Tumour necrosis factor α -308G/A gene polymorphism: lack of association with knee osteoarthritis in a Turkish population

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Abstract

Objective

To study the association between TNFα-308 G/A polymorphism and susceptibility to and severity of knee osteoarthritis in a Turkish population.

Methods

Genomic DNA was obtained from 151 patients with knee osteoarthritis and 84 ethnically matched healthy controls. Polymerase chain reaction-restriction fragment length analysis was used to identify G/A polymorphism at position -308 in the promoter region. Genotype distributions and allelic frequencies of TNFα-308 G/A polymorphism were compared between osteoarthritis patients and controls. Thereafter, this association was investigated between patients and controls of the same sex. In addition, the standard Kellgren-Lawrence grading score and the Turkish version of the Western Ontario and McMaster Universities Osteoarthritis Index were used to assess the radiological and functional severity of the disease and their relationship with the TNFα-308 gene polymorphism was investigated.

Results

Genotype distribution and allelic frequencies of -308 G/A polymorphism in the TNFα gene did not differ significantly between patients with knee osteoarthritis and controls ($p>0.05$). Moreover, there were no significant differences between patients and controls of the same sex ($p>0.05$). In addition, no association was observed between the radiological and functional severity of the disease and TNFα-308 G/A polymorphism ($p>0.05$).

Conclusion

These findings suggest that the examined polymorphism in the TNFα gene does not contribute to susceptibility to or severity of knee osteoarthritis in the Turkish population.

Key words

Gene polymorphism, knee osteoarthritis, TNFα.

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Introduction

One of the most common skeletal diseases, osteoarthritis (OA) is characterized by the progressive loss of articular cartilage in synovial joints, and it is a major cause of decreased activity in daily living and decreased quality of life after middle age (1). OA has a high prevalence, which is expected to increase in the coming years (2). Although it has been reported that many risk factors are associated with OA, such as age, previous injury, obesity, diet, hormone therapy, and smoking habits, the pathogenesis of OA is still not clear (3). Previously OA was often described as a noninflammatory arthropathy. However, recent studies have revealed that an inflammatory process played a role in the pathogenesis of OA (4). Proinflammatory cytokines are now implicated as important mediators in the disease (5). It seems that particularly TNFα and IL1-β are prominent and of major importance for cartilage destruction (6, 7). TNFα and IL1-β can stimulate their own production and induce chondrocytes and synovial cells to produce other cytokines, such as IL-8, IL-6, and leukocyte inhibitory factor. They also stimulate proteases and prostaglandin E2 production. Moreover, it has been shown that TNFα induces osteoclastic bone resorption *in vitro*, a phenomenon likely to play a role in the remodelling of OA subchondral bone (8, 9). Levels of these mediators are increased in articular cartilage and synovial fluid of OA (10-12).

Many studies have suggested that primary OA has an important genetic component and that OA is a polygenic disease controlled by the expression of genetic factors (3). Cytokine genes also have a relevant role in regulating the catabolic/anabolic balance of articular cartilage, and recent studies have confirmed the association of the interleukin (IL)-1 gene cluster and IL-1 receptor antagonist (IL-1RA) with OA (14-15). However, in our previous study, we found no evidence of an association of polymorphisms in the IL1A, IL1B and IL1RA genes with the susceptibility to or the severity of knee osteoarthritis in a Turkish population (16).

It has been hypothesized that TNFα, produced by macrophages and monocytes, plays a key role in driving the acute inflammatory response. The gene encoding TNFα is located in the major histocompatibility complex class III region of chromosome 6 (6p21.3). Previous studies have suggested that variability in single-nucleotide polymorhisms (SNPs) in the promoter and coding regions of TNFα gene may modulate the magnitude of the secretory response of this cytokine (17, 18). There are several polymorphisms of the gene coding for TNFα, one of which is -308 G/A polymorphism. This is a SNP in which a guanine turns into adenosine at base pair -308 in the promoter region, which results in altered TNFα expression (19-21). It has been claimed that TNFα -308G/A polymorphism is also associated with increased susceptibility to and severity of a variety of illnesses, such as cerebral malaria, inflammatory bowel disease, systemic lupus erythematosus and ankylosing spondylosis (22-26). In addition, this polymorphism has recently been reported to be involved in psoriatic arthritis, rheumatic heart disease, juvenile idiopathic arthritis, and rheumatoid arthritis (27-34).

In view of the literature, one may think that interindividual differences in cytokine production may have a genetic basis, and that a proinflammatory cytokine profile may play a role in susceptibility to or severity of diseases (35). To our knowledge, there have not been any studies on the role of the TNFα promoter polymorphism in the knee OA. Therefore, for the first time, we attempted to determine whether TNFα (-308 G/A) gene polymorphism was a genetic marker of susceptibility to or severity of knee OA in a Turkish population.

Materials and methods

Participants

We recruited 151 patients presenting with primary knee OA to the outpatient clinic of Physical Therapy and Rehabilitation Department of Mersin University Hospital, Mersin, Turkey. The diagnosis of OA in all patients was based on the criteria of the American College of Rheumatology, which include primary OA with any symptoms and signs of OA, and radiographic signs of OA according to the Kellgren-Lawrence

grading. Patients with autoimmune diseases, patellofemoral osteoarthritis or osteoarthritis secondary to other conditions (including inflammation, sepsis, metabolic abnormalities and trauma) were excluded.

The Turkish version of the Western Ontario and McMaster University Osteoarthritis (WOMAC) Index, a validated disease-specific questionnaire, dealing with the severity of joint pain (5 questions), stiffness (2 questions) and physical functioning (17 questions) (36) was used to determine functional severity in all patients. Anterioposterior and lateral radiographs of all patients were obtained. Radiographs were graded by one expert reader according to the Kellgren-Lawrence grading system, a five-point scale (stage 0-4). In addition, venous blood samples were taken, and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were determined.

Eighty-four controls presenting to the outpatient clinics at the Mersin University Hospital were recruited. Eligible participants were aged ≥40 years and had never had any signs or symptoms of OA, other types of arthritis or joint diseases (pain, swelling, tenderness, or restriction of movement) at any sites based on their medical history and a through examination conducted by an experienced physiatrist. Radiographs were not obtained for most control subjects. The control subjects had no relationship with the patients and no family history of OA.

All subjects were Caucasians from the southern part of Turkey. The study was approved by the ethical committee of the Medical Faculty of Mersin University and informed consent was obtained from all subjects.

DNA extraction and analysis

Venous blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) containing tubes. DNA was extracted from whole blood with salting out procedure (37).

Genotypic analysis of the TNFα-308 G/A polymorphism

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assays was used to determine TNFα-308 G/A polymorphism. The oligonucleotide primers were used to determine -308 G/A polymorphism within TNFα gene (38). The primers, forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and reverse 5'-TCCTCCCTGCTCCGATTCCG-3' were used to amplify TNFα gene. PCR was performed in a total volume of 25 μl containing 100 ng DNA, 100 μm dNTPs, 20 pmol of each primer, 1.5 mM $MgCl_2$, 1x PCR buffer with $(NH_4)SO_4$ (MBI Fermentas, Vilnius, Lithuania), 10 % DMSO and 2U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). Initial denaturation lasted for 2min at 95°C; denaturation for 45s at 35 cycles and 95°C, annealing for 1 min at 60°C, extension for 90 s at 72°C and final extension for 7 min at 72°C. Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). After the amplified PCR products were digested with restriction endonuclease 10 U Nco I (MBI Fermentas, Vilnius, Lithuania) for 14 h at 37°C, the genotyping of the TNFα gene was performed with fragment separation at 120 V for 40-50 min on a 3.5 % Agarose gel containing 0.5μg/ml ethidium bromide. A 100 bp marker (50 bp DNA Ladder, MBI Fermentas) was used as a size standard for each gel lane. The gel was visualized under UV light with a gel electrophoresis visualizing system (Vilber Lourmat). The Nco I restricted products of TNFα-308 G/A, GG, GA and AA genotypes had band sizes of 87bp/20bp, 107bp/87bp/20bp and 107bp respectively. Genotyping was based upon independent scoring of the results by two reviewers who were unaware of case/control status.

Statistical analyses

Descriptive statistics were expressed in mean ± SD and frequencies (numbers and percentages) in tables. Multiple logistic regression models were used to compare the distribution of genotypes between cases and controls and to determine the association between TNFα-308 G/A polymorphism and knee OA. To adjust differences in age, BMI, and gender between the groups, these three variables were included into the model as covariate and thus relations between genotypes and the disease were corrected in accordance with covariate effects. Odds ratios (ORs) for this model with the corresponding 95 % confidence intervals (95 % CIs) were also computed. Alleles were counted to determine their frequencies, and their proportions in samples were calculated. Pearson chi-squared test was used to compare the distribution of allelic frequencies between the control and OA groups. Fisher's exact test was used to check the allele distribution in each group for deviations from Hardy-Weinberg equilibrium. One way ANOVA, Kruskal-Wallis and Pearson chi-squared tests were used to determine the relations between genotypes of TNFα-308 G/A polymorphism and clinical, laboratory and radiological characteristics of knee OA.

All statistical analyses were performed with SPSS software, version 12. $P<0.05$ was considered significant.

Results

The demographic characteristics of the study population are shown in Table I. There were significant differences between the groups in age and body mass index (BMI), but there was not a significant difference in gender. The mean age of the patients and control group were 61.7±9.2 (range: 41-86 years) and 52.3±6.9 (range: 41-75 years) years respectively (p=0.0001). The mean BMI was 30.0±4.3 kg/m² in patients with

Table I. Characteristics of the study population.

Characteristics	Patients (n=146)	Control (n=84)	*p*-values
Age, mean ± SD, years	61.7 ± 9.2	52.3 ± 6.9	0.0001*
BMI, mean ± SD, kg/m²	30.0 ± 4.3	27.3 ± 6.0	0.0001*
Female/Male ratio, n	118/33	59/25	0.169

Table II. Genotype distribution of TNFα gene -308 G/A polymorphism between groups.

TNFα -308	Patients (n, %)	Controls (n,%)	*p*-values	OR	95 % CI
GG*	121 (80.1)	72 (85.7)	0.537	(-)	(-)
GA	26 (17.2)	12 (14.3)	0.533	1.3	0.5-3.1
AA	4 (2.6)	0 (0.0)	0.338	3.5	0.2-48.7
TNFα -308	Female patients (n %)	Female controls (n %)	*p*-values	OR	95 % CI
GG*	95 (80.5)	49 (83.1)	0.709	(-)	(-)
GA	20 (16.9)	10 (16.9)	0.860	1.0	0.4-2.8
AA	3 (2.5)	0 (0.0)	0.412	3.0	0.2-44.9
TNFα -308	Male patients (n %)	Male controls (n %)	*p*-values	OR	95 % CI
GG*	26 (78.8)	23 (92.0)	0.498	(-)	(-)
GA	6 (18.2)	2 (8.0)	0.238	4.7	0.3-63.3
AA	1 (3.0)	0 (0.0)	1.0	IR	IR

OR: Odds ratio; 95% CI: 95% confidence interval; IR: Inefficient results (simple size is too small); * Reference genotype.

Table III. Allelic frequency distribution of TNFα gene -308 G/A polymorphism between groups.

Alleles	Patients (n %)	Controls (n%)	*p*-values
G	268 (88.7)	156 (92.9)	0.150
A	34 (11.3)	12 (7.1)	0.150
Alleles	Female patients (n %)	Female controls (n %)	*p*-values
G	210 (89.0)	108 (91.5)	0.456
A	26 (11.0)	10 (8.5)	0.456
Alleles	Male patients (n %)	Male controls (n %)	*p*-values
G	58 (87.9)	48 (96.0)	0.123
A	8 (12.1)	2 (4.0)	0.123

Table IV. The relationship between TNFα -308 genotypes and clinical, laboratory findings of patients of with knee OA.

	GG	GA	AA	*p*-values
WPS	14.4 ± 3.7	13.1 ± 3.0	15.7 ± 4.6	0.293
WSS	4.8 ± 2.3	4.6 ± 1.8	5.7 ± 2.0	0.679
WPFS	47.3 ± 14.3	45.7 ± 11.5	48.5 ± 14.5	0.897
ESR (mm/h)	23.6 ± 16.3	19.2 ± 28.6	33.2 ± 22.5	0.393
CRP (mg/L)	3.1 ± 2.8	3.1 ± 4.0	7.7 ± 11.5	0.643

WPS: WOMAC pain subscale; WSS: WOMAC stiffness subscale; WPFS: WOMAC physical function subscale.

knee OA and 27.3±6.0 kg/m^2 in the control group (p=0.0001). In addition, the male/female ratio was 33/118 in patients and 25/59 in controls (p=0.169). The study population was adjusted for age (in years) and BMI (kg/m^2) using multiple logistic regression model. The distribution of genotypes both in the patients with knee OA and in the controls did not show deviations from Hardy-Weinberg equilibrium (p>0.05). The genotype distributions and allele frequencies of TNFα-308 G/A polymorphism were compared between the OA patients and the control group. The genotype distributions of 151 patients with knee OA and 84 control subjects were 121GG, 26GA, 4AA and 72GG, 12GA, 0AA respectively. The G allele was found in 92.7% of the control subjects and in 88.7% of the patients, while the A allele was detected in 11.3% of the patients with knee OA and in 7.1% of the controls. There were no significant differences between the groups in both the genotype distributions and allele frequencies of TNFα -308 gene polymorphism (p>0.05, Tables II and III). Thereafter, we compared genotype distributions and allele frequencies between patients and control individuals of the same sex for the TNFα-308 G/A polymorphism. There were no significant differences between OA patients and controls of the same sex (p>0.05). The relationships between the genotype distribution of TNFα -308 gene polymorphism and clinical characteristics (such as WOMAC pain, stiffness and physical function scores), results of the laboratory investigations (ESR, CRP), and K&L radiological scores are shown in Tables IV and V. In addition, there was not a significant difference between genotype distribution of the TNFα-308 gene polymorphism and clinical features, laboratory findings and radiological scores of patients with knee OA.

Discussion

This is the first study to evaluate the role of TNFα -308 G/A polymorphism in the pathogenesis of osteoarthritis. In the present study, we found no relation between TNFα -308 G/A polymorphism and the pathogenesis of osteoarthritis, which suggested that the TNFα -308 G/A polymorphism were not markers of genetic susceptibility to knee OA in the Turkish population. Furthermore, this study also suggested that TNFα -308 G/A polymorphism were not associated with the severity of functional, radiological and laboratory features of knee OA.

The observed lack of association between -308 G/A polymorphisms in the TNFα gene and susceptibility to or severity of knee osteoarthritis may simply indicate that this polymorphism has a minor or no role in the susceptibility to or severity of knee osteoarthritis. Another possibility is that this polymorphism in the TNFα gene may be responsible for the pathogenesis of osteoarthritis, but this influence might

Table V. The relationship between TNFα -308 genotypes and radiological findings of patients with knee OA.

K&L score	GG	GA	AA	*p*-values
Grade 1 (%)	19.8	29.4	50	0.395
Grade 2 (%)	43	35.3	0	0.395
Grade 3 (%)	29.1	35.3	50	0.395
Grade 4 (%)	8.1	0	0	0.395

have been too small to be detected in the present study sample, and a larger sample size may be required.

Alternatively, a possible association may have been weakened by disease heterogeneity, environmental factors or gene-environment interactions. In fact, linkage disequilibrium is strong in this area, and it may be difficult to study the role of single nucleotide polymorphisms in isolation (39). Moreover circulating TNFα levels are regulated at different stages: gene transcription, post-transcription control of mRNA stability, cleavage of the membrane form to liberate the soluble form, and the expression of receptors (40).

This study has some limitations. Firstly, the sample size was small, which may limit statistical power to detect any existing association. Secondly, radiographs were not taken for all control subjects. Therefore, we cannot exclude the presence of asymptomatic osteoarthritis in the controls, which weakens the statistical power of the study as well. Thirdly, we examined only one polymorphism because of its relatively high frequency. However, several additional polymorphisms in the TNFα gene may exist and these polymorphisms possibly contribute to the susceptibility to or severity of knee osteoarthritis. Fourthly, the study population was not similar in terms of age and BMI, but we adjusted both groups for age and BMI using multiple logistic regression method. Although these drawbacks exist in the formation of the study protocol, this is nevertheless the first study in the literature performed to determine whether TNFα (-308 G/A) gene polymorphism was a genetic marker of susceptibility to, or severity of knee OA in a Turkish population.

In conclusion, the obtained data suggest that the -308 G/A polymorphism in the TNFα gene is not associated with susceptibility to or severity of knee osteoarthritis in the Turkish population and, therefore, cannot be regarded as a major cause of osteoarthritis. This finding will require replication in different ethnic populations with larger samples.

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