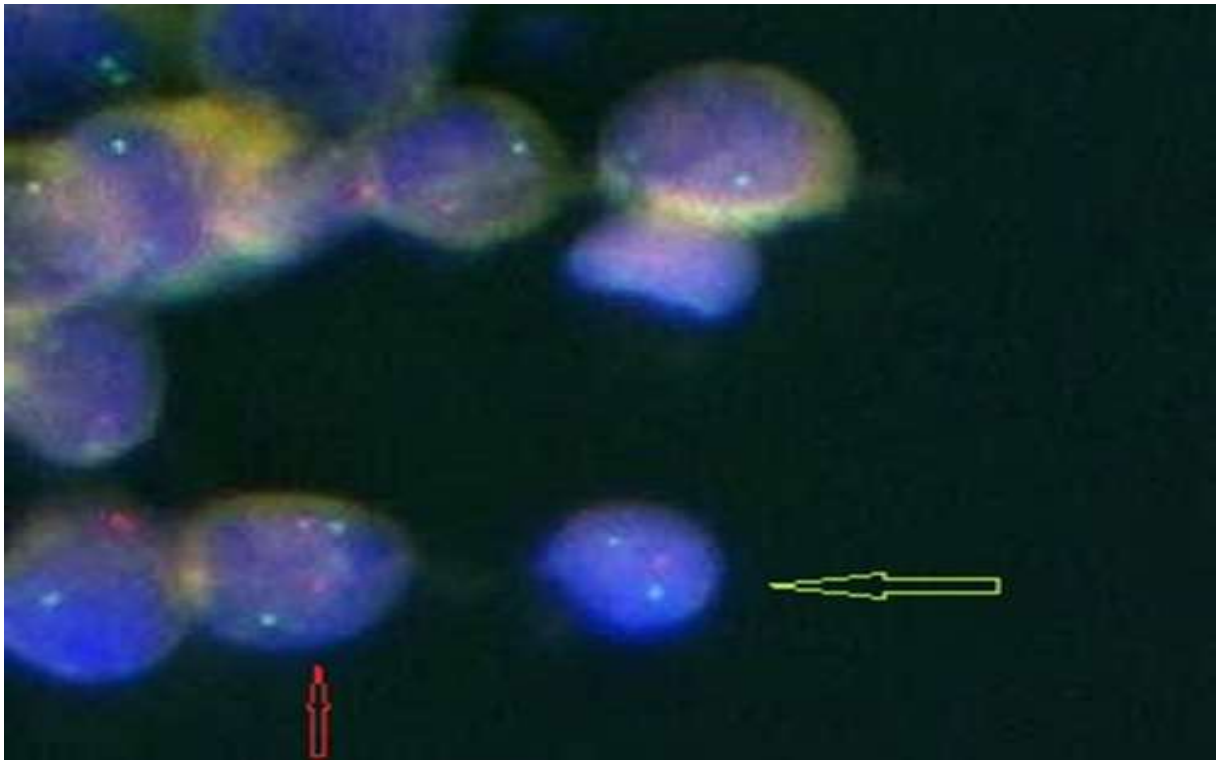

Figure 6. FISH analysis using XL DLEU/LAMP probe detect deletions on chromosome 13q, show leukemic cells with monoallelic deletion of chromosome 13, the orange (spectrum: orange)-labeled probe detects the minimal deleted region, the green (spectrum: green)-labeled probe detects a terminal DNA segment of the long arm of chromosome 13. To facilitate its identification Chromosomes are counterstained by DAPI. one orange signal and two green signals (green arrow) on power 60x. red arrow represent normal FISH pattern with two orange signals and two green signals

Significant negative correlation between percent of trisomy cells and Hb level. No other significant correlation was found (P> 0.05) (Table 7), (Figure 7).

Table 7. Correlation between percent of deleted cells for chromosome 11 and chromosome13 and trisomy cells for chromosome 12 to hematological parameters

Characteristic	Percentage of del Ch 11		Percentage of tri Ch 12		Percentage of del Ch 13	
	r	p	r	p	r	p
Hemoglobin (Hb) g/dL	-0.080	0.815	-0.553	0.040*	-0.060	0.780
Platelet count 10^3/µl	-0.360	0.277	0.126	0.667	-0.400	0.053
WBC count10^3/µl	0.385	0.242	0.362	0.204	0.144	0.501
Atypical lymphocyte/ 100 Ly	0.480	0.135	0.229	0.431	-0.240	0.259
Lymphocyte count	0.409	0.212	0.367	0.197	0.194	0.363
Prolymphocyte %	-0.267	0.428	-0.267	0.356	-0.377	0.070

Del: deletion, Ch: chromosome, tri: trisomy

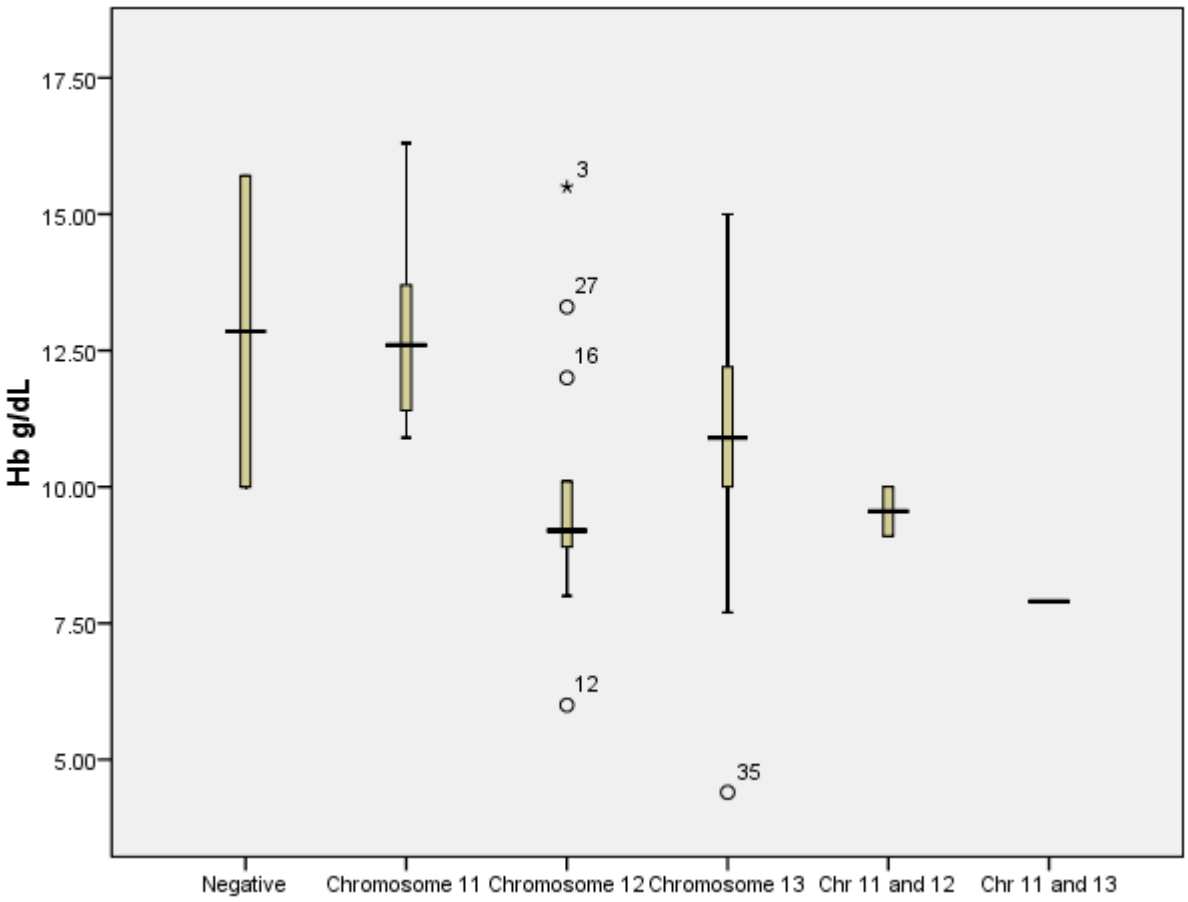

Figure 7. Comparison of hemoglobin level to chromosomal abnormalities

Discussion

CLL is the accumulation of monoclonal B cells in the bone marrow and peripheral blood [(1)]. Identification of specific chromosomal aberrations is an important tool for diagnosis and risk stratification in CLL patients [(15-18)].

In this study, the median age of the patients was 64 years and the range was 45-76 years. It is well known that this disorder is a disease of elderly. This age was close to the results obtained by other Iraqi studies in 2013, 2014 and 2016, 2019 [(19-22)] and a study in Pakistan in 2015 [(23)]. Whereas the median age at presentation, in western countries was higher; it was reported to be 70 years [(24,25)]. This difference can be attributed to the difference in population structure, environmental variations, genetic predisposition between Iraq and western countries, and difference in life expectancy.

Regarding the physical characteristic of CLL patients included in this study, it was noticed that the most frequent presenting signs were pallor, lymphadenopathy and hepatosplenomegaly, hepatomegaly, splenomegaly respectively. Those results were comparable to other Iraqi studies [(26,27)], also, western study reported that lymphadenopathy was more common than splenomegaly and hepatomegaly [(28)].

In the current study, about 43 out of 50 patients (86%) were in score 4 and 7 out of 50 patients (14%) were in score 3 and the diagnosis was confirmed by immunohistochemistry. It is in acceptance with Rodrigues et al. study [(29)].

Regarding the modified Rai clinical stages of CLL patients in this study, 40% of patients were in intermediate risk, 60% of patients were within high-risk group as well as three diploid cytogentic patients were in high-risk group.

This fact reveals that more than halve of patients enrolled in the current study had advanced stage of the disease. An Iraqi study reported higher percentage of patients within high-risk group [26], similar to this study, whereas a western study showed lower percentage of patients fell within high-risk group and higher percentage of patients fell within intermediate risk group, probably due to better awareness of patients seeking early medical services so the patients will be diagnosed earlier [29].

Regarding hemoglobin level; the median Hb level was 10.8 g/dl with IQR of 3.6 and the range was from 4.4-16.3 g/dl, which is close to many Iraqi and non-Iraqi studies [30,31]. In general, anemia may reflect progression of bone marrow failure. In the present study, 56% of patients had Hb less than 11 g/dl whereas 44% had Higher Hb; and this goes with the high frequency of patients in the high-risk group.

Regarding platelet count, the current study revealed that the median of platelet count was 129 $\times 10^9$/L IQR (93.25) and the range was 25.3 -324 $\times 10^9$/L. Similar results were reported by many Iraqi studies [26,32]. Moreover 31 out of 50 (62%) patients had thrombocytopenia. Those results were comparable to another study [33]. Thrombocytopenia was usually attributed to bone marrow failure and reflecting the degree to which bone marrow has been replaced with leukemic cells, thus the high percent of thrombocytopenic patients in the present study reflect high percent of CLL patients in high-risk group.

Leukocyte count is a well-known continuous prognostic variable, where by increasing counts confer a poorer outcome [33-35]. The present study showed that WBC count was more than the upper limit of normal in 95 % of our cases, the leukocyte count was in the range of 7-196 $X10^9$/L and the median WBC count was 24 $\times 10^9$ with IQR (11.8). Those results match Korejo et al in 2020 study [36].

In the current study the median level of ALC was 7.25 $\times 10^9$/L with IQR of (10.88) and the range was 5.5-161 $X10^9$/L, which was comparable with an Iraqi study [37]. ALC has been used as a prognostic factor and high lymphocyte count of more than 50,000/L has been linked to poor prognosis [38].

Regarding prolymphocyte percent, the median of prolymphocyte was (1%) and the range was (1-8%), as we did not include cases with increased circulating prolymphocytes >10% meeting criteria for CLL/PLL (prolymphocytic leukemia). It agrees with the findings of Frater et al study who stated that high percent of prolymphocyte associated with poor prognosis [39].

Identification of specific chromosomal aberrations is an important tool for diagnosis and risk stratification in CLL patients [40]. In the current study, 48 out of 50 CLL patients (96%) present with chromosomal aberrations and two patients were negative for three FISH probes which is in acceptance with Skowronska et al. who found 92% of CLL cases had genomic abnormalities [14].

The median of Hb level and median platelet count in those two patients who were negative for the three chromosomal abnormalities were higher than patients with chromosomal abnormalities and this was similar to other studies [41,40]. Moreover, negative chromosomal aberrations cases showed higher median of WBC counts compared to positive cytogentic abnormalities cases.

The current study did not find any significant correlation between the percent of the studied chromosomal aberration in CLL cells and hematological parameters. Only the percent of trisomy 12 in the cells showed a significant negative correlation with Hb concentration. This was similarly observed by Tabernero et al. study [42]; this presumably due to that trisomy clone had proliferation advantage with respect to non-trisomy cells, thus those leukemic cells will grow rapidly in the bone marrow and result in impaired hematopoiesis. This impairment had resulted in low Hb concentration, low platelet count, high WBC count and ALC in trisomy 12 cases compared to those in patients with del 11 and deletion 13. This clarifies the adverse effect of trisomy 12 in CLL patients.

Lastly the current study showed no relation between percent of deleted or trisomy cells and CD38 or light chain restriction expression. However, it was noted that the highest percent

of cases with positive CD38 were in patient carrying trisomy 12 where 73.3% of them carry CD38 compared to those with deletion 13 and deletion 11 where 56.5% and 41.6% of the patients were expressing CD38 respectively. Also, the three patients with diploid abnormalities were expressing CD38. The expression of this bad prognostic marker in those patients could explain the adverse prognosis of trisomy 12 and diploid aberration in CLL patients [43].

The lack of surface light chain in CLL is rare fact but in this study, it was observed in 35 out of 50 CLL patients. In contrast, 32 of 396 cases (8.1%) of CLL were reported by Matutes et al. to have no surface light chain [9]. This variability may be related to different factors including patient selection, inclusion criteria, and geographical differences.

The absence of immunoglobulin expression on malignant B cells can result from defects at any level from gene transcription to translocation of fully assembled proteins to the cell surface; that mean there is marked derangement of surface immunoglobulin synthesis which denote more aggressive disease [44].

From this study, it can be concluded that the frequency of deletion chromosome 13 in a sample of Iraqi adult CLL patient was the most common cytogenetic aberrations, followed by trisomy chromosome 12 and chromosome 11 deletion and deletion chromosome 13 was associated with better hematological parameters than trisomy chromosome 12. Thus, we may propose that del ch. 13 may ameliorate the hematological parameters, whereas trisomy chromosome 12 was associated with poor prognostic parameters.

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Author contribution

Dr. Hashim conducted the study, collected the data and performed the statistical analysis and drafting the manuscript. Dr. Abdulateef contributed in the designing, organization and finalization of manuscript.

Conflict of interest

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Tamoxifen as Empirical Treatment in Males with Primary Infertility and Normal Gonadotropic Hormonal Levels: A Prospective Study

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Abstract

Background One of the most commonly employed medical therapies for idiopathic male infertility are antiestrogens. Tamoxifen as estrogen receptor blockers have been used as empiric treatments for male infertility, acting through blocking estrogen receptor at the hypothalamus and pituitary levels and so preventing the important negative feedback of estrogens to the pituitary and hypothalamus as a result that leading to gonadotropin releasing hormone (GnRH) stimulation and pulsatile release of luteinizing hormone (LH)/follicle-stimulating hormone (FSH).

Objective To assess tamoxifen role in the treatment of men with primary infertility who are normogonadotropic. as it is widely prescribed drug for that purpose as well as to evaluate possible patient's criteria for a better response to tamoxifen.

Methods One hundred twenty-three patients with idiopathic primary infertility and normal FSH, LH, and testosterone serum levels were enrolled in this prospective study. All patients received 20 mg tamoxifen daily as a single dose for at least 3 months, and semen analysis (total sperm concentration, total number of progressive motile sperms and percentage of normal sperm morphology) as well hormones levels were evaluated, with the values being compared with those before treatment.

Results Overall, there was no statistically-significant improvement in semen parameters 3 months after treatment (P>0.05); however, patients with pretreatment lower normal FSH level of (1.7-6.85 mIU/ml) and those with pretreatment sperm concentration of (five to ten million per ml) showed statistically significant improvement in sperm concentration after tamoxifen therapy (p=0.044 and p=0.012 respectively).

Conclusion Tamoxifen is an acceptable treatment for idiopathic male infertility. A better response can be expected in patients with pretreatment lower normal FSH level of (1.7-6.85 miu/ml) and sperm concentration of 5-10 million per ml. It might be used as first line treatment to enhance sperm concentration, giving its availability and lower cost in comparison to other hormonal therapies.

Keywords Male Infertility, tamoxifen, empirical treatment

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List of abbreviations: A.Tamox = After tamoxifen treatment, B.Tamox = Before tamoxifen treatment, FSH = Follicle-stimulating hormone, GnRH = Gonadotropin releasing hormone, LH = Luteinizing hormone, SFA = Seminal fluid analysis, SPSS = Statistical package for social sciences

Introduction

Idiopathic male infertility is an idiopathic oligoasthenoteratozoospermia, in another words, the male has an unexplained impairment in semen parameters. On the other hand, unexplained male infertility is recognized

as infertility of unknown origin with normal sperm parameters [1].

Idiopathic male infertility has an effect on nearly 10−15% of men in their reproductive age [2]. Furthermore, the most advantageous strategies for treating idiopathic male infertility have remained unclear [3], as the pathological mechanisms behind this infertility status is unknown.

Various empirical approaches have been applied to treat idiopathic male infertility, which should be evaluated in the context of evidence-based medicine. In general, empirical treatment used in male idiopathic infertility can be divided into two groups depending on their mechanism of action: hormonal treatment and antioxidant supplementation; hormonal medications represented by gonadotropins, estrogen receptor blockers and aromatase inhibitors [4].

One of the most commonly employed medical therapies for idiopathic male infertility are antiestrogens [5]. Tamoxifen as an estrogen receptor blocker has been used as an empiric treatment for male infertility, acting through blocking estrogen receptor at the hypothalamus and pituitary levels, thus preventing negative feedback of estrogens on the pituitary and hypothalamus, leading to gonadotropin releasing hormone (GnRH) stimulation and pulsatile release of luteinizing hormone (LH)/follicle-stimulating hormone (FSH) [6].

However, up to date, there is no universal model to evaluate or identify the ideal patient for such therapy. In addition, there is no agreement regarding the optimal medical therapy and a considerable uncertainty exists as to the perceived effects on fertility [7].

Methods

Prospective study was done in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad for the period from November 2019 to July 2021.

One hundred twenty-three patients were selected as follows:

- Male patient with history of primary infertility.
- Multiple previous seminal fluid analysis (SFA) showed subnormal parameters regarding sperm concentration, sperm motility and sperm morphology, each alone or as combination.
- They should have normal FSH, LH and testosterone levels.
- No history of any chronic disease.
- No history of chronic medication abuse including exogenous testosterone.

All participants agreed and signed an informed consent after sufficient explanations. Physical examination done to ensure presence of testes, confirming normal size, and no clinically significant varicocele is present.

SFA with serum FSH, LH and testosterone levels were collected prior to the treatment.

Patients then fallowed up for at least 6 months, regular checkup was arranged with time interval of 3 months. All patients received 20 mg tamoxifen daily as a single oral dose. Semen analysis (sperm concentration, total progressive motile of sperms and percentage of normal sperm morphology according to WHO 2010 standards) as well as hormones levels were evaluated, with the values being compared with those before treatment by calculating mean value and P value which was estimated by utilizing paired t-test using statistical package for social sciences (SPSS) version 27 (2019).

Results

In general, there was an improvement in the mean values of seminal fluid parameters (sperm concentration, total progressive motile sperms and percentage of normal sperm morphology), however, improvement was not statistically significant as shown in table (1), but it was near significant in regard to sperm concentration parameter.

Table 1. Mean of seminal fluid parameters before and after treatment

SFA parameters (n=123)	B.Tamox Mean±SD	A.Tamox Mean±SD	P value
Sperm concentration (million/ml)	6.35±2.8	7.36±2.3	0.054
Total motile sperm per ejaculate	4.5±1.6	4.75±2.37	0.080
Normal sperm morphology percentage	15.4±0.06	16.0±0.05	0.963

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

Since the results showed an improvement in sperm concentration with tamoxifen that approached statistical significance, secondary analysis was performed by sub classification of the studied group according to sperm concentration prior to treatment into 3 subgroups; from 1 sperm to 5 million, from 5 million and one to 10 million and from 10 million and one to 15 million sperm concentration per ml. It has been found that the second group (5 million and one to 10 million sperm concentration per ml) got statistically significant improvement as illustrated in table (2).

Table 2. Response of studied group participants according to their baseline sperm concentration levels

Sperm concentration (million/ml)	B.Tamox Mean±SD	A.Tamox Mean±SD	P value
<5 (n=34)	2.64±0.9	2.63±0.75	0.960
5 to >10 (n=45)	8.4±1.3	10.08±2.8	0.012
10 to15 (n=44)	11.84±1.4	12.08 ±1.4	0.260

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

When hormonal levels were compared before and after tamoxifen for the 3 subgroups, there was an increase in serum levels of FSH, LH and testosterone following tamoxifen treatment by 19.4%,17.8% and 29.4% respectively, but yet all remained within normal ranges, none of these increments was statistically significant as demonstrated in table (3).

Table 3. Response of studied group participants according to their initial serum hormones levels

Hormone	B.Tamox Mean±SD	A.Tamox Mean±SD	P value
FSH (mIU/ml)	5.90±1.2	7.05±1.1	0.570
LH (mIU/ml)	5.65±0.9	6.73±1.4	0.200
Testosterone (ng/ml)	3.83±1.1	4.95±0.9	0.080

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

For more a precise evaluation, seminal fluid parameters before and after treatment were gauged against the baseline FSH, LH and testosterone level, which were divided into two categories, lower and upper normal levels.

Regarding serum testosterone and LH there was no significant difference in seminal fluid parameters for those of initial lower normal levels in comparison to patient with initial upper normal levels as shown in table (4) and table (5) respectively. Although patients with upper normal LH levels showed an increase in sperm concentration and total motility that approached statistical significance.

Table 4. Response of studied group participants according to their initial serum testosterone levels

SFA parameters	Testosterone level					
	2.27-6.29 ng/ml (n=69)		P value	6.3-10.3 ng/ml (n=54)		P value
	B.Tamox Mean±SD	A.Tamox Mean±SD		B.Tamox Mean±SD	A.Tamox Mean±SD	
Sperm concentration (million/ml)	7.56±1.9	9.13±1.5	0.150	7.45±2.1	7.9±2.0	0.100
Total motile sperm per ejaculate	3.38±0.8	4.2±0.75	0.100	4.25±0.9	4.51±1.0	0.240
Normal sperm morphology percentage	14.9±2.1	16.2±1.9	0.130	15.0±1.4	15.2±1.75	0.360

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

Table 5. Response of studied group participants according to their initial serum LH levels

SFA parameters	Serum LH level					
	1.1-4.0 mIU/ml (n=57)		P value	4.1-7.0 mIU/ml (n=66)		P value
	B.Tamox Mean±SD	A.Tamox Mean±SD		B.Tamox Mean±SD	A.Tamox Mean±SD	
Sperm concentration (million/ml)	8.04±2.9	9.37±2.3	0.080	7.05±1.9	8.1±2.1	0.060
Total motile sperm per ejaculate	3.4±0.9	4.0±0.6	0.210	4.0±0.6	4.45±0.9	0.070
Normal sperm morphology percentage	14.1±2.2	14.45±1.9	0.850	15.1±2.0	16.0±2.6	0.360

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

On the other hand, subgroup of patients with initial lower normal serum FSH levels showed a significant improvement in concern to sperm concentration but not for the other parameters as demonstrated in table (6).

Table 6. Response of studied group participants according to their initial serum FSH levels

SFA parameters	Serum FSH level					
	1.7-6.85 mIU/ml (n=60)		P value	6.85-12.0 mIU/ml (n=63)		P value
	B.Tamox Mean±SD	A.Tamox Mean±SD		B.Tamox Mean±SD	A.Tamox Mean±SD	
Sperm concentration (million/ml)	7.74±1.3	9.66±1.9	0.040	7.38±1.2	7.9±1.3	0.075
Total motile sperm per ejaculate	3.75±0.5	4.0±0.7	0.780	4.25±0.4	4.45±0.3	0.310
Normal sperm morphology percentage	15.3±3.1	17.2±2.8	0.180	14.7±2.9	16.9±1.3	0.210

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

In addition, the response to treatment was gauged against patients age; younger patients (20-30 years old) having a better response, the difference between the two age groups was not statistically significant as seen in table (7).

Table 7. Response of studied group participants according to their age

SFA parameters	Patients' age					
	20-30 years (n=66)		P value	31-40 years (n=57)		P value
	B.Tamox Mean±SD	A.Tamox Mean±SD		B.Tamox Mean±SD	A.Tamox Mean±SD	
Sperm concentration (million/ml)	7.85±1.2	9.25±0.9	0.167	7.45±1.4	8.0±1.2	0.660
Total motile sperm per ejaculate	3.38±1.1	4.25±1.3	0.090	4.13±1.1	4.58±1.4	0.070
Normal sperm morphology percentage	15.4±2.1	16.6±2.2	0.140	15.1±1.9	16.3±1.8	0.480

B.Tamox= before tamoxifen treatment, A.Tamox= after tamoxifen treatment

Discussion

Tamoxifen by acting as antiestrogens is frequently recommended medical treatment in idiopathic male infertility [(5)]. Kotoulas et al. concluded that tamoxifen is a valuable effect on sperm density was associated with tamoxifen treatment but no considerable effect was found in regard sperm motility and morphology [(8)]. Even more, Moein et al. showed that sperm recovery in testis samples can be enhanced by treating patients diagnosed with nonobstructive azoospermia with anti-oestrogenic drugs like tamoxifen and also can increase the chance of pregnancy by microinjection [(9)]. In contrast, more recent study may not agree with that as concluded by Sadeghi et al. [(10)]. Because of that, controversy re-evaluation of tamoxifen as empirical therapy is common research filed, some researchers suggested even an adverse effect of tamoxifen

on sperm quality and sexual accessory glands as Motrich et al. found [11]. The current study did not become aware of measurable adverse effect of tamoxifen on seminal fluid parameters.

Dabaja and Schlegel observed a significant increase in sperm concentration and percent of sperm motility along with significant elevation in the serum FSH and testosterone levels [12]. Kadioglu TC et al experimented a similar result regarding FSH and testosterone levels [13], in the existing study improvement in sperm concentration was observed as well in some patients and it seems to be related to pretreatment serum FSH level, while it is correct that increment in serum FSH level was noticeable but it was not statistically significant.

Kadioglu et al. noticed that patients presented with initial lower FSH levels had a significant improve concerning sperm counts in comparison to those of initial higher FSH levels [13]. A finding that goes hand by hand with present study but no significant improvement in sperm motility was established as they did. Limitations include that it is non-randomized, non-blinded, short follow-up study that is done in single center

In conclusion, tamoxifen is an acceptable treatment for idiopathic male infertility. A better response can be expected when the patient presented with pretreatment FSH level of (1.7-6.85) mIU/ml as well as in those patients of pretreatment sperm concentration of five to ten million per ml. A better response might be expected in younger patients as well. And Tamoxifen might be used as first line improve sperm concentration, giving its availability and lower cost in comparison to other hormonal replacement therapies.

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Conflict of interest

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