



ORIGINAL ARTICLE

Medicine Science 2022;11(3):995-9

Examination of IL-6 -174 gene polymorphism in patients diagnosed with primary knee osteoarthritis: A case-control study

Mehmet Vakif Keskinbicki¹, Zekeriya Oztemur², Serdal Arslan³, Hayati Ozturk²,
 Seyran Kilinc², Okay Bulut², Ozhan Pazarci⁴

¹Dr. Ersin Arslan Training and Research Hospital, Department of Orthopedic and Hand Surgery, Gaziantep, Turkey

²Cumhuriyet University School of Medicine, Department of Orthopedic and Traumatology, Sivas, Turkey

³Mersin University School of Medicine, Department of Medical Biology, Mersin, Turkey

⁴Adana City Training and Research Hospital, Department of Orthopedic and Traumatology, Adana, Turkey

Received 21 February 2022; Accepted 15 March 2022

Available online 08.07.2022 with doi: 10.5455/medscience.2022.02.045

Copyright@Author(s) - Available online at www.medicinescience.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



Abstract

Degenerative osteoarthritis (OA) is the most common joint disease. However, its etiology has not been clearly understood. We aimed to investigate the association between the IL-6-174G/C gene variant responsible for regulating IL-6 functions and primary knee osteoarthritis (PKOA). Patients older than 40 years who presented between February 2012 and April 2013 and were diagnosed with PKOA and had grade 3 (i.e., moderate) or 4 (i.e., severe) disease constituted the case group. Healthy volunteers formed the control group. Genomic DNA was extracted from blood leukocytes in both case and control groups, and the IL6-174 G/C genotypes were determined. Two groups were compared regarding demographic, clinical, radiological findings, and IL-6-174 genotyping results. Both case and control groups included 90 patients each. No statistically significant difference was found between the two groups regarding gender distribution, patient age, and body weight. The frequency of the mutant base pairs GC and CC in the IL-6-174 promoter region were significantly higher in controls than in cases. Women with genotypes including C alleles were genetically protected against the clinical disease. Similar findings were not detected in the male patient group. The IL6-174 G/C gene polymorphism does not increase the risk of PKOA. However, the mutant C allele can be protective against PKOA in female patients. Studies conducted on larger patient populations from different ethnic backgrounds are needed to confirm our findings since our sample size is relatively small.

Keywords: Osteoarthritis, interleukin 6, genotype, polymorphism, gene, DNA

Introduction

Degenerative osteoarthritis (OA) is the most frequently encountered joint disease [1]. It involves joint cartilage erosion, contraction of joint spacing, osteophyte formation around the joint, sclerosis in subchondral bone, morphological and biochemical changes in the joint capsule and synovial membrane. Degenerative OA has a relatively high incidence in the general population, and it is known that its incidence increases with age. However, despite its high incidence, its etiology has not been clearly understood yet. On the other hand, complicated biomechanical, biochemical, metabolic, and genetic factors are involved in its etiopathogenesis.

In the light of these findings, it was suggested that some biochemical and genetic markers could be used in the early diagnosis of osteoarthritis [2]. Therefore, cases with high-risk factors for degenerative OA can be treated early during the clinical course of the disease. Some clinical studies reported that genetic factors and cytokines had a significant role in the etiopathogenesis of degenerative OA [3]. Cytokines were shown to contribute to several infectious, chronic inflammatory, autoimmune and malignant diseases. Interleukin-6 (IL-6) is one of the pro-inflammatory cytokines involved in OA pathogenesis. Significantly increased synovial fluid IL-6 mRNA levels were detected in patients with OA [4]. Also, increased serum levels of IL-6 were shown to be associated with radiographic progression of knee OA [5]. It was also suggested that polymorphisms in the genes encoding cytokines could alter their expressions and provide a basis for genetic predisposition to osteoarthritis [6]. Among the gene polymorphisms associated with OA, the best-characterized one is a single nucleotide polymorphism at position -174, upstream

*Corresponding Author: Ozhan Pazarci, Adana City Training and Research Hospital, Department of Orthopedic and Traumatology, Adana, Turkey
E-mail: dr.pazarci@gmail.com

of the transcription start site involving the substitution of cytosine (C) for guanine (G). This gene polymorphism was examined in a wide variety of diseases, including degenerative arthritis [7]. This study aimed to investigate the association between the IL-6-174G/C gene variant responsible for regulating IL-6 functions and primary knee osteoarthritis (PKOA).

Materials and Methods

Approval of the Local Research Ethics Committee of our Cumhuriyet University was obtained before initiating the study (no: 15.02.2012-02/30).

Patients older than 40 years who presented to our institution between February 2012 and April 2013 with the complaint of knee pain and were later diagnosed with PKOA as per the American College of Rheumatology (ACR) diagnostic criteria of OA, and had grade 3 (i.e., moderate) or 4 (i.e., severe) disease according to Kellgren-Lawrence system for classification of OA constituted the target population of this study. These patients formed the case group after the application of inclusion and exclusion criteria. Also, healthy volunteers who had no clinical symptoms and radiological signs of PKOA were included (Table 1). These patients formed the control group. Patients who received steroid or hyaluronic acid injection to the knee during the last year, patients with chronic rheumatic or inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus, familial Mediterranean fever, ankylosing spondylitis, gout, pseudogout, psoriatic arthritis, patients with a previous history of knee trauma or surgery, those with ipsilateral hip or ankle arthrosis, slipped capital femoral epiphysis, hemophilia, neurological and neuromuscular system diseases and diseases which may cause secondary knee OA, patients with malignancies and those who were pregnant were excluded. Demographic data of each individual included in the study were recorded. Comparative knee radiographs were taken as upright anterior-posterior and 30-degree flexion knee radiographs.

Table 1. Demographic and clinical data of the case and control groups

	Case-n-(%)	Control -n-(%)	P value	Odds Ratio
Total	90	90		
Age (Mean±SD) years	57.91±11.23	56.38±12.65	0.341	
Weight (Mean±SD)kg	80.30±13.00	78.53±11.30	0.332	
Gender				
Female	60	48		
Male	30	42	0.068	0.57(0.31-1.04)
Grade				
3	40			
4	50			

Two observers evaluated all radiographs as per Kellgren- Lawrence (KL) scale. A 2-cc venous blood sample was taken from all study participants and transferred to an EDTA tube. These tubes were stored at -80°C.

IL6-174 G/C genotyping: In both case and control groups, genomic DNA was extracted from blood leukocytes using a standard phenol-chloroform method. The IL6-174 G/C genotypes

were determined using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay. The PCR was carried out in a 25-ml vol containing 0.2 mM of each primer (F:5'-CAGAAGAAGCTCAGATGACTG-3' and R:5'GTGGGGCTGATTGGAAACC-3'). All genotypes were determined as per the method described by Pereira et al [8].

Statistical analysis: Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 13.0) software. The statistical significance of the risk coefficient (i.e., Odds Ratio) was calculated by Pearson's χ^2 test. The Fisher's Exact Test was used for values less than 5. The p-value was considered significant when it was less than 0,05.

Results

Our analysis revealed that the case and control groups included 90 patients each; while there were 60 females and 30 males in the case group, the control group consisted of 48 females and 42 males. Two groups were similar regarding female-male patient ratios (p:0.068). The mean patient age was 57.9±11.2 and 56.4±12.6 in the case [41-89] and control [41-83] groups, respectively (p:0.34). The mean body weight was 80.3±13 in the case and 78.53±11.3kg in the control group (p:0.332).

No statistically significant difference was found between the two groups regarding gender distribution, patient age, and body weight. The IL-6 genotypes and allele distributions in the two groups are shown in Table 2. While the genotype of the IL-6-174 promoter region included GG in 66, GC in 20, and CC in 4 patients in the case group, it included GG in 51 individuals, GC in 29 individuals, and CC in 10 individuals in the control group. Thus, the frequency of the mutant base pairs GC and CC were significantly higher in controls than in cases (p=0.045).

Table 2. IL-6 genotypes and allele distributions in the case and control groups

Genotype distribution	Case-n- (%)	Control-n- (%)	p value	Odds Ratio (95% Confidence interval)
IL-6 -174	90(100)	90(100)		
G	152	131	Ref	
C	28	49	0.007	0.49(0.29-0.83)
GG	66	51	Ref	
GC	20	29	0.067	0.533(0.271-1.049)
CC	4	10	0.045	0.309(0.092-1.043)
GC+CC	24	39	0.019	0.476(0.254-0.889)
Female				
GG	49	26	Ref	
GC	9	15	0.016	0.318(0.123-0.826)
CC	2	7	0.017	0.152(0.029-0.783)
Male				
GG	17	25	Ref	
GC	11	14	0.777	1.155(0.424-3.146)
CC	2	3	0.683	0.980(0.148-6.505)

In the OA group, 24 patients had GC and CC genotypes, while they were present in 39 cases in the control group (p=0.019). While we did not detect a significant difference between the

patients with G/C polymorphism in the two groups regarding the presence of clinical disease, we found that the rate of knee OA was significantly lower in patients with C allele in the IL-6-174 position (i.e., mutant gene). The comparative analysis based on allele distributions in different genders revealed that the number of women with heterozygote (i.e., GC) and homozygote (i.e., CC) C alleles was 9 and 2 in the OA group. These figures were 15 and 7 in the control group. Our analysis also showed that women with genotypes including C alleles (i.e., GC or CC) were genetically protected against the clinical disease ($p=0,016$ for GC and $p=0,017$ for CC). Similar findings were not detected in the male patient group.

Discussion

Degenerative OA is the most common joint disease in the adult population [1]. It is characterized by focal loss or damage of articular cartilage, particularly in load-bearing joints. Its incidence increases with age. Numerous studies showed that OA was a disease with polygenic inheritance, and cytokine genes regulating the balance of construction and destruction in articular cartilage were involved in its pathogenesis.

In cases with IL-6-174 gene polymorphism, the mutant C allele induces IL-6 transcription in vitro by a mechanism similar to IL-1 and endotoxins and leads to cartilage damage [7,9]. After discovering single nucleotide polymorphisms, several variants of the IL-6 gene were identified, and it was suggested that -174 G/C polymorphism was associated with OA. [10]. Pola et al. [7]. showed that non-stimulated increased IL-6 levels could be related to the C-allele. In patients with severe joint cartilage degeneration, the serum and synovial fluid levels of several cytokines, including IL-6, were higher than those in healthy individuals, and these high cytokine levels were believed to be associated with the disease [8]. These studies also demonstrated that widespread G/C polymorphisms in the -174 position of the promoter region of the IL-6 gene increase the expression and levels of IL-6 and lead to chronic inflammatory diseases and arthritis.

Our findings are inconsistent with the findings reported in these studies, and they suggest that IL-6-174 G/C polymorphism may not be the only reason for the elevation of IL-6 levels in the synovial fluid and serum. Some studies reported that the serum and synovial fluid IL-6 levels could also be affected by the levels of other cytokines such as IL-1, Tumor Necrosis Factor-alpha (TNF α) [11-13]. It was also shown that the C allele forming in the cases with IL-6-174 G/C polymorphism increases the transcription of IL6 in vitro by a mechanism similar to IL-1 or endotoxins [11,12]. It was also demonstrated that cartilage damage occurs as a result of this process. After discovering single nucleotide polymorphisms (SNPs), several variations of the IL-6 gene were described, and it was suggested that the IL-6-174 G/C polymorphism could be associated with OA [7]. Some reports demonstrated that in patients with advanced articular cartilage degeneration, the blood and synovial fluid levels of some cytokines, including IL-1, TNF α , IL-6, and other acute-phase proteins, were found to be higher than the levels in healthy individuals, and these relatively high cytokine and acute phase protein levels were associated with disease formation in these cases [9,10].

In another study investigating the association of the cytokines

including IL-6 with OA, the authors concluded that IL-6 was the most significantly upregulated hub gene in the protein-protein interaction network, and the level of IL6 in the synovial fluid decreased after sodium hyaluronate treatment [8]. They also found that serum and synovial fluid IL-8 levels were higher in patients with OA and RA than in healthy individuals. Based on these findings, it can be proposed that IL6 plays a role in the pathogenesis of OA. A study investigating the association between OA and IL-1 gene polymorphism showed that this gene polymorphism might also play a role in knee and hip OA [7,9,10,13]. In line with this, Moxley et al. [14] confirmed the relationship between IL-1 gene polymorphism and small joint OA of the hand.

Previously published studies showed that some polymorphisms in the IL-1 and IL-1 receptor antagonist genes were involved in the OA of the hip joint [14,15].

An experimental study utilizing human recombinant IL-6 and IL- (hr IL-6/hr IL 1 β) showed that hr IL-6 could inhibit cell proliferation and proteoglycan synthesis in the chondrocytes of rabbit knee cartilage tissue [16]. These researchers also stated that the addition of hr IL-1 β to the milieu led to increased stimulated proteoglycan degradation and paved the way for degenerative arthritis.

Various studies showed that IL-6 took a critical role in inflammatory processes, especially in those related to joints such as cartilage damage, joint degeneration, joint prosthesis loosening, generalized OA, chronic rheumatic and inflammatory diseases, small joint OA of the hand where familial passage is not infrequent [17-21]. This finding was supported by the identification of elevated serum and synovial fluid levels of IL-6 in these patients. Some of these studies reported that OA could be induced in vitro by increasing the IL-6 levels in the joint, and this process could be facilitated by the addition of IL-1 β [20,21]. These reports also stated that IL-1, TNF α , SNPs forming on the IL-6 gene altogether contributed to the elevation of IL-6 levels, and SNPs were the most important cause of a non-stimulated increase in IL-6 levels. It was suggested that SNPs could lead to elevated IL-6 levels and may pave the way for both generalized OA and early degenerative OA in heavy load-bearing joints such as the knee joint [20,21].

The researchers examined the association between primary hip joint OA and IL6-174 G/C polymorphism [7]. These investigations found that the rate of GG genotype was higher in the case (i.e., OA) group than in the control (i.e., healthy individuals) group. This finding was consistent with ours. The same researchers also found that the C allele was more frequent in the healthy controls, and they could be genetically protected from primary hip joint OA. In a meta-analysis including 1101 patients with hip OA, 1904 patients with knee OA, and 2511 healthy controls, the authors did not correlate IL6 gene polymorphism with hip and knee OA [4]. Our findings are in line with the results of this study. In another meta-analysis including 3331 patients with PKOA and 3133 healthy controls, no association was found between IL-6-174 G/C polymorphism and knee OA [22]. Both meta-analyses also emphasized that the effects of the same genetic factor could be different in individuals from different ethnic backgrounds [4,22].

Our study aimed to analyze the relationship between PKOA and IL6 -174 G/C gene polymorphism in patients with grade 3 or 4

PKOA as per the Kellgren-Lawrence system for classification of OA. We did not find a statistically significant correlation between IL-6-174 G/C gene polymorphism and knee OA. Our results suggested that GC or CC genotypes of the polymorphism in the IL6 -174 G/C promoter region were not correlated with OA. On the other hand, these genotypes had protective effects on women against this disease.

Pola et al. [7] analyzed the relationship between primary hip OA and IL-6-174 G/C gene polymorphism, and they reported that GG genotype was significantly more prevalent in patients with the disease than in the patients in the control group. However, in line with our findings, they did not detect a significantly increased disease risk in this patient group ($p=0,09$). They also reported that the rate of C allele was relatively more prevalent in healthy individuals, and these patients might be genetically protected against primary hip OA ($p=0,02$). These authors stated that the same genetical mutation could lead to different outcomes in different ethnic groups, and thus studies conducted in patients from different ethnic backgrounds were needed.

The differences between the findings of these studies may be due to the differences in the ethnicities of the patient groups, as suggested by Valdes et al. [4,12,22] In a meta-analysis investigating the association between IL-6 gene polymorphism and hip and knee OA, Valdes et al. [22] included 2511 healthy controls, 1101 patients with hip OA and 1904 patients with knee OA and they did not find a relationship between this gene polymorphism and these disorders. This finding is in line with ours.

In our study, IL6-174 G/C genetic mutation did not alter the risk of PKOA. This result could be because synovial fluid levels of IL-6 could also be affected by SNPs developing in the promoter region of IL6. Additionally, it is known that IL-1 can trigger cartilage cell apoptosis and pave the way for knee joint OA as a result of increasing serum and synovial fluid IL-6 and TNF α levels.

Our study has some limitations which need to be considered while evaluating its findings. First, the study population is relatively small. Second, it did not include patients and healthy controls from different ethnic backgrounds. As there are examples in the literature, there is a need for animal studies in experimental knee osteoarthritis models [23].

Conclusion

Despite the weaknesses mentioned above, we conclude that IL6-174 G/C gene polymorphism does not increase the risk of PKOA. However, the mutant C allele can be protective against PKOA in female patients. Since our study cohort was relatively small, our findings should be confirmed by studies conducted on larger patient populations from different ethnic backgrounds.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

Approval of the Local Research Ethics Committee of our Cumhuriyet University was obtained before initiating the study (no:15.02.2012-02/30).

References

- Di Cesare PE, Haudenschild DR, Samuels J, et al. Pathogenesis of Osteoarthritis. In: Kelley's Textbook of Rheumatology. Elsevier. 2013.p.1617-35.
- Arden N, Nevitt MC. Osteoarthritis: Epidemiology. Vol. 20, Best Practice and Research: Clinical Rheumatology. Best Pract Res Clin Rheumatol; 2006.p.3–25.
- Garnero P, Piperno M, Gineyts E, et al. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: Relations with disease activity and joint damage. Ann Rheum Dis. 2001;60:619–26.
- Kaneko S, Satoh T, Chiba J, et al. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. Cytokines, Cell Mol Ther. 2000;6:71–9.
- Livshits G, Zhai G, Hart DJ, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The Chingford Study. Arthritis Rheum. 2009;60:2037–45.
- Bettica P, Cline G, Hart DJ, et al. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: Longitudinal results from the Chingford study. Arthritis Rheum. 2002;46:3178–84.
- Pola E, Papaleo P, Pola R, et al. Interleukin-6 gene polymorphism and risk of osteoarthritis of the hip: A case-control study. Osteoarthr Cartil. 2005;13:1025–8.
- Pereira DS, Garcia DM, Narciso FMS, et al. Effects of -174 G/C polymorphism in the promoter region of the interleukin-6 gene on plasma IL-6 levels and muscle strength in elderly women. Brazilian J Med Biol Res. 2011;44:123–9.
- Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000;275:18138–44.
- Kämäräinen OP, Solovieva S, Vehmas T, et al. Common interleukin-6 promoter variants associate with the more severe forms of distal interphalangeal osteoarthritis. Arthritis Res Ther. 2008;10:21
- Spector TD, Cicuttini F, Baker J, et al. Genetic influences on osteoarthritis in women: A twin study. Br Med J. 1996;312:940–4.
- Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis: Classification of osteoarthritis of the knee. Arthritis Rheum. 1986;29:1039–49.
- Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology. 2002;39:237-46.
- Moxley G, Han J, Stern AG, et al. Potential influence of IL1B haplotype and IL1A-IL1B-IL1RN extended haplotype on hand osteoarthritis risk. Osteoarthr Cartil. 2007;15:1106–12.
- Meulenbelt I, Seymour AB, Nieuwland M, et al. Association of the Interleukin-1 Gene Cluster With Radiographic Signs of Osteoarthritis of the Hip. Arthritis Rheum. 2004;50:1179–86.
- Murata M, Trahan C, Hirahashi J, et al. Intracellular interleukin-1 receptor antagonist in osteoarthritis chondrocytes. Clin Orthop Relat Res. 2003;285–95.
- Jikko A, Wakisaka T, Iwamoto M, et al. Effects of interleukin-6 on proliferation and proteoglycan metabolism in articular chondrocyte cultures. Cell Biol Int. 1998;22:615–21.
- Malik MHA, Jury F, Bayat A, et al. Genetic susceptibility to total hip arthroplasty failure: A preliminary study on the influence of matrix metalloproteinase 1, interleukin 6 polymorphisms and vitamin D receptor. Ann Rheum Dis. 2007;66:1116–20.
- Gordon A, Kiss-Toth E, Stockley I, et al. Polymorphisms in the interleukin-1 receptor antagonist and interleukin-6 genes affect risk of osteolysis in

- patients with total hip arthroplasty. *Arthritis Rheum.* 2008;58:3157–65.
- 1996;14:67–73.
20. Bennermo M, Held C, Stemme S, et al. Genetic predisposition of the interleukin-6 response to inflammation: Implications for a variety of major diseases? *Clin Chem.* 2004;50:2136–40.
 21. Mohtai M, Gupta MK, Donlon B, et al. Expression of interleukin-6 in osteoarthritic chondrocytes and effects of fluid-induced shear on this expression in normal human chondrocytes in vitro. *J Orthop Res.* 2004;22:1033–40.
 22. Valdes AM, Arden NK, Tamm A, et al. A meta-analysis of interleukin-6 promoter polymorphisms on risk of hip and knee osteoarthritis. *Osteoarthr Cartil.* 2010;18:699–704.
 23. R. Sevimli, A.S. Erenler, A.B. Karabulut, N. Akpolat, H. Geçkil. Microbial chondroitin sulfate in experimental knee osteoarthritis model investigation of chondroprotective effect. *Eur Rev Med Pharmacol Sci.* 2021;25:5402-11.

Copyright of Medicine Science is the property of Society of Turaz Bilim and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.