

CORRELATION OF WOMAC INDEX AND CYTOKINE VALUES IN PATIENTS WITH OA KNEE

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Received: 01.02.2022.

Accepted: 01.03.2022.

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ABSTRACT

Osteoarthritis (OA) of the knee (gonarthrosis) is a degenerative rheumatic disease, which is the most common rheumatic disease. Numerous factors, such as mechanical, enzymatic, and biological, are responsible for the development of this complex, multifactorial disease. The connection between inflammation and the development of knee OA is being investigated more and more. Cytokines are thought to be one of the main causes of disturbances in normal cartilage metabolism.

This study, designed as a prospective cohort study included 60 patients with knee OA. The examinees were recruited from total population of patients with knee OA, hospitalized or treated as outpatients at Department of Physical Medicine and Rehabilitation, University Clinical Centre Kragujevac. After the consent was obtained, anamnestic data were collected, blood samples were taken and functional testing was done together with anthropometric measurements. The values of the following cytokines were determined from a blood sample: IL-6, TNF- α , IL-10, IL-17. The average age of patients was 69.10 ± 9.06 years, most of whom were females (78,3%). Both IL-6 and TNF- α correlated positively with WOMAC scores. WOMAC index subscales showed different association with cytokines. In our studies the level of cytokines in the serum of patients with OA knee is show not correlation with radiographic image.

The present study demonstrated that the concentrations of proinflammatory cytokines (TNF- α , IL-6) can be correlated with WOMAC scores of knee OA patients. The results indicate that concentrations of TNF- α and IL-6 may affect knee joint function in patients with knee OA.

Keywords: Knee osteoarthritis, WOMC index, cytokines, TNF- α IL-6, IL-10, IL-17.



DOI: 10.2478/sjecr-2022-0036



INTRODUCTION

Osteoarthritis (OA) of the knee is a degenerative rheumatic disease, which is the most common rheumatic disease. The changes in the OA of the knee primarily affect the articular cartilage, we must not forget that the changes also occur in the synovium and subchondral part of the bone (1, 2, 3, 4). Numerous factors, such as mechanical, enzymatic, and biological, are responsible for the development of this complex, multifactorial disease. The connection between inflammation and the development of knee OA is being investigated more and more (4, 5). In healthy individuals, there is a balance between wear and tear and cartilage regeneration. Cytokines are thought to be one of the main causes of disturbances in normal cartilage metabolism (4). In the process of inflammation chondrocytes become the target of catabolic cytokines (IL-1 β , tumor necrosis factor (TNF) - α , IL-6), so cytokines are important in the development of inflammation in OA of the knee (6, 7, 8). As the OA knee complex is a disease of probably several different cytokines, in the form of a cytokine network it acts together in initiating the pathogenesis of the OA knee (9).

Tumor necrosis factor (TNF) - α is considered to be the major cytokine involved in the pathophysiological processes that occur in OA of the knee. Increased concentrations of TNF - α in synovial fluid, synovial membrane, cartilage and subchondral bone layer have been demonstrated in patients with knee OA (10).

IL-6 is considered a cytokine that strongly activates the immune system and enhances the response to inflammation (7,10). The effect of IL-6 together with other cytokines causes a decrease in the production of type II collagen, and is considered a key cytokine that causes changes in the subchondral part of the bone. With the synergistic effect of IL-6 and TNF- α , they lead to accelerated osteoclast formation, and thus bone reabsorption (10).

IL-10 has numerous immune functions, inhibits the synthesis of proinflammatory cytokines IL-2, IL-4, TNF- α , has the ability to affect the suppression of antigen presenting cells, affects the differentiation of B cells (11). IL-10 can affect chondrocyte proliferation and differentiation thereby contributing to enchondral bone formation (8). Thus, it is known that IL-10 has a chondroprotective effect in chondrocytes and cartilage tissue, not only anti-inflammatory, anti-catabolic and anti-apoptotic effect. Increased values of TNF - α and IL-10 can be interpreted as a compensatory attempt to slow the onset of inflammation in OA (8).

There is a growing number of reports in clinical and animal studies that support a role for IL-17 cytokines in OA. IL-17 can affect cartilage destruction, bone remodeling, and pain sensitization (5, 12).

Many tests are used today to evaluate the functional capabilities of patients with OA of the knee, the most well-

known and most commonly used test being the WOMAC index.

There are few studies comparing cytokine values with the functional capacity of patients with OA of the knee.

AIM

The main aim of the research is to examine the connection between cytokine values and the functional ability of patients with OA of the knee. In line with this basic aim of the study, secondary objectives are to determine the association between cytokine values and radiographic characteristics, C reactive protein (CRP) values as well as disease duration, Body Mass Index (BMI) of patients with OA of the knee.

MATERIAL AND METHODS

This study, designed as a prospective cohort study, was conducted in the Department of Physical Medicine and Rehabilitation of the University Clinical Center in Kragujevac (UCC) in the period from February to December 2018. The protocol of this study was approved by the Ethics Committee of UCC Kragujevac. A total of 60 respondents for the study were recruited from the total population of patients with OA of the knee who were treated on an outpatient or inpatient basis in the Department of Physical Medicine and Rehabilitation of the University Medical Center "Kragujevac".

Inclusion criteria were: subjects of both sexes aged 40-70 years, with a confirmed diagnosis of OA of the knee according to the criteria of the American College of Rheumatology (13), Kellgren and Lawrence (KL) radiographic osteoarthritis grade 2 and 3 to moderate radiographic osteoarthritis (14), pain in the knee joint for 3 months and longer. All respondents signed a consent form to participate in the study. Criteria for exclusion from the study were as follows: subjects who had implanted knee endoprosthesis, knee joint injury during the last 6 months, secondary rheumatoid or septic arthritis or systemic disease involving the knee joint, active OA of the knee with intra-articular effusion, as well as subjects who in parallel intervention studies.

Before joining the study, all respondents first signed an informed consent to participate in the study. After that, anamnestic data were collected, and then a blood sample was taken from the subjects and functional testing (WOMAC questionnaire) and anthropometric measurement were performed.

Sample collection procedure: in the Service for Physical Medicine and Rehabilitation of the University Medical Center Kragujevac, a blood sample was taken from the peripheral vein according to the valid standards. After taking the blood, the tubes were sent to the immunological laboratory of the Faculty of Medical Sciences for analysis. The values of the following cytokines were determined from a blood sample: IL-6, TNF- α , IL-10, IL-17.



Measurement of TNF- α , IL-6, IL-17, IL-10 in serum samples. The blood samples of from all patients were collected before any therapeutic application. After the blood samples were taken, blood clot was cut and centrifuged for separating the serum. Serum samples were kept at -80 °C before testing. Serum levels of molecules of interest were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits specific for human cytokines (R&D Systems, Minneapolis, MN, for TNF- α , IL-6, IL-17, IL-10, according to the manufacturer's instructions (15). Concentrations of cytokines were determined by interpolation of a standard curve. Results were expressed as picogram per milliliter of patient serum.

The WOMAC questionnaire (Western Ontario and McMaster Universities) is a three-dimensional disease-specific instrument used in a patient with OA of the knee or hip, measuring pain, stiffness, physical function during typical daily activities. The questions refer to the patient's condition during the last 48 hours. The patient completed the WOMAC questionnaire by selecting one of the offered answers (marking with X). It consisted of three parts: the first part of the questionnaire included questions about the severity of knee pain (5 questions in total). The second part of the questionnaire included questions related to joint stiffness (2 questions). The third part of the questionnaire included questions related to the function of the patient, ie difficulties during the performance of everyday life activities (17 questions) (16).

Anthropometric evaluation included the measurement of body weight (kg) and body height (cm). Body weight was measured using a standing scale, and body height on an anthropometer. Both measures are taken to calculate the BMI (Body Mass Index) (kg / m²).

The statistical program SPSS version 20 for Windows (SPSS Inc.) was used for all statistical analyzes. Descriptive statistics methods (mean, standard deviation) were used to analyze the socio-demographic characteristics. Spearman Correlation coefficient was used to examine the association between the WOMAC, KL scale, CRP and TNF- α , IL-6, IL-17, IL-10. Level of $p > 0.05$ was used to determine statistical significance.

RESULTS

Patient demographics

Our study included 60 patients with knee OA, most of whom were females 47 (78,3%). The average age of patients was 69.10 $\pm$ 9,06 years, with average length of hardship 9.36 $\pm$ 5.95 years. The average BMI was 29.69 $\pm$ 4.97. Majority of patients, 32 (53,3%) had 2 degree by K/L score. Table 1 shows the patient demographics.

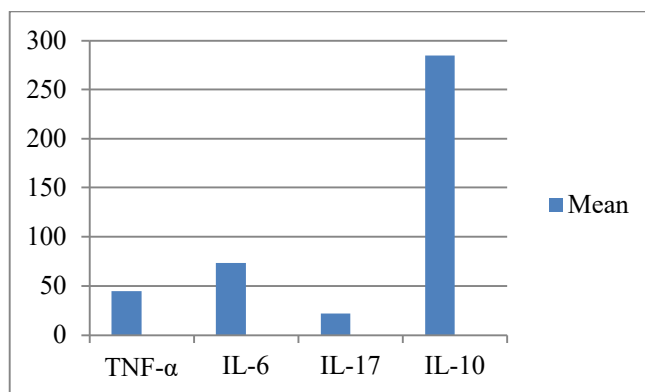
Table 1. Demographic characteristics

Demographic characteristics	
Age (years), mean $\pm$ SD	69.10 $\pm$ 9.06
Males/females (%)	13 (21.7) / 47 (78.3)
Body mass index (BMI) (kg/m ²), mean $\pm$ SD	29.69 $\pm$ 4.97
Length of disease, mean $\pm$ SD	9.36 $\pm$ 5.95
K/L score	
2	32 (53,3%)
3	28 (46,7%)

Blood Samples

Graph 1 shows the serum cytokine concentration (TNF- α 44,17 ($\pm$ 86,03), IL-6 73,51 ($\pm$ 63,44), IL-10 284,78 ($\pm$ 584,11). IL-17 21,74 ($\pm$ 47,70))

Graph 1. Serum cytokine values



Correlation Analysis

Mean WOMAC scores in the OA patients 52,47 $\pm$ 11,51 (pain 11,14 $\pm$ 2,50; stiffness 3,05 $\pm$ 1,31; difficulties 38,24 $\pm$ 8,66). Correlation analyses of variables are given in table 3. Both IL-6 and TNF- α correlated positively with WOMAC scores. WOMAC index subscales showed different association with cytokines. The most statistically significant association was between cytokines and functionality. In our studies the level of cytokines in the serum of patients with OA knee is show not correlation with radiographic image (table 2). The CRP value was correlated with the WOMAC index, but did not show an association with cytokine values (table 2). The duration of the discomfort was not correlated with cytokine values (table 2).



Table 2. Correlation of WOMAC index and KL score, CRP, BMI

variables		TNF- α	IL-6	IL-17	IL-10
WOMAC total	Spearman* P	-0,439 0,000	-0,404 0,001	0,224 0,070	-0,44 0,726
WOMAC pain	Spearman* P	-0,542 0,000	-0,459 0,000	0,230 0,64	-0,020 0,874
WOMAC stiffnes	Spearman* P	-0,235 0,057	-0,188 0,130	0,142 0,254	-0,109 0,384
WOMAC difficulties	Spearman* P	-0,411 0,001	-0,386 0,001	0,236 0,056	-0,031 0,805
K/L score	Spearman* P	-0,097 0,436	-0,030 0,809	-0,124 0,322	-0,050 0,693
CRP	Spearman* P	-0,099 0,430	0,000 0,999	-0,189 0,128	0,166 0,184
BMI	Spearman* P	-0,092 0,463	-0,042 0,737	-0,003 0,980	-0,127 0,311

DISCUSSION

There are more and more studies that study the effects of cytokines on OA knee. In our study we investigated the serum concentration of proinflammatory cytokines (IL-6, IL-17, and TNF- α) and anti-inflammatory cytokines (IL-10) in patients with knee OA and associated those with the functional ability of the patient.

Numerous studies have examined the association between pain and cytokine values. There are few studies that have examined the functionality of patients with OA of the knee and the value of cytokines. By analyzing the results of our study, some differences were found between IL in terms of their correlation with the WOMAC score and its subscales. An association between WOMAC index values and TNF- α and IL-6 has been shown. Although IL-10 is an anti-inflammatory cytokine that essentially regulates the production of proinflammatory cytokines, no correlation has been found between its value and the value of the WOMAC index. Numerous studies have shown a positive association between pain, as the main symptom of knee OA, and cytokine values. One of these studies followed IL-17 level in synovial fluid was positively correlated with WOMAC pain score in OA knee patients (17). However, we also found a study showing a negative correlation between WOMAC pain and IL-17 (18). IL-17 can stimulate bone reabsorption, some studies have shown increased values of IL-17 in patients with rheumatoid arthritis. (17, 19). Also IL-17 can affect synovial cells, macrophages, osteocytes to produce TNF- α and IL-6, the major proinflammatory cytokines in OA (17). Based on previous research, IL-17 can be considered a key factor in causing inflammation in OA. The analysis of the results of our study did not find a correlation between the total WOMAC index and the value of IL-17, but showed a correlation between the WOMAC index subscale related to functionality and IL-17.

Beyond being simply a biomarker of inflammation, CRP may reflect or contribute to molecular mechanisms of disease states. Research have suggested that lower levels of CRP - as seen in chronic, subclinical inflammatory states might enhance inflammation by activating endothelium and proinflammatory cytokine synthesis. IL-6 and IL-1 are known to promote hepatocyte CRP production., studies have demonstrated that IL-6 is the chief regulator of CRP production by hepatocytes (20). Several studies have shown a correlation between synovial fluid IL-6 levels and CRP but not serum IL-6 levels (21, 22). Our results were consistent with the results of a previous study, the CRP value was not correlated with cytokine values. Babaei et al in their study showed a negative correlation between IL-10 values and CRP measured from synovial fluid (23). In our work, there was also a negative correlation between CRP and IL-10, which is explained by the fact that high values of IL-10 associated with reduced values of CRP are consistent with the anti-inflammatory and protective characteristics of IL-10.

Comparing the results of different studies, we came to the conclusion that the results differ significantly. In some studies, an association between radiographic image severity and IL-6, IL-17 values from both synovial fluid and serum was shown (10,24). However, synovial fluid (SF) levels of IL-6 have been shown not to correlate with OA knee (KL) (25). In our study, no relationship was found between interleukin values and radiographic image severity. Some studies have shown that IL-6 levels may increase in early-stage OA. As elevated IL-6 values are associated with early-stage OA of the knee, this may be useful in explaining why there is no correlation between KL scores and IL-6 values in our patients because we followed only patients who were on the KL scale at score 2 and 3. Although some studies have shown that some ILs are associated with cartilage destruction, (26) some other studies have shown that TNF- α , measured from



synovial fluid, was not correlated with the weight of the radiographic image (19).

BMI and interleukins were not correlated in our study, which was in accordance with the results of other studies.

The present study demonstrated that the concentrations of proinflammatory cytokines (TNF- α IL-6) can be correlated with WOMAC scores of knee OA patients. The results indicate that concentrations of TNF- α and IL-6 may affect knee joint function in patients with knee OA. All cytokines monitored in our study were not correlated with radiographic evaluation. Although the level of CRP may affect the synthesis of proinflammatory cytokines, our results did not show a correlation between CRP and cytokines.

The limitation of this study is that cytokine values were determined only from serum and not from synovial fluid. Subsequent studies could bridge the gap between functionality in patients with knee OA and serum cytokines and synovial fluid cytokines.

The clinical contribution of this study is that we have shown that cytokines, in addition to causing inflammation in the knee joint in OA, can also affect the functionality of patients.

ACKNOWLEDGMENT

The authors would like to express gratitude to the Faculty of Medical Science, University of Kragujevac for Grant No. JP 05/18.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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