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The Role of Innate Immune Response And IL-6 IN Osteoarthritis Patients in Basrah Province

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Abstract

Background: Osteoarthritis (OA) has been observed as degeneration disease of joint cartilage and the underlying bone affecting millions of people worldwide. OA has traditionally been classified as a non-inflammatory arthritis but with evidences accumulation indicates the role of immune response and inflammation in OA onset and progression.

Aim of study: To shed the light on role of innate immune response, CRP and IL-6 level in the development of OA in Basra province.

Patients and methods: 55 osteoarthritis patients (11 males and 44 females) and 70 healthy individuals as control (24 males and 46 females) were participate in this study. Total and differential WBCs count in whole blood in addition to the levels of all ESR, CRP and IL-6 were measured in serum for all participants.

Results: The results were recorded a significant increasing in total WBCs count, neutrophils and monocyte in addition to the level of all inflammatory markers (ESR, CRP and IL-6) in osteoarthritis patients as compared with healthy control. While lymphocytes and platelets count record an increase in OA patients but insignificant . A significant positive correlation was found between IL-6 level in serum and each of CRP, ESR, WBCs and the platelet. In addition to a significant correlation was found between CRP and both of WBCs and ESR.

Conclusion: The results indicates the active participation of inflammatory response in development of osteoarthritis in addition to the importance role for IL-6 as considered multifunctional cytokines effect different immunological parameters in the body and has adverse effect in development of OA.

Keywords: Osteoarthritis, inflammatory response, CRP, ESR, IL-6

Introduction

Osteoarthritis (OA) is a chronic and dynamic disorder characterized by a progressive degeneration of articular cartilage , bone remodeling leading to joint space narrowing (JSN) associated with pain, and loss of function ([1] ,[2],[3]) .Any joint can be affected by OA but more frequently the weight-bearing joints particularly knees and hips. ([3])

Numerous risk factors have been related to that disease including genetic predisposition, age, sex, obesity, reproductive status (e.g., postmenopausal) in females, individuals variation in physical activity and a history of previous joint trauma. ([1],[3],[4],[5])

Initially, OA involves a low-grade inflammatory disease (innate immune response) before a mild degree of adaptive immunity. ([6] ,[7],[8]) During tissue damage, damage-associated molecular pattern (DAMP) which are group of endogenous molecules, provide signals to the innate immune cells (like macrophages and mast cells) mediating a protective response and enhancing both wound healing and tissue repair. Mast cells regulate vascular permeability, facilitating leukocyte infiltration ([8])while macrophages display a plasticity function depending on the environment where they are present. Upon prolonged stimulation by DAMP molecules and chronic activation , macrophages can lead to more producing of proinflammatory cytokines that make OA joints worst. ([9], [10])

Several proinflammatory cytokines have been found to play a role in OA progression, one of them is IL-6. IL-6, is an important pro-inflammatory cytokine produced by many non-immune tissues and by many immune and nonimmune cells like T cells, B cells, monocytes, macrophages as well as fibroblasts and osteoblasts ; in vivo IL-6 is considered as a central mediator for regulating immune and inflammatory responses. ([7],[11],[12])

Healthy chondrocytes produce IL-6 in low amount without stimulation but upon exposure to certain cytokines during inflammation like TNF-α and IL-1β, chondrocytes will increase its production([3],[7]). The exact mechanism action of IL-6 in OA is not yet understood , some *in-vitro* studies showed that IL-6 will inhibit type II collagen production by rabbit articular chondrocytes ([13]) and the combination of IL-6 with IL-1β and TNF will regulate matrix metalloproteinases (MMP-1) production in human and bovine chondrocytes as

well as it can induce proteoglycan degradation([14]). The increasing level of IL-6 in synovial fluid and plasma of OA patients is positively correlated with the severity of radiographic findings ([15]) . IL-6 is considered the principal stimulator of acute-phase protein synthesis through hepatocyte stimulation. ([11])

Another sensitive systemic marker for inflammation during OA is C- reactive protein (CRP).CRP is one of the most important acute phase proteins produced from many sites within the human body, mostly from the liver. ([16]) During acute inflammatory disease, CRP test can be considered as a diagnostic tool as well as a good indicator for the response to the treatment in addition it can assess whether the infection is existing in active stage or not. ([4],[12])

Many studies showed that elevated CRP level in OA patient's sera have been associated with disease progression as well as with the severity of pain and its level will dramatically increase with the elevation of circulating pro-inflammatory cytokines levels like Interleukin (IL) 6 and IL-1. ([4],[17], [18],[19]) .CRP level will rise above its normal limit within 6 hours after getting infection and peaks at 48 hours and its concentration will regulate by the body immunity. ([20])

The aim of the present study was conducted to evaluate innate immune response components, inflammatory markers IL-6, CRP and ESR in patients with KOA in compared to apparently healthy control in Basra province).

Material & methods

Studied groups

This case–control study was conducted for the period from June 2019 to February 2020. One hundred twenty-five individuals were involved in the study with age range 30-65 years.

The patient group comprised 55 patients (11 males and 44 females) attended the Rheumatology and medical Rehabilitation clinic in both Al-Sader Teaching Hospital and Al-Fayhaa Teaching Hospital of Basra province. All OA patients included in the study have either unilateral or bilateral knee OA. They were examined clinically and diagnosed and classified depending on Kellgren-Lawrance classification of knee OA([21]).Any patient with secondary OA or having any systemic illness like hypertension, metabolic syndrome, endocrine disease, cancer or rheumatic diseases was excluded.

The control group included 70 (age- and sex-matched) apparently healthy individuals (24 males and 46 females). Sociodemographic information and family history of OA were enquired about using a special questionnaire designed for the study's purpose.

Body weight and height were measured and body mass index (BMI) were calculated using the formula BMI equals weight/Height^2 in (kilogram/meter^2).

The National Institutes of Health (NIH) classified subjects according to their BMI as:

Normal weight: 18.5-24.9 kg/m^2

Overweight: 25-29.9 kg/m^2

Obese: ≥ 30kg/m^2 ([10])

Blood samples

Five milliliters of blood were drawn from all patients enrolled in this study and then divided into two tubes ;(3) ml of blood were kept in EDTA tubes for estimation the WBCs counts and ESR. The remaining (2) ml of blood were kept in vacuum tube without anticoagulant for serum isolation which was used for measurement of CRPs and IL-6.

Hematological test

- **Determination of total and differential WBCs count & ESR**

WBCs counts (total and differential) were done by using automated hematology analyzer (Coulter counter, Sysmex xt-2000i Japan) in Al-Sader Teaching Hospital and Al-Fayhaa Teaching Hospital. Erythrocyte Sedimentation Rate (ESR) was estimated using westergrens method and the value was expressed in (mm/hr)

Serological test

- **Measurement of serum IL-6**

IL-6 level was measured in the serum of all people enrolling in the study by (ELISA) kit (myBioSource/USA) for in vitro quantitative measurement of human IL-6 and this test was done according to manufacturer's instruction.

- **Measurement of CRPs**

Serum level of CRPs for all individuals was detected by using of Cobas Integra 400 Plus automated analyzer (Roche Diagnostics, Rotkreuz, Switzerland) in both hospitals.

Statistical analysis

All the results were recorded as (mean ± SD). For comparison between the two groups, independent sample T-test was used. Analysis was done by using SPSS software, version 24 for windows

Results

Fifty-five KOA patients and 70 healthy controls were enrolled in the study. The demographic characteristic features of them were illustrated in (Table 1) which showed there was no significant differences between the studied groups in relation to their age which they were very close to each other.

During the study most of the participants are females 90 (72%) from these females 59 (65.6 %) have menopause (31 cases and 28 controls). A significant difference was recorded in BMI between KOA patients and healthy controls.

Table 1. Characteristics of the studied groups. Means were compared by t-test

	OA Cases =55 (mean ±SD)	Controls= 70 (mean ±SD)	P value
Age	54.7±9.8	53.9 ± 9.9	0.787
BMI (kg/cm2)	33 ±6.5	29 ±3.4	0.000

* Note: values are presented as mean and standard deviation (SD). Kg: kilogram. Cm: centimeter.

* P > 0.05 is not significant; P<0.01 is a highly significant

According to the Body Mass Index (BMI) results, it was found that all KOA patients had increase in weight; 19 (34.5%) were overweight and the remaining 36 (65.5%) were obese. All of obese KOA patients are over 50 years of age and 30 of them were female. In 26 obese OA patients, both legs were affected.

Table (2) shows a comparative analysis of blood parameters between the two groups. A statistically significant increase in total WBCs count, neutrophils and monocyte was found in OA patients compared to the controls, while the mean levels of lymphocyte and platelets count were insignificant increased in OA patients than the controls.

Table 2. Association between study groups and different hematological parameters

	OA Cases=55 (mean ±SD)	Controls=70 (mean ±SD)	P value
WBCs	7.48 ± 2.1	6.32 ± 1.34	0.008
Neutrophil	56.16 ± 6.8	41.1± 8.6	0.017
Monocyte	7.9 ± 1.73	6.8 ± 1.25	0.029

Lymphocyte	35.2 ± 8.8	34.32 ± 6.6	0.88
Platelet	261 ± 66.7	232 ± 65.6	0.731

* P<0.05 is significant; * P<0.01 is a highly significant

All the studied inflammatory markers, including acute phase proteins (CRPs), ESR and IL-6 were increased significantly in the OA patients and their results were presented in table (3).

Table 3. Association between studied groups and inflammatory markers

	OA Cases =55 (mean ±SD)	**Controls= 70 (mean ±SD)**	**P value**
CRPs	6.57 ± 5.06	2.3 ± 0.82	0.000
ESR	18.3 ± 8.2	4.11 ± 0.8	0.000
IL-6	80.5 ±41 .2	44 ± 18.4	0.000

* P<0.01 is a highly significant

Current study found that CRP level was significantly increased in obese KOA patients compared to overweight patients (p= 0.022) , while ESR and IL-6 levels although being higher in obese patients compared to overweight patients, the difference was statistically insignificant.
In KOA patients group, a correlation was found between IL-6 level in serum and each of CRP (r = 0.606) ,ESR (r = 0.237) , WBCs (r=0.140) and platelet (r=0.106) but no correlation was noted with BMI. Also, in the same group, a correlation was found between CRP and both WBCs (r =0.264) and ESR (r =0.182) (Figure 1)

Discussion

Although osteoarthritis is considered an age-related degenerative joint disease-causing irreversible damage to the cartilages, it was found that the immune system, in all its branches, is involved in the OA pathogenesis. Many factors like metabolic, genetic, or mechanical are responsible for the initiation of cartilaginous injury leading to the release of several cartilage specific auto-antigens, which can stimulate the immune response involved in the synovial inflammation and the production of proinflammatory cytokines ([10] ,[22.23]).
The main general factors which were investigated in current study that affect the development of OA are age, obesity, sex and hormonal status. OA is widely distributed in elderly. Moreover, obesity was found to be an important susceptible factor responsible for development of KOA, as all KOA patients had an increase in weight and 65.4 % of them were obese and this was agreed by many studies ([24] , [25], [26]) This could be attributed to either the reduction of movement, due to OA, causing weight gain as OA is the most common chronic disease in later life, or the subject may already be obese, and this obesity will reduce the physical activity and increase the forces at weight-bearing joints ([25], [26]). In related to the sex and hormonal state , this study reported that OA was more distributed among females than males particularly in postmenopausal women, a result that is consistent with many studies ([1], [5],[12]) . This may be attributed to the decreased level or lack of estradiol in postmenopausal women which may increase the risk of OA because estrogen has anti-inflammatory effects ([12]).
The first changing signs recorded during hematological analysis in current study were the significant elevation in total WBCs including neutrophils , monocytes and lymphocytes in KOA patients as compared with healthy controls for the both sex .These results were in agreement with Zhang *et al* (2015)([27]) and Amer *et al* (2018) ([28]) . This usually occurs as a result of first body's responses that are derived from the innate immunity which will be followed by the adaptive immunity ([29]) . Neutrophils are important part of innate immune cells which participate actively in the development of knee osteoarthritis as upon activation during acute inflammation, they will release proteolytic enzymes and reactive oxygen intermediates which can cause tissue destruction and joint damage([11]) In addition to that, neutrophils in patients with active OA will start spontaneous release of inflammatory cytokines like (TNF-α , IL-1 and IL-6) that drive the differentiation and activation of other cells (dendritic cells and macrophages) ([23]) .In addition these cytokines together stimulate osteoclast formation which has adverse effect as it cause bone resorption and will activate the production of MMPs that affect the surrounding cartilage ([7])
The increase in monocytes is associated with non-antigen-specific responses that cause monocytes migration from the blood vessels and differentiation into macrophages. During OA progression, macrophages become more activated by different mediators and release pro-inflammatory cytokines which are responsible for removal and cleanup of debris and microbes before remodeling can occur. In addition to that, macrophages are also responsible for the down regulation of inflammation and tissue repair initiation ([29]). In the other hand, the increase in lymphocytes is correlated with their role in the development of adaptive immune response by differentiating into B and T cells (as they are present in high numbers in OA patients than in healthy controls) in addition to their role in production of inflammatory cytokines (IL-1β and TNF- α) that play an important role in the breakdown of the joint's cartilage ([29],[30]).

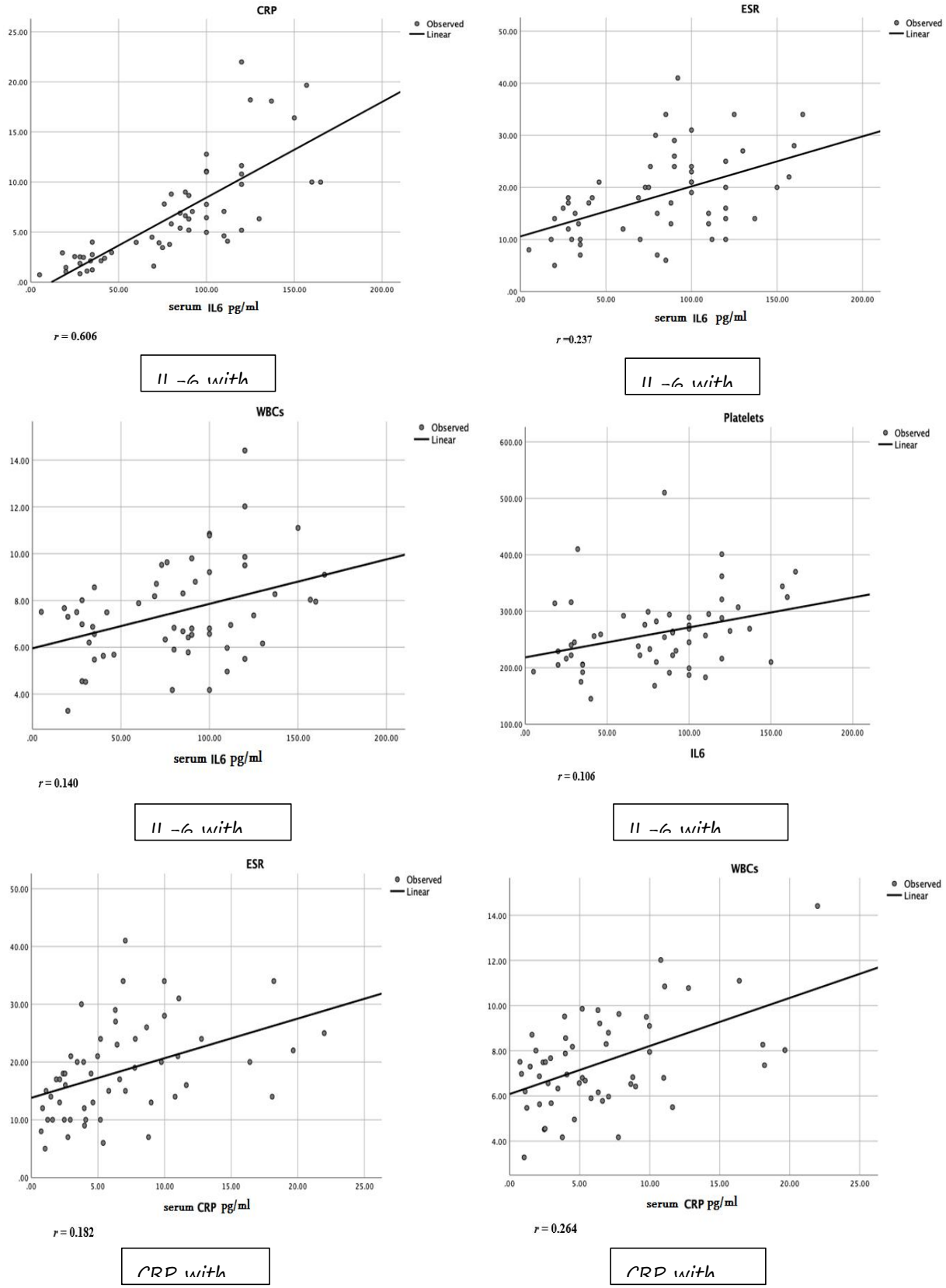

Figure 1. Correlation of IL-6 and CRPs in serum of 55 OA patients with different parameters

The result of this study reported that platelets count increase in patients with KOA and that agreed with Amer *et al* (2018)[28] and Kwon *et al* (2020)([31]). Platelet is an important factor affecting OA as it will release different chemokines and cytokines during its activation that participate in attracting leukocytes as well as promoting adhesion to damaged vascular endothelium during the inflammatory response and this process is be regulated through the action of IL-6 and thrombopoietin ([31], [32]). *In vivo* administration of IL-6 appears to has a role in generation of megakaryocyte

(Mk) and platelets at time of acute inflammation ([32])

The most important inflammatory indicator focused on during this study was IL-6 which showed a significant increase in KOA patients than in healthy controls and that agreed many studies ([12],[25],[33]) . During a longitudinal study that lasted for (2.9-year) targeting elderlies, Stannus and his team found that circulating IL-6 had a role in the pathogenesis of knee OA and could induce cartilage destruction starting from acute inflammation by stimulating neutrophil migration toward chronic by increasing monocytes recruitment as well as activating osteoclasts (which are the key cells involved in erosions of arthritis) and stimulating the production of matrix metalloproteinases (MMP) from synovial cell and chondrocytes ([11],[25],[33]).

There are many non- inflammatory factors contributed to rise in IL-6 concentration, one of them is the age even in the absence of infection, stress, or trauma. IL-6 will increase and that is due to several factors causing down-regulation of IL-6 gene expression like estrogen and testosterone which decrease with age progression ([34],[35],[36]) Decrease estrogen level in postmenopausal women is associated with increased level of IL-6 as the binding of estrogen receptors with nuclear factor inhibitors will block IL-6 genes expression ([12]).

The second factor is obesity, as adipose tissue is an important source of IL-6 and CRP in the absence of inflammation ([25] ,[37]).

The next most frequently used diagnostic indicators for OA investigated in this study were ESR and CRP both of them were significantly increased in OA patients and that agreed with many studies ([2],[12],[19],[28]).CRP concentration decreases several days after the body's inflammation is controlled earlier than ESR which continues to remain high for more time provided early evidence of response to antibiotic than ESR ([2], [12], [28]).

Sipe in 1995 and Paik JK *et al* in 2013 reported a slight increase in serum CRP concentration with age that may be act as predisposing for inflammatory response development as CRP has been observed to interact with white blood cells ; increase phagocytic activity and chemotaxis of monocytes ([2]. [35]).

Conclusion

OA develops as a result of many predisposing factors, the most important are age, obesity and immune response. IL-6 is a multifunctional cytokine affecting different immunological parameters in the body and has an adverse effect in development of OA as a strong correlation was found between the elevation of IL-6 and many parameters in the body indicating an important role for IL-6 in the regulation of OA development and progression.

This study requires more development by studying the gene predisposing factor for OA and studying the treatment that reduce the proinfammatory cytokines.

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