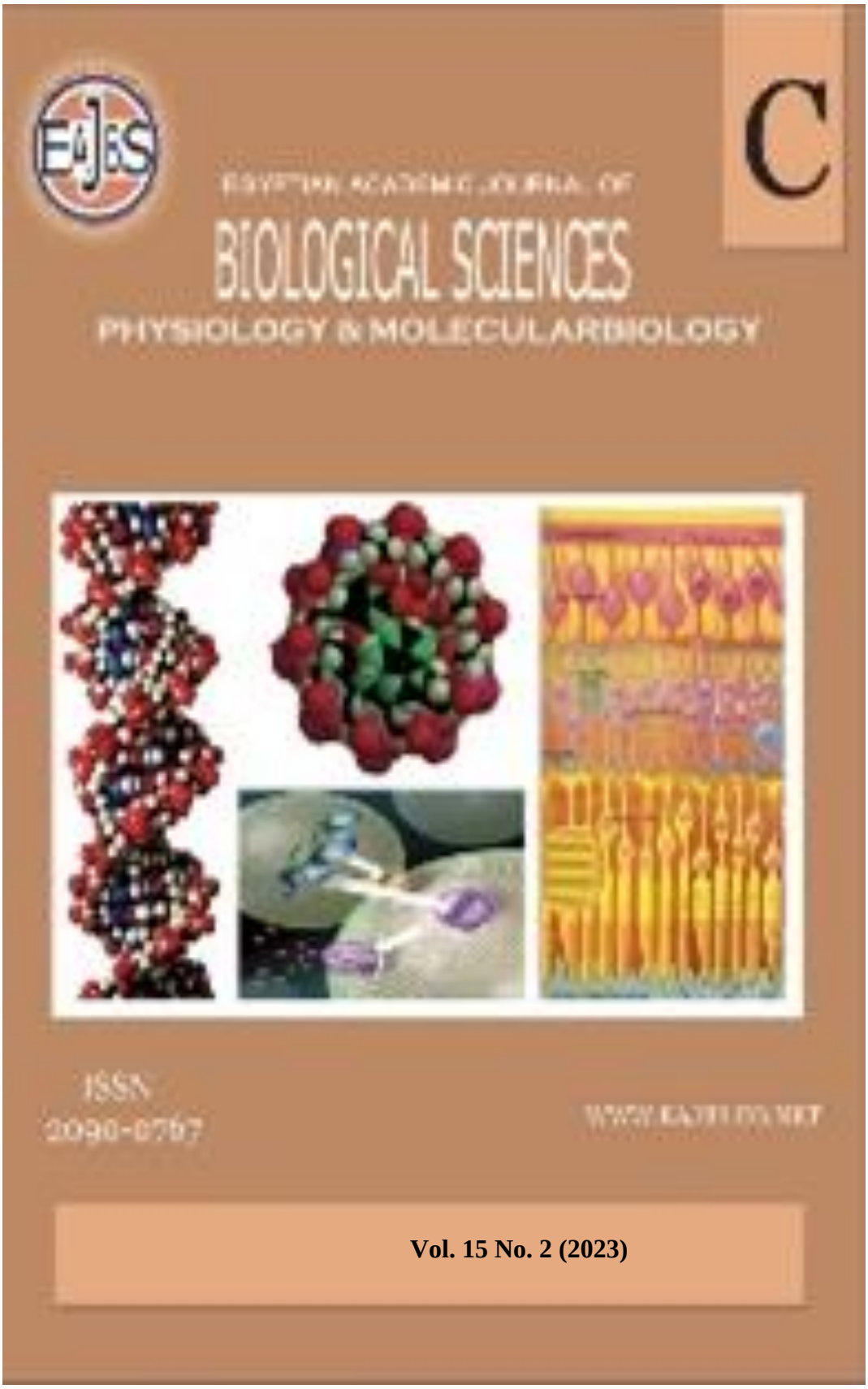

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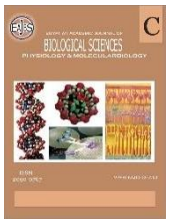

Study the Association of IL-21, IL-17, CD163, and hs-CRP with Activity of Knee Osteoarthritis Among Iraqi Patients

Alrawi, Yaqeen Abood[1] and Huda Rafaa Sabbar[2]
[1]Department of Biology, medical college, Anbar university, Baghdad 2016.
[2]Department of Microbiology, College of Medicine, Alanbar University.
***E-mail:** yaqeenalrawi93@gmail.com ; Huda1973@uoanbar.edu.iq

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ABSTRACT

Objectives: The current work was designed to assess the roles of IL-21, IL-17, CD163, and hs-CRP in the activity of osteoarthritis of Iraqi patients**. Patients and Methods:** Sixty (60) patients with age groups ranging from (45 -70) years suffering from knee osteoarthritis were involved in this work**.** Any gender with age > 45 years with knee pain. Patient's diagnosis with KOA according to the American College of Rheumatology (ACR) reviewed criteria of early diagnosis of KOA. Data collection Age, gender, body mass index, disease duration, disease severity and medication were collected from all patients. Sixty (60) apparently healthy volunteers without a previous history of knee osteoarthritis and any of the exclusion criteria were selected from our population as the control group. Three milliliters (3ml) of peripheral blood were obtained via venipuncture and poured into a plain gel tube to separate the serum. Serum specimens were kept in tubes at -20°C to be used for detection of these markers by Sandwich ELISA technique. **Results**: The mean levels of hs-CRP, CD163, and IL-17 were significantly higher in Knee osteoarthritis patients when compared with healthy controls (5.71 mg/dl vs 0.84 mg/dl, P= 0.001; 60.81 ng/ml vs 48.53 ng/ml, P= 0.001; and 177.2 pg/ml vs 30.29 pg/ml, P= 0.003, respectively). On the other hand, no significant difference ($P \geq 0.05$) was found in the mean levels of IL-21 between the two studied groups. There is a relation only between IL-17 and hs-CRP with the severity of the disease, this is indicated by P-value (0.001 and 0.009) respectively.**Conclusion**:This study concludes that levels of some immunological markers were elevated in osteoarthritis patients as IL-17, hs-CRP and CD163. IL-17 and hs-CRP levels were higher in grades 4 and 3 than in grade 2 and, therefore can be considered as an indicator of the severity of this disease.

INTRODUCTION

The most common degenerative disorder seen in elderly people is Osteoarthritis; OA is a major contributor to severe daily-life impairment. There are many different etiologies for osteoarthritis, which are identified by pathological alterations in the structure of the joint, including degradation of cartilage, synovial inflammation, and subchondral sclerosis with osteophyte production. Chronic pain, joint instability, stiffness, and radiographic joint space constriction are the main clinical signs (Wang *et al* 2017). Post-traumatic Osteoarthritis can result from traumatic sports injuries at any age, even though it primarily affects the elderly. Phenotyping patients by detecting a marker in the blood, rather than by subjecting them to arthrocentesis, would obviously be more conducive to their cooperation (Helmick *et al.*, 2008).

The role of inflammation is not well understood and there is an ongoing debate to determine if the inflammatory reaction triggers the change of Osteoarthritis, or instead, the inflammation is secondary to the Osteoarthritis changes. Different from inflammatory arthritis, inflammation in Osteoarthritis is chronic and low-grade inflammation, involving mainly innate immune mechanisms (Ayhan *et al.*, 2014). Osteoarthritis and rheumatoid arthritis are both diseases of joints, but they have very different etiologies. Osteoarthritis is a disease assumed to result from wear and tear over time, whereas rheumatoid arthritis is an autoimmune disease where the body's immune system attacks joint tissues (Richards *et al.*, 2016). Higher levels of IL-17A were observed in the synovial fluid and serum of cases with knee OA in comparison with the healthy control group, and this difference was associated with pain, function, and disease severity (Wang *et al*., 2017). When OA patients' inflamed synovia was compared to non-inflamed synovia, a high concentration of IL-17A was discovered, which was linked with the rise of TGF-β1, IL-23 and IL-6 (Deligne *et al.,* 2015).

In Rheumatoid arthritis, there is an association between the IL-21 and IL-17 levels in patients' sera and synovial fluid, allowing the Th17 cells' development that support the ongoing immune response. Since its levels are connected with those of IL-6, lowering IL-6 also lowers IL-21 levels (Spolski *et al.,* 2014). However, it is still unclear how OA coordinates with the underlying immunologic process. In patients with degeneration of cartilage, the majority of synovial fluid samples contain IL-21. Higher amounts of IL-21-follicular helper T-cells and IL-21 were located in a more recent review, and these results linked with the OA severity as measured by Western Ontario and McMaster (WOMAC) scores and C - reactive protein levels, indicating that IL-21 may act as a biomarker for OA (Shan *et al.,* 2017).In the presence of the hemoglobin-haptoglobin complex, the protein CD163 has a high-affinity scavenger receptor, whereas, in the absence of haptoglobin, it has a lesser affinity for hemoglobin alone. A marker for cells from the lineage of monocyte/macrophage is CD163. The expression of CD163 on the cell surface is restricted to the lineage of monocyte/macrophage and is induced by glucocorticoids and certain cytokines (IL-10 and IL-6), which are anti-inflammatory signals. Lipopolysaccharide (LPS), TNFα, and IFNγ are pro-inflammatory mediators that suppress gene expression and CD163 cell surface (Daghestani *et al.,* 2015).

According to one study, OA synovial tissue had more CD163 and mononuclear cells than rheumatoid arthritis synovial tissue (Tsuneyoshi Y *et al.,* 2012). Numerous signals, such as LPS and phorbol 12-myristate 13-acetate (PMA) cause the ectodomain of CD163 to be cleaved by a metalloprotease. This causes soluble CD163 to be shed from the surface of macrophages. (Van Gorp *et al.,* 2010). Researchers found that biomarkers, which are soluble macrophages, indicated the frequency of activated macrophages and mediated the relationship between pain in OA knees and structural progression (CD163).

C-reactive protein has historically been used to identify "non-inflammatory" diseases like OA from systemic inflammatory disorders like RA. In addition, the invention of hsCRP, which can reveal levels of CRP that are an order of magnitude lower than earlier approaches, low-level spikes in CRP have been discovered in diseases where it is low-grade, a local component of inflammation. Even though inflammation is simply a biomarker, it may reflect or contribute to the underlying biological components of disease condition (Pearle *et al.,* 2007). Some data showed that hs-CRP may have prognostic and clinical significance, despite its limited value as a diagnostic marker of

OA since it is a nonspecific measure of inflammation (Stürmer *et al.*, 2004). In OA, both disease progression and clinical severity have been linked to elevated hsCRP levels in sera. It was shown that a high level of hsCRP can suggest events that occur before radiographic progression in OA but ultimately contribute to it. Furthermore, it has been found that increased hs-CRP is linked to the intensity of pain in OA. However, it's still unclear how high hs-CRP levels are associated with the activity of disease (Stürmer *et al.*, 2004).

MATERIALS AND METHODS

Sixty (60) patients with an age group ranging from (45 -70) years suffering from knee osteoarthritis were included in this work.

The gender, age, body mass index, disease duration, disease severity, and medication were reported from all patients. The selection of each case was established by the clinical examination done by a rheumatologist, and the samples were obtained during the period extended from October 202l to April 2022.

Sixty (60) apparently healthy volunteers without a previous history of knee osteoarthritis and any of the exclusion criteria were chosen from the population as the control group. Blood specimens were taken from the patients and control groups, three milliliters (3ml) of the blood was obtained via venipuncture and poured into a plain gel tube after sterilization of the skin with antiseptic material.

The blood specimen was centrifuged at 3000 rpm for 5 minutes to separate the serum. Serum samples were kept in tubes at -20°C to be used for detection of the level of immunological parameters.

Sandwich-ELISA method is used for the detection of these immunological parameters as construction methods of kits.

RESULTS

The mean age in the patients' group was 59.81 ± 6.98 years versus 58.8 ± 5.80 years for the control group and the highest proportion of the patient group aged 45-56 years 21 patients (35.6%) while 24 (40%) of the control group were found in the age group of (56 – 60) years.

In this study, there was no statistically significant difference ($P \geq 0.05$) was found in the age between the two groups, as shown in (Table 1).

Table 1: Comparison between study groups according to grades.

Variable	Study Groups		P- Value*
	Knee OA group n= 60	Control Group n= 60	
Age (Years)			
45 _ 56	22 (36.7)	15 (25.0)	**0.133**
56 – 60	13 (21.6)	24 (40.0)	
61 – 65	10 (16.7)	6 (10.0)	
> 65	15 (25.0)	15 (25.0)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 levels.

Regarding grades of knee osteoarthritis, 30 patients (50%) were in grade two, 14 (23.3%) in grade three, and 16 (26.7%) in grade four as in Table (2).

Table 2: Distribution of the patients according to grades.

Clinical Characteristics	No. (n= 60)	Percentage (%)
Grades of Knee OA		
Grade 2	30	50.0
Grade 3	14	23.3
Grade 4	16	26.7

Comparison of Biomarkers in The Studied Groups:

The comparison in the mean value of certain biomarkers showed that there was a statistically significant difference (P<0.05) between the patients and control groups in the levels of hs-CRP, IL-17, and CD163. The mean values of hs-CRP, CD163, and IL-17 were (5.71 mg/dl vs 0.84 mg/dl, P= 0.001; 60.81 ng/ml vs 48.53 ng/ml, P= 0.001; and 177.2 pg/ml vs 30.29 pg/ml, P= 0.003, respectively). On the other hand, non-significant difference (P ≥ 0.05) was found in the mean levels of IL-21 between the two studied groups. This study showed no significant difference in IL-21 this may be due to all patients being at an advanced stage of the illness and the samples used in this study were blood rather than synovial fluid, as shown in (Table 3).

Table 3: levels of hs-CRP, CD163, IL-17 and IL-21 in patients and controls.

Biomarker	Study groups		P - Value*
	Knee OA group Mean ± SD	Control Group Mean ± SD	
hs-CRP (mg/dl)	5.71 ± 4.66	0.84 ± 0.63	**0.001**
CD163 (ng/ml)	60.81 ± 19.42	48.53 ± 16.56	**0.001**
IL-17 (pg/ml)	177.2 ± 41.7	30.29 ± 21.93	**0.003**
IL-21 (pg/ml)	35.08 ± 22.23	42.62 ± 14.37	**0.369**

Relation of Markers with Grades:

There is a relation only between IL-17 and hs-CRP levels and the severity of the disease, this is indicated by P- value (0.001 and 0.009 respectively as shown in Table (4).

Table 4: The relationship between hs-CRP, CD163 and IL-17 with knee OA grades.

Grades of knee OA (hs-CRP)		
Grade 2	2.04 ± 0.87	**0.001**
Grade 3	6.97 ± 1.93	
Grade 4	11.51 ± 7.55	
Grades of Knee OA (CD163)		
Grade 2	60.67 ± 21.51	**0.349**
Grade 3	66.49 ± 19.50	
Grade 4	56.10 ± 14.37	
Grades of Knee OA (IL-17)		
Grade 2	36.29 ± 15.20	**0.009**
Grade 3	208.2 ± 23.06	
Grade 4	414.4 ± 71.74	

* Significant difference between two independent means using Students-t-test, or among more than two independent means using the ANOVA-test at 0.05 levels.

Relation of Markers with Age:

There is no relation between hs-CRP, IL-17 and CD163 levels and the age of the patients, this is indicated by P- value (0.736 ,0.729 and 0.884) respectively as shown in Table (5).

Table 5: The relationship between hs-CRP, CD163 and IL-17 with knee OA age.

ients Characteristics	**Hs-CRP (mg/dl) Mean ± SD**	**P - Value***
Age (Years)		
45 – 50	4.96 ± 2.34	**0.736**
51 – 55	6.43 ± 3.51	
56 – 60	4.08 ± 3.55	
61 – 65	6.14 ± 3.59	
> 65	5.72 ± 3.46	
Patients Characteristics	**CD163 (ng/ml) Mean ± SD**	P – Value
Age (Years)		
45 – 50	58.61 ± 13.62	**0.729**
51 – 55	63.04 ± 21.53	
56 – 60	62.08 ± 22.62	
61 – 65	65.28 ± 16.74	
> 65	59.23 ± 19.12	
Patients Characteristics	**IL-17 (pg/ml) Mean ± SD**	P - Value*
Age (Years)		
45 – 50	102.1 ± 81.28	
51 – 55	99.2 ± 52.33	**0.884**
56 – 60	79.48 ± 59.14	
61 – 65	100.3 ± 68.59	
> 65	111.2 ± 147.3	

There is no significant difference between all markers and age.

Relation of Markers with Gender:

There is a relation only between hs-CRP levels and gender, this is indicated by the P-value (0.018) as shown in Table (6).

Table 6: The relationship between hs-CRP, CD163 and IL-17 with knee OA gander.

Patients Characteristics	**Hs-CRP (mg/dl) Mean ± SD**	**P - Value***
Gender		
Male	3.55 ± 2.91	**0.018**
Female	7.09 ± 6.52	
Patients Characteristics	CD163 (ng/ml) Mean ± SD	P – Value
Gender		
Male	57.39 ± 21.32	**0.286**
Female	62.93 ± 18.13	
Patients Characteristics	IL-17 (pg/ml) Mean ± SD	P - Value*
Gender		
Male	62.67 ± 92.54	**0.089**
Female	48.49 ± 50.92	

DISCUSSION

Biomarkers are essential as an outcome measure in population-based epidemiological research, in addition to being employed as a goal measure in clinical trials for treatment as a tool to describe KOA (Sawitzke 2013). Furthermore, especially in young adult cohorts who improve our awareness of the pathophysiology and risk factors for OA, these markers can serve as an alternative for early diagnosis of OA (Antony *et al.,* 2021).

In response to infections and tissue damage, interleukin 6 (IL-6) is rapidly and briefly produced, and it may be the cause of the patient's elevated levels of hs-CRP in this study. Acute phase responses, hematopoiesis, and immunological responses are all aided by IL-6, making it an important component of host defence. After IL-6 is created in a local lesion during the first stage of inflammation, it goes via the bloodstream to the liver, where it rapidly induces different acute phase proteins, like C-reactive protein (hs-CRP). Because of this, OA patients tend to have higher hs-CRP levels than the general population (Tanaka *et al.,* 2014).

Regarding CD163, Li et al found that the proportion of CD163+ cells in synovial fluid and peripheral blood was significantly different between OA and fracture patients. After observing a significant rise in serum sCD163 levels in the OA group compared to the control group, Mardanpour and colleagues got a conclusion that early OA patients' plasma levels of CD 163 macrophages accurately indicated the involvement of this crucial biomarker in the development of the disease and joint damage (Mardanpour *et al.,* 2018).

Similarly, the Ohashi et al study, in which reported that expression of CD163, is significantly elevated in patients with knee osteoarthritis compared with healthy controls (Ohashi *et al.,* 2022).

Interferon-Gamma (IFNγ), lipopolysaccharide (LPS) and TNFα, all reduce CD163 expression, whereas macrophage colony-stimulating factor, IL-10, IL-6, and glucocorticoids all increase it. Moreover, CD163 levels were found to be elevated in synovia and per articular tissues in or near vessel walls. Therefore, the discovery of CD163 macrophages in OA synovia suggests that CD163 may play an immunoregulatory role in this illness (Mardanpour *et al.,* 2018).

Regarding the level of IL-17, these findings were consistent with those reported by Kamel *et al.,* study, which found that patients with knee OA had significantly higher serum levels of IL-17A than healthy controls (Kamel *et al.,* 2022). This finding was also consistent with the Wan et al study, which also reported that serum IL-17 levels were measured using an ELISA kit. whereby individuals with early, middle, and late stages of knee OA had levels of IL-17 that were substantially higher than those of the healthy participants (Wan *et al.,* 2018).

Another similarity was found in a study conducted by Liu *et al.,* who revealed that IL-17 levels were significantly higher in OA patients compared with controls (Liu *et al.,* 2015). According to the findings of the Askari *et al.,* study, serum levels of IL-17 were statistically greater in OA patients than in healthy controls, suggesting that IL-17 plays a substantial role in the pathology of OA and in the onset of pain (Askari *et al.,* 2016).

The production of tumor necrosis factor-alpha (TNF-α), IL-17A (Th17 cells) and IFNy (Th1 cells) (Th1 and Th17 cells, monocytes/IL-17 specific receptor, expressed on epithelial cells, endothelial cells and fibroblasts) by pro-inflammatory CD4+ T-helper cells is particularly important in the rise of IL-17. IL-17 also triggers the synthesis of several important substances, such as prostaglandin E2, monocyte chemotactic protein-1 (MCP-1), IL-8, IL-6, IL-1β, and TNF-α promote granulocyte production and neutrophil

Chemotaxis, IL-17 also participate in bone and cartilage damage (Rosu *et al.,* 2012, Ban *et al.,* 2019).

The findings of this study revealed that serum CRP levels in patients with grade four had significantly higher hs-CRP levels compared to patients with grade two or grade three (11.51 mg/dl vs 2.04 and 6.97 mg/dl, P= 0.001) this may due to CRP might be a reflection of or a cause of the illness states' biological processes.

The most well-known biochemical marker for joint disease activity is hs-CRP. Their assays enable the identification of patients with low levels of inflammation since they are substantially more sensitive than conventional assays at detecting CRP levels. Therefore, these hs-CRP assays may be more helpful in predicting the progression and development of OA (Wen *et al.,* 2018).

The mean levels of CD163 of patients with osteoarthritis were not significantly different ($P \geq 0.05$) according to their sociodemographic and clinical characteristics. As compared to other studies, Mardanpour and colleagues in their study observed a similar finding, in which there was no correlation existed between grades of disease and level of CD163 (Mardanpour K *et al.,* 2018). Another different finding was reported in Ohashi *et al.,* study, in which findings suggest that CD163 expression is significantly associated with higher resting pain scores with no relation to other parameters included in their study (Ohashi Y *et al.,* 2022). However, a contrary conclusion was reached by Wang and colleagues, who compared the distribution of CD163 macrophages across synovium and peripheral blood of patients with varying stages of knee OA (Wang *et al.,* 2019).

Prior research found that advanced stages of OA were accompanied by increased expression of existing monocytes and macrophages (Mardanpour *et al.,* 2018). In this study, there's no relation with a grade this may be due to that a large proportion of patients in this study occur in grade 2.

According to this study, patients with grade four had mean levels of IL-17 that were substantially higher than those in patients with grade two or grade three (414.4 vs 36.29 and 208.2, P= 0.009). The outcomes of the Kamel et al study showed that greater IL-17A levels were substantially correlated with longer duration, greater pain scores, worse quality of life, severe disability, and advanced damage in individuals with primary knee OA (Kamel *et al.,* 2022). The level of IL-17 in the serum of OA patients and healthy controls was studied as well by Wan and his co-authors. Their findings demonstrated a favorable correlation between the rise in serum IL-17 levels and the severity of knee OA (Wan *et al.,* 2018). In agreement, Liu et al. studied the association between IL-17 level, pain score, and radiographic severity of knee arthritis. They found that while IL-17 level is associated with the degree of knee OA discomfort; there is no clear relationship between it and the radiographic severity. It might be a new probable biochemical marker that reflects the intensity of pain from osteoarthritis (Liu *et al.,* 2015). The biological elements that influence the cytokines in the articular cartilage are getting more and more attention recently. Research on OA therapy and prevention has a major focus on cytokines. Since it possesses characteristics resembling those of cytokines and because it regulates interferon alpha (IFN-α) prompted IL-10 secretion in triggering T-cell proliferation, IL-17, which is only secreted by TH17 cells, has attracted a lot of interest. In the pathophysiology and therapy of OA, IL-17 has attracted much study attention (Liu *et al.,* 2015). Autoimmune diseases are significantly influenced by IL-17 pathways. Inflammation can be avoided by precise and effective control of IL-17 signalling. It is able to control chronic inflammation and host defense, which result in autoimmune reactions and tissue damage (Reinert-Hartwall 2015).

This study showed no significant difference in IL-21. This may be due to all patients being at an advanced stage of the illness and the samples used in this study were blood rather than synovial fluid (Kapoor *et al.*, 2011).

This study concludes that levels of some immunological markers were elevated in osteoarthritis patients as IL-17, hs-CRP and CD163 and may be used as indicators of disease activity.

Ethical Statements:

This study was approved by the Medical Ethics Committee of the University of Anbar, Al-Anbar Governorate, Ramadi, Iraq (approval number 206, Dec, 29, 2022) in accordance with the Declaration of Helsinki. Written informed consents were obtained from all study participants.

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