Analysis of TNF-alpha G308A and C857T Gene Polymorphisms in Turkish Patients with Obstructive Sleep Apnea Syndrome

Obstrüktif Uyku Apne Sendromu Olan Türk Hastalarda TNF-Alfa G308A ve C857T Gen Polimorfizmlerinin İncelenmesi

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ABSTRACT Objective: Tumor necrosis factor-alpha (TNF-alpha) is an important indicator of inflammation. Recent studies have demonstrated a relationship between inflammation and obstructive sleep apnea syndrome (OSAS). The aim of this study was to investigate the association between TNF-alpha G308A and C857T gene polymorphisms and OSAS in Turkish patients. **Material and Methods:** Sixty-nine patients who were diagnosed with OSAS and 42 control subjects were included in the study. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect TNF-alpha G308A and C857T gene polymorphisms. The level of significance for statistical analysis was set at $p<0.05$. **Results:** The distribution of genotypes was not significantly different between subjects with a definite diagnosis of OSAS and the control group ($p> 0.05$). However, the mean body mass index of the OSAS group was significantly different from that of the control group ($p<0.05$). **Conclusion:** To our knowledge, this study is the first to analyze the relationship between OSAS and TNF-alpha G308A and C857T gene polymorphisms in Turkish patients. Our results do not support an association between OSAS and TNF-alpha G308A and C857T gene polymorphisms.

Key Words: Polymorphism, single nucleotide; sleep apnea, obstructive

ÖZET Amaç: Tümör nekroz faktörü-alfa (TNF-alfa), yangının önemli bir göstergesidir. Son çalışmalar, yangı ve obstrüktif uyku apne sendromu (OUAS) arasında bir ilişki olduğunu göstermiştir. Bu çalışmanın amacı, Türk hastalarda, TNF-alfa G308A ve C857T gen polimorfizmleri ile OUAS arasında bir ilişki olup olmadığını araştırmaktır. **Gereç ve Yöntemler:** OUAS tanısı almış 69 hasta ile 42 kontrol birey çalışmaya dahil edildi. TNF-alfa G308A ve C857T gen polimorfizmlerini belirlemek için polimeraz zincir reaksiyonu kısıtlayıcı fragman boyu polimorfizmi [polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)] yöntemi kullanıldı. İstatistiksel analizler için anlamlılık düzeyi $p<0,05$ olarak belirlendi. **Bulgular:** Genotiplerin dağılımı OUAS ve kontrol grupları arasında anlamlı derecede farklı değildi ($p>0,05$). Ancak, OUAS grubunun ortalama beden kitle endeksi ile kontrol grubunun beden kitle endeksi arasında anlamlı bir farklı vardı ($p<0,05$). **Sonuç:** Bu çalışma bildiğimiz kadarıyla Türk hastalarda OUAS ile TNF-alfa G308A ve C857T gen polimorfizmleri arasındaki ilişkiyi araştıran ilk çalışmadır. Elde ettiğimiz sonuçlar, OUAS ve TNF-alfa G308A ve C857T gen polimorfizmi arasında bir ilişkiyi desteklememektedir.

Anahtar Kelimeler: Çok biçimlilik, tek nükleotid; uyku apnesi, tıkayıcı

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Obstructive sleep apnea syndrome (OSAS) is characterized by repeated episodes of apnea-hypopnea that are caused by a narrowing or blocking of the upper airway during sleep. The relationship between OSAS and cardio-vascular complications and thromboembolic incidents has been the subject of considerable research. OSAS is now considered

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an independent risk factor for hypertension and cardiovascular mortality.[1-4]

There is considerable and increasing evidence implicating inflammation in OSAS. A polymorphism caused by a guanine to adenine conversion at position -308 in the promoter region of the tumor necrosis factor alpha (TNF-alpha) gene has been reported to modulate TNF-alpha production in both in vivo and in vitro studies.[4-6] TNF-alpha may also play a role in the regulation of pro-inflammatory cytokines during sleep. Excessive daytime sleepiness promotes increases in TNF-alpha levels. Furthermore, intermittent hypoxia has been reported to be a major indicator of serum TNF-alpha levels.[4-7,8] However, studies have shown that the presence of the A allele in the TNF-alpha gene alters adipose tissue metabolism, leading to increased TNF production by doubling the TNF-alpha gene expression.[9,10]

The TNF-alpha locus is located within the highly polymorphic major histocompatibility III (MHC III) gene region of chromosome 6p21.3. This gene contains several single nucleotide polymorphisms (SNPs). In the Caucasian population, the most commonly varied SNP positions are at 308, 857, 863 and 1031 bp upstream of the TNF-alpha transcription start site. Various studies have demonstrated that these polymorphisms may have an impact on TNF-alpha gene expression.[11-13]

Several studies have investigated the relationship between OSAS and polymorphisms in the TNF-alpha gene, and the results of those studies suggest that the G308A polymorphism might be correlated with OSAS.[4,6] However, Popko et al.[9] were not able to confirm this relationship. Generally, only the G308A polymorphism has been studied in relation to the development of OSAS, and because the effects of other TNF-alpha gene polymorphisms are not clear, further research is required. To the best of our knowledge, the only genetic study of Turkish patients suffering from OSAS was conducted by Yakut et al. to investigate the potential link between ACE I/D gene polymorphism and OSAS, but no studies have investigated the relationship between OSAS and TNF-alpha polymorphism.[14]

The aim of this study was to investigate the impact of polymorphism in the TNF-alpha gene on the development of the disease in Turkish patients suffering from OSAS.

MATERIAL AND METHODS

STUDY SUBJECTS

This study involved 111 subjects who were classified based on the apnea-hypopnea index (AHI), as determined by standard polysomnography at the Department of Pulmonary Medicine. The number of apnea and hypopnea episodes was divided by the total sleep time to obtain the AHI score. Patients with an AHI ≥10 were considered to have OSAS. Subjects with an AHI <5 were included in the control group. Subjects with AHI scores between 5 and 10 were not included in the analysis in order to maintain a clear distinction between the subjects with and without OSAS.[4] Among the subjects tested, 69 were assigned to the OSAS group, and 42 were assigned to the control group. Demographic characteristics (age and gender) and body mass index (BMI) were noted for all individuals. The study was approved by the local Ethics Committee.

DNA ISOLATION AND GENOTYPING OF TNF-ALPHA (G308A AND C857T)

Blood samples were obtained from patient and control groups and were collected in EDTA tubes. Genomic DNA was extracted from whole blood using a DNA isolation kit (Dr. Zeydanlı Life Sciences, Ltd., Turkey) according to the manufacturer's instructions.

The genotypic analysis of the TNF-alpha G308A and C857T polymorphisms was performed using a modified version of a previously described polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.[15] Briefly, C857T genotyping was run using the forward primer 5′-AAGTCGAGTATGGGGACCCCCCGTTAA-3′ and the reverse primer 5′-CCCCAGTGTGTGGCCATATCTTCTT-3′, which enabled the use of the restriction enzyme HincII. The polymorphic region containing the NcoI restriction site at G308A was amplified using primers 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-TC-

CTCCCTGCTCCGATTCCG-3′. PCR products were digested with the restriction enzymes HincII and NcoI at 37°C for 16 hours and were analyzed on a 4% agarose gel. When C was present at codon 857, the 131 base pair (bp) PCR product was cleaved into two fragments of 106 and 25 bp (Figure 1). When C was present at codon 308, the 107 bp PCR product was cleaved into two fragments of 87 and 20 bp.

STATISTICAL ANALYSIS

The normal distribution of age and BMI variables was tested using the Shapiro-Wilk test. Age and BMI variables were represented as the mean±standard deviation. These variables were compared between the OSAS and control groups using independent samples t tests. Sex and the G308A and C857T gene polymorphism variables were represented as frequencies and relative percent values. These variables were compared between the OSAS and control groups using the Pearson's chi square tests with Yates correction and Fisher's exact tests. All statistical analyses were performed in SPSS 13.0 (Chicago, IL). Statistical significance was set at $p<0.05$.

RESULTS

The mean ages of the 69 subjects in the patient group (52 males and 17 females) and the 42 subjects in the control group (29 males and 13 females) were 49.75±11.32 and 50.55±10.65, respectively. There was no significant difference in age or sex between the groups (p=0.715, p=0.613, respectively). However, BMI was significantly different in the OSAS group and the control group (p=0.002) (Table 1).

TABLE 1: Clinical characteristics of the obstructive sleep apnea syndrome group and the control group.

	OSAS patient group n=69	Control group n=42	p value
Sex			
Female, n (%)	17(24.60)	13(31.00)	0.613
Male, n (%)	52(75.40)	29(69.00)	
Age (years)±SD	49.75±11.32	50.55±10.65	0.715
BMI (kg/m²)±SD	31.09±4.05	28.50±4.34	0.002

BMI: Body mass index;
OSAS: Obstructive sleep apnea syndrome;
SD: Standard deviation.

The distribution of the G/G and G/A genotypes of the G308A polymorphism in the TNF-alpha gene was not significantly different between the OSAS and control groups (p=0.744). The distribution of the C/C, C/T and T/T genotypes were similar in the OSAS and control groups (TNF-alpha C857T C/C: 50.72%, C/T: 44.93% and T/T: 4.35% in the patient group; C/C: 52.38%, C/T: 42.86% and T/T: 4.76% in the control group; p=0.979, p=0.987 and p=1, respectively) (Table 2).

For the TNF-alpha G308A polymorphism, the frequency of the A allele was 13% in the OSAS patient group and 11% in the control group. For the TNF-alpha C857T polymorphism, the frequency of the T allele was 27% in the OSAS patient group and 26% in the control group. The allelic frequencies of both polymorphisms were thus similar in both groups (Figure 2). The A allele frequencies for TNF-alpha G308A found in the patient and control groups of previously published studies were shown in Table 3.

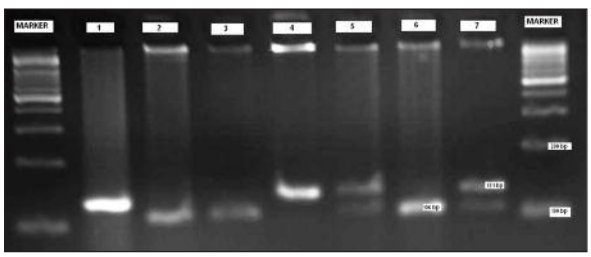

FIGURE 1: Image of TNF-alpha gene (C857T) polymerase chain reaction (PCR) products after HincII enzyme digestion, resolved on a 4% agarose gel. The MARKER lane shows a 100 bp DNA ladder; lane 1, uncut PCR product (no restriction enzyme added); lanes 2, 3, and 6, C/C genotype (106 bp, 25 bp); lane 4, T/T genotype (131 bp); lanes 5 and 7, C/T genotype (131 bp, 106 bp, 25 bp).

DISCUSSION

OSAS is frequently associated with inflammation and elevated levels of pro-inflammatory cytokines in the circulation. TNF-alpha and interleukin-6 (IL-6) levels increase in OSAS, independent of patient obesity, and the circadian rhythm in the secretion of TNF-alpha is disturbed. Furthermore, other regulators of inflammation, such as intracel-

TABLE 2: Distributions of the TNF-alpha (G308A) and (C857T) allele frequencies in the obstructive sleep apnea syndrome group and the control group.

	OSAS patient group n=69	Control group n=42	p-value
G308A gene polymorphism			
G/G genotype	51(73.90)	33(78.60)	0.744
G/A genotype	18(26.10)	9(21.40)	
C857T gene polymorphism			
C/C genotype	35(50.72)	22(52.38)	0.979
C/T genotype	31(44.93)	18(42.86)	0.987
T/T genotype	3(4.35)	2(4.76)	1.000

Table cell values indicate n (%). OSAS: Obstructive sleep apnea syndrome.

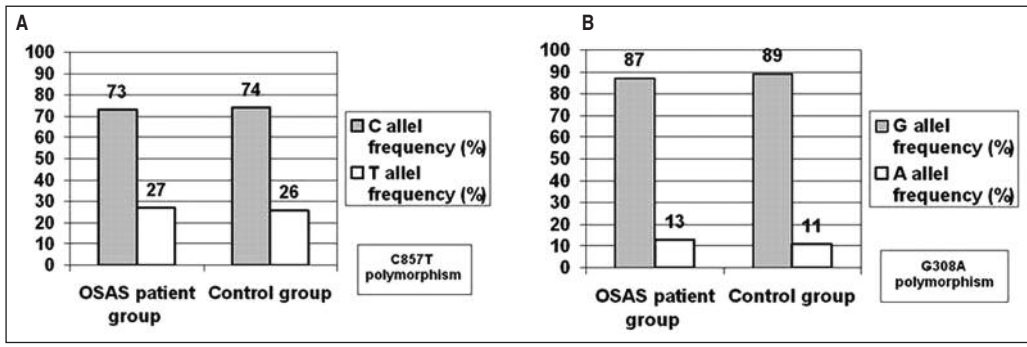

FIGURE 2: Distribution of the allele frequencies of both TNF-alpha polymorphisms in the obstructive sleep apnea syndrome patients and control groups.
A: C857T polymorphism. The gray bars represent the frequency of the C allele, and the white bars represent the frequency of the T allele in both groups.
B: G308A polymorphism. The gray bars represent the frequency of the G allele, and the white bars represent the frequency of the A allele in both groups.

TABLE 3: TNF-alpha G308A A allele frequencies in obstructive sleep apnea syndrome patients and control groups from published studies.

Study	Number of OSAS patients	A allele frequency of OSAS patients (%)	Number of control subjects	A allele frequency of control group (%)
Bhushan et al.[4]	104	29	103	13
Riha et al.[6]	103	28	190	18
Popko et al.[9]	102	14	77	12
This study	69	13	42	11

OSAS: Obstructive sleep apnea syndrome.

lular adhesion molecule-1 and C-reactive protein (CRP), are also elevated. TNF-alpha, CRP and IL-6 appear to exert their harmful effects by inducing endothelial dysfunction. TNF-alpha causes apoptosis in endothelial cells and triggers pro-coagulant activity and fibrin build up. TNF-alpha also increases the production of reactive oxygen species and decreases myocardial contractility.[6,16-18]

Several studies have investigated TNF-alpha gene polymorphism in OSAS patients.[4,6,9] Riha et al. reported an association between the TNF-alpha gene polymorphism G308A and OSAS in 103 patients.[6] Bhushan et al. suggested that the TNF-alpha gene polymorphism might increase the risk of inflammation in obese Asian Indians with OSAS, reporting that the A308 allele frequency of

TNF-alpha was twice as high in patients with OSAS than in individuals who did not suffer from OSAS.[4] In contrast, Popko et al. did not detect any significant difference in the A308 allele frequency between groups of patients with and without the disease.[9] In that study, this polymorphic variant was shown to affect the promoter region of TNF-alpha, leading to a higher rate of gene transcription than was observed for the -308G allele. Polymorphism in the regulatory region of a gene may lead to variations in gene expression.

Here, we reported the first prospective patient-based study of Turkish OSAS patients. There were no associations between the TNF-alpha G308A and C857T gene polymorphisms and OSAS risk (Table 2). Our study is important because it is the first to include Turkish OSAS patients as well as being the first to study the C850T polymorphism in conjunction with OSAS. In this study, the frequencies of the A allele of -308 in the patient and control groups were similar (13% and 11%, respectively) (Figure 2). These rates are similar to those reported by Popko et al. but lower than those reported by Bhushan et al. and Riha et al (Table 3).[4,6,9] The difference in allelic frequencies may be due to the ethnic and geographic backgrounds of the populations in each study.

Several studies have reported that the TNF-alpha C857T gene polymorphism may increase the risk of the development of inflammation-related diseases, such as psoriatic arthritis, multiple sclerosis and chronic fatigue syndrome.[19-21] However, the TNF-alpha C857T gene polymorphism has not been studied in OSAS patients, although the G308A polymorphism has been investigated. Thus, we investigated the relationship between OSAS and the TNF-alpha C857T polymorphism, which has been linked to inflammation. In our study, the genotypic distribution of TNF-alpha C857T was not significantly different between OSAS patients and the control individuals (Table 2). The allele frequencies in the patient and control groups were very similar, with 27% and 26%, respectively.

Interestingly, the average BMI in the two groups showed a significant difference (Table 1). The BMI of the OSAS group was higher than that of the control group. The mean BMI in our group of OSAS patients was similar to that reported in other polymorphism studies.[6,22,23]

While no significant relationship was identified between OSAS and the TNF-alpha G308A and C857T gene polymorphisms, the number of cases in our study was small relative to those of the studies conducted in other populations.

Thus, the conclusions of this report should be supported by further studies in a larger number of Turkish patients with OSAS. Further research to investigate the relationship between OSAS and other polymorphisms that affect cytokine expression would also be valuable.

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