Research Article

Detection of biomarkers in serum and synovial fluid of Iraqi patients with knee osteoarthritis

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ABSTRACT

Osteoarthritis is believed to be the most predominant incessant joints ailment. The rate of osteoarthritis occurrences is raising a direct result of the maturing populace; it is moreover associated with a combination of both modifiable and non-modifiable hazard factors encompasses; absence of activity, weight gain, occupational injury , bone thickness, hereditary inclination, trauma, and gender. Clinical highlights that lead to treatment are agony and loss of capacity, involving life style modification, medical treatment, and surgical methodologies. The determination of osteoarthritis by clinicians is developed late in the disease process, perhaps past the point where it is possible to expect a lot of help from disease-modifying drugs. In spite of endeavors over the previous decades to create biomarkers for osteoarthritis, as yet image studies and biochemical markers examinations should be enhanced and perhaps stretched out with increasingly explicit and delicate strategies to dependably depict illness forms to analyze the ailment at a beginning period, to categorize sufferers as indicated by their anticipation, to pursue the course of ailment and therapy. In future, superior meaning of the diseases expected by outlining various phenotypes of the sickness, therapy focused more specifically at these phenotypes may prompt enhanced results.

Objective: The main objective of the present paper is the early diagnosis of osteoarthritis by detection of biomarkers in serum and synovial fluid in Iraqi patients suffering from knee osteoarthritis.

Patients and Methods: This study involves 100 participants; 50patients suffer from knee osteoarthritis and 50 healthy controls. Platelets numbers (PLT), level of erythrocyte sedimentation rate (ESR), level of C - reactive protein (CRP) and level of C3 complement were collected and studied.

Results: A significant increase in the level of biomarkers; platelet number (PLT), erythrocyte sedimentation rate (ESR), C - reactive protein (CRP) and C3 complement were demonstrated in patients with osteoarthritis compared to healthy individual. These increments demonstrate a noteworthy positive relationship between the state of patient and the severity of the disease.

Conclusion: These results provide an evidence for the significant role of biomarkers in the determination of the early stages of the disease and thus obtaining better results and improving the quality of life of the patient.

Keyword: knee osteoarthritis, synovial fluid, PLT, ESR, CRP, C3 complement

INTRODUCTION

Osteoarthritis (OA) is a persistent progressive and often irreversible joint disease which leads to handicap and weakness in joint function particularly between older patients [1]. Osteoarthritis shows up as a worldwide medical issue that includes all inclusive community [2].This degenerative and dynamic joint ailment influences around 250 million individuals overall [3] and in excess of 27 million individuals in the United States [4-5]. Despite its commonness, the pathogenesis and development of OA are still past seeing, basically because of confusing elements in human epidemiology; which involves; singular variety, exercise, nutrition state and medicinal history [6]. Immune cells assume a significant part in the formation of the disease. However, there is a weak relationship between OA symptoms, radiological lesions and our capacity to recognize early immune malady. Better comprehension of these natural instruments, may prompt better medicines for OA patients [7-8]. Knee OA constitutes a heterogeneous group of conditions prompting common histological and radiological changes.

Primary knee OA is a degenerative disorder brought about by the biochemical breakdown of the meniscus (hyaline) in the synovial joints [9]. The commonness of knee OA has been accounted for to increment in females during premenopausal age and stays high all through menopause. The explanation behind this has been uncovered in several studies that the loss of estrogen at the time of menopause increases the risk of women developing osteoarthritis [10]. Synovial fluid "joint fluid": is a thick fluid found in the cavities of the mobile joints or synovial joints. The joint is encased in a stringy joint container lined by the synovial film. The synovial layer contains specialized cells called synoviocytes. The smooth auricular ligament and synovial liquid lessen contact between the bones during joint movement. Notwithstanding giving grease in the joints, synovial liquid provides nutrients to the auricular ligaments and reduce the shock of joint compression that occurs during activities such as walking and jogging [11-12].

PATIENTS AND METHODS

A hundred persons (from the third to the seventh decade of life), fifty patients with OA diseases, admitted in Baquba Teaching Hospital and Specialized Clinics for Fractures and Joint Diseases in Diyala, Iraq and fifty healthy individual as control from September 2018 to June 2019, were included in the study. This case-study encompasses of 100 individual selected Depending on specific determination, afterward subdivided into two categories:

Group A: Involve 50 individuals suffering from osteoarthritis.

Group B: Include 50 healthy persons as control group for comparison.

A- **Diagnostic methods**

RESULTS

All patients are evaluated by:

B- **Collection and preparation of samples**

1) Venous blood samples (5 milliliters) were pulled from all individual participating in this study; these samples were placed in a clean test tubes and left for 15 minutes at room temperature for coagulation. The serum was extracted by placing samples in a centrifuge at 3000 r.p.m. then serum was subdivided and placed into new and clean test tubes (500 micro liters of serum in each test tube) and kept at temperature of – 20º C until utilized for biochemical analysis. To avoid melting and freezing of samples, serum was utilized once.

2) Auricular fluid samples (2-3 milliliters) were pulled from all individual participating in this study; these samples were placed in clean test tubes having anticoagulant. The synovial fluid was extracted by placing samples in a centrifuge for 10 minutes at 3000 r.p.m. then samples was subdivided and placed into new and clean test tubes and kept at temperature of – 20º C until utilized for biochemical analysis. To avoid melting and freezing of samples, serum was utilized once. The measured parameters include: (level of PLT, ESR, CRP, and C3 complement).

Statistical analysis

Statistical analysis was done utilizing the statistical package for social science program no. 21 as for illustrative factors. X^2 test was done for comparison and describing numbers and percentages. Mean and standard deviation were utilized to portray numerical formula with variables. More so, T test was used for comparison between 2 groups and for comparison between more than 2 groups we utilized ANOVA test. *P* value (<0.05) was considered the cutoff point of significance.

Table (1): Distribution of study samples depending on personal characteristics

		Positive family history	Stable life	obesity	Other disease
Patients	No.(50)	41	42	46	43
	%	82%	84%	92%	86%
Control	No.(50)	33	34	18	15
	%	66%	68%	36%	30%

(*) implies significant difference ($P< 0.05$)

Table (1) demonstrates the distribution of all participants (patients and controls) according to personal characteristics, showing that positive family history which is one of the risk factors, while stable life for patient group compared with control group was found to be not linked with the development of symptomatic osteoarthritis. Same thing was applied to obesity and other disease compared controls. The results of knee OA appeared in significant at *P*- value < 0.05.

Table (2): Measurement of biochemical markers; platelet number (PLT), erythrocyte sedimentation rate (ESR), C - reactive protein (CRP) and complement protein (C3) in all participants according to gender

Parameters (Units)	Gender	Group A Patients/ No. (50)	Group B Control/ No. (50)
PLT1000/mm³	Male	107322.2±145100.2	289.77±62.2
	Female	(165516.9±16111.4)*	300.71±77.9
ESR h/mm	Male	19±18.6	14±4
	Female	(29±21.4)*	15±6
CRP mg/l	Male	14.2±16.5	43±1.7
	Female	(22.7±24.1)*	44±2.0
C3 mg/l	Male	114.8±24.2	111.5±26.4
	Female	(133.8±39.6)*	120.7±65

(*) implies significant difference ($P< 0.05$)

data was presented as (Mean ± SD). Table (2) demonstrate a high increment in the levels of; Platelet number (PLT), erythrocyte sedimentation rate (ESR), level of C - reactive protein (CRP) and level of C3 complement protein in Iraqi female patients with OA in comparison with both male participants of the same group and control group of both gender.

Table (3): Measurement of Complement protein (C3) in serum and synovial fluid for patients suffering from osteoarthritis according their age

Groups/Age	Parameters (Units)	
	C3 in serum mg/l	C3in SF mg/l
20-29	115.2±25.4	0±0
30-39	128.2±24	40.5±43.3
40-49	120.3±41.9	66±32.8
50-59	142.9±34.5	78.5±36.7
60-69	(150±46.9)*	(79.5±16.7)*
70-79	(173.5±27)*	(82±3.2)*

(*) implies significant difference ($P< 0.05$)

data was presented as (Mean ± SD).Table (3) demonstrates a significant increase in the level of complement protein (C3) among OA patients who were in the sixth and seventh decade of life and this increase was in both samples (blood and synovial fluid).. (4): Measurement of biochemical markers; platelet number (PLT), erythrocyte sedimentation rate (ESR), C- reactive protein (C-RP), and complement protein (C3) in patients according to the state of knee osteoarthritis (KOA

Table (4) demonstrate the increments in biomarkers (PLT, ESR, C-RP) in patients according to the state of knee osteoarthritis, where there is a significant increase in the level of both (PLT and ESR) in very severe condition. While in severe condition there is a significant increase in the level of (C-RP) only.

Groups / State of KOA	Parameters (Units)		
	PLT 1000 / mm³	ESR mm / h	C-RP mg / l
Very severe	(390333.3±67099.4)*	(46±21)*	20.8±7.05
Severe	279360±66710	40.5±24.2	(42.1±27.1)*
Moderate	309590±94760	36.4±24.2	32.4±14.5

(*) implies significant difference ($P< 0.05$)

data was presented as (Mean ± SD)

Table (5): Grades of KOA with and without pain according to radiographic descriptions

Groups/ Grade of KOA	Radiographic descriptions	KOA with pain%	KOA without pain%
Grade 0	No osteophyte, normal joint space.	2%	1%
Grade 1	Possible osteophyte, doubtful joint space narrowing.	10%	5%
Grade 2	Definite osteophyte, possible joint space narrowing.	18%	6%
Grade 3	Moderate osteophyte and sclerosis, definite joint space narrowing.	28%	3%
Grade 4	Large osteophyte with marked joint space narrowing, sclerosis.	15%	1%

(*) implies significant difference ($P < 0.05$).

Table (5) demonstrates grades of KOA with and without pain according to radiographic descriptions. This table shows that most KOA patients are presented with pain as the grade increase from zero to four compared to KOA patients without pain.

DISCUSSION

From table (1) of this case control study, there is an agreement with the studies of Jenny, et al. [13] and Kristiann, et al [14] that shows Stable life behaviors appear to be unrelated to the development of symptomatic osteoarthritis. In this work, stable life seems to be associated with the development of KOA, but statistically it was not important. Regular exercise, in addition to facilitating weight control, alone or in combination with a diet, improves many of the metabolic abnormalities associated with both obesity and other joint problems. Result in table (2) of the present study shows agreements with Aminet al [15]. The researcher showed high ESR and C-RP, and pointed to the association of PLT with ESR, where the decrease in the number of platelets lead to slow in the speed of ESR, and in another study by Pricilaet al [16] shows that platelets are affected by progesterone and estrogen hormone in females, so the number of platelets changes during the different stages of a woman's life before and after menopause (menopause) compared to males. The researcher pointed out that osteoarthritis is an infection that leads to high platelets, especially in females who are more susceptible to the disease. Gabriel et al [17] demonstrate the presence of complementary protein C3 in the lining of the uterine membranes and high effectiveness in females with knee osteoarthritis. From table (3) there is an agreement with the study of Morgan et al. [18] which found that supplemental C3 increased by 65% in the serum and synovial fluid of patients aged from 50-71 years with knee osteoarthritis in comparison with younger patients and healthy individual. Also in similar study by Gkretsiet al. [19] patients with knee osteoarthritis were assessed for the level of C3 supplementation in serum and synovial fluid. This study shows an increase in C3 concentration in both serum and synovial fluid in patients aged from 60-81 years compared to younger patients. Results of the present study are agreed with the study carried out by Page, C. [20] as the rise of (PLT) and (ESR) in the very severe state of patients with KOA. The researcher pointed out that (PLT) is mainly involved in the repair of damage and inflammation and its role is highly regulated in the functional response of the aggregation and formation of fine particles at the site of auricular cartilage damage and explained that ESR increases with age and the severity of very severe cases. While Ramonda et al. [21] reported that increased C-RP concentration correlates with the severity of the condition, it rises when knee osteoarthritis develops in patients and can be considered a predictor of diagnosis. Our results agreed with Geilan et al. [22] that show Egyptian patients with KOA rejected mainly by pain component of the KOA questionnaire, disease duration and bilateralism were predictors for lower scores in all questionnaire domains, knee stiffness for four subscales. Hypertension, knee deformity and K-L classification were good predictors for lower scores of two subscales.

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