RESEARCH ARTICLE

The relationship between tumor necrosis factor α-308 G/A polymorphism and serum tumor necrosis factor α levels in patients with migraine without aura

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ABSTRACT

Objective: The aim of this study is to investigate the association of tumor necrosis factor (TNF) α-308 G/A genotype with migraine risk and the effect on serum TNF-α levels in patients with migraine without aura.
Methods: A total number of 70 patients with the diagnosis of migraine without aura and 65 control subjects were included in this study. TNF-α level was studied from two separate blood samples taken from the patient group during an attack and attack-free period separately and TNF-α-308 G/A genotype was studied once while one blood sample was taken for studying the TNF-α-308 G/A and TNF-α level genotype from the control group.
Results: The mean TNF-α level during an attack and attack-free periods were 13.58±2.84 pg/mL and 12.91±3.39 pg/mL, respectively in patients with migraine (n=70). This difference was statistically significant (p=0.033). The mean serum TNF-α level during an attack was 14.37±3.4 pg/mL in the migraine patients with G/A genotype (n= 38) and 12.79±1.54 pg/mL in those with A/A genotype (n=30); the difference between the two groups was statistically significant (p=0.034). The effect of genotype distribution on migraine development was statistically significant (p=0.003).
Conclusion: TNF-α-308 G/A polymorphism is related to migraine in Turkish patients and TNF-α level increases during an attack compared with attack-free periods in patients with migraine without aura. Further studies are required for determining the relationship between TNF-α308 G/A polymorphism and TNF-α levels in the serum.

Keywords: TNF-α, migraine, TNF-α-308 G/A polymorphism

Aurasız migrenlilerde tümör nekroz faktörü α-308 G/A polimorfizmi ve serum tümör nekroz faktörü α düzeyleri arasındaki ilişki

ÖZET

Amaç: Bu çalışmanın amacı, tümör nekroz faktör (TNF)-α 308 G/A genotipinin migren ile ilişkisini ve aurasız migrenlilerde serum TNF- α düzeyi üzerine etkisini araştırmaktır.
Yöntem: Bu çalışmaya aurasız migren tanılı 70 hasta ile migreni olmayan 65 kişiden oluşan kontrol grubu dahil edildi. Hasta grubunda TNF-α seviyesi biri atak biri ataksız dönemde olmak üzere alınan iki ayrı kan örneğinden ayrı ayrı çalışılmış, TNF-α 308 G/A genotipi ise bir kez çalışılmıştır. Kontrol grubunda da TNF-α seviyesi ve TNF-α 308 G/A genotipi birer kez çalışılmıştır.
Bulgular: Migren (n=70) grubunda ataklı ve ataksız dönemde TNF-α seviyesi sırasıyla 13.58±2.84 pg/mL ve 12.91±3.39 pg/mL idi. Bu fark istatistiksel olarak anlamlıydı (p=0.033). G/A genotipi olan migren hastalarında (n=38) atak sırasında ortalama serum TNF-a seviyesi, 14.37±3.4 pg/mL ve A/A genotipi olanlarda (n=30) ise 12.79±1.54 pg/mL idi. İki grup arasındaki fark (p=0.034) istatiksel olarak anlamlıydı. Ayrıca genotip dağılımının migren gelişimi üzerindeki etkisi istatistiksel olarak anlamlı olarak bulundu (p=0.003).
Sonuç: TNF-α 308 G/A polimorfizmi, Türkiye'deki migren hastaları ile ilişkilidir ve aurasız migrenli hastalarda TNF- α seviyesi ataklı dönem ile ataksız dönemle karşılaştırıldığında artmaktadır. TNF-α 308 G/A polimorfizmi ile serumdaki TNF-α seviyeleri arasındaki ilişkinin belirlenmesi için daha fazla çalışmalar gerekir.

Anahtar kelimeler: TNF-α, migren, TNF-α 308 G/A polimorfizmi

INTRODUCTION

Migraine is a highly prevalent paroxysmal neurological disorder with as much as 17% of the population and three times higher in females than males [1]. In the migraine pathophysiology, the vascular theory has largely been abandoned and the integrated vascular theory has been adopted under the light of recent data [2]. In this theory, as a result of neural events, blood vessels in anatomical structures which are sensitive to pain become dilated, leading to trigeminal nerve activation and pain [2-6]. Migraine is clinically

classified generally into two subtypes based on presence of aura symptoms such as hallucination, paresthesia, weakness and disturbed vision [7].

Although the pathological mechanism of migraine is unclear, evident data strongly suggests the impact of inflammation. Pro and anti-inflammatory cytokines [2,5,8] play an important role in pain modulation, and also contribute to sensitization of the trigeminal nerve [2,5]. Cytokines are the pain mediators in neurovascular inflammation and they are also the generators of pain in migraine [5].

Cytokines produced by a wide range of cell types, serves as humoral immunomodulatory mediators and exert their biological functions through their receptors [9]. They act in a complex regulatory network. In addition, severity of chronic inflammatory diseases have been affected from various genetic and epigenetic factors [9]. Especially the polymorphisms within the regulatory region of genes which code for cytokines often affect the expression levels and can serve as disease modifiers [9].

The tumor necrosis factor (TNF) family modulates the inflammatory pathway [10] previous clinical trials suggested an association with headache [5, 11]. The TNF-α gene is located between HLA-B and HLA-DR on human chromosome 6. The polymorphisms at positions −238 and −308 are the best characterized promoter polymorphisms defined in TNF-α gene and effect the production of protein at the transcriptional level [10]. These polymorphisms have been linked to various chronic diseases including migraine in several recent reports [12-15].

Moreover, Rainero et al. showed the relationship between TNF-α 308 G/A gene polymorphism and migraine [11]. TNF-α, IL-1β, and IL-6 leads to hyperalgesia by triggering prostanoid release, by increasing nerve growth factor (NGF) and bradykinin receptor expression, or by modulating the activity of sympathetic fibers [16-18]. And also, TNF-α increased the transcription of calcitonin gene-related peptide, which is a treatment target in recent drugs in migraine [10, 19]. However, there are also conflicting results in the relation between TNF-α and migraine. In meta-analysis overall susceptibility to migraine was not found with TNF-α genotype but also suggested relation in different ethnic groups and migraine types [20-22].

In this study, we aimed to evaluate the association between TNF-α 308 G/A polymorphisms and migraine and compare with the serum TNF-α.

MATERIALS and METHODS

The patient group was composed of 70 patients presenting to neurology outpatient clinic of tertiary center; these patients (i) were diagnosed with migraine without aura according to the International Headache Society (IHS) criteria [25], (ii) were between 18 and 55 years of age, and (iii) had no infectious, immunological, or inflammatory disorders, and accepted to participate in our study by signing the informed consent form approved by local ethics committee. This study was carried out between July 2007 and May 2009. The period of migraine headache was considered as migraine attack, and the period without a migraine-type headache for at least 7 days was considered an attack-free period.

The study group included 70 subjects having migraine without aura and 65 age and sex match healthy volunteers as control.

Ethical approval

This study was approved by Başkent University Institutional Review Board and Ethics Committee (Project no: KA07/114; 11/07/2007).

TNF-α level

In order to determine serum TNF-α level the blood sampling was repeated twice for the patient group as; during the migraine attack and 7 days after the migraine attack while once for the control group: All blood specimens were collected in to non-additive tubes, following centrifugation at 3000 rpm, they were kept at -70°C until the day of analysis. Serum TNF-α level were measured with ELISA method using Bio Source Invitrogen, Camarillo, CA kit. The sensitivity of the TNF-α enzyme immunoassay kit was 1.7 pg/mL.

Genotyping

3 mL of peripheral blood samples were drawn from the antecubital vein of all participants. Genomic DNA (hereinafter, DNA) was extracted from peripheral blood leukocytes by means of a high pure polymerase chain reaction (PCR) template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). Genotypes of TNF-α-308 G/A gene polymorphism were determined by restriction fragment length polymorphism analyses after PCR with appropriate primers according to the slightly modified procedures previously described [24].

A 220-bp PCR product was cut with NcoI to reveal the TNFA −308A, −308G polymorphism. The uncut product (220 bp) showed the presence of the A allele. If the PCR product was cut into 2 fragments (as 201 and 19 bp), it revealed the G allele [24].

Statistical analysis

The normality of the continuous variables was tested with Shapiro-Wilk test. The homogeneity of variances was tested with Levene test. As the parametric assumptions were not met, the means of two independent groups were compared with Mann-Whitney U test and two related groups were compared with Wilcoxon test. The results were expressed as mean± standard deviation. The categorical variables were analyzed with likelihood ratio test and Fisher's exact test since the frequency of some cells of the cross table were too low to perform Chi-Square test. The results were expressed as percentages. A $p<0.05$ was considered statistically significant. The data set was analyzed with SPSS software package (SPSS version 15.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Seventy patients with the diagnosis of migraine without aura according to IHS criteria [23] and 65 age- and sex-matched subjects as the control group were included in the study. Fifty-four (77.1%) of the migraine patients and fifty (76.9%) of the control subjects were female. The mean ages of the patient and control groups were 33.1±7.9 and 32±8.2, respectively.

TNF-α 308 G/A polymorphism was studied and, 38 (54.3%) of migraine patients had G/A, 30 (42.9%) had A/A, and 2 (2.9%) had G/G genotype. Among the control group, 52 (80%) had G/A and 13 (20%) had A/A genotype. None of the control group subjects had G/G genotype. The frequency of the A/A genotype is higher in migraine patients than control group and this difference was statistically significant (p=0.003) (Table 1).

The genotype distribution of the patients studied for TNF-α 308 G/A gene polymorphism was analyzed using the Hardy-Weinberg equation. As none of our control subjects had G/G genotype, the number of our subjects was not appropriate for the Hardy-Weinberg analysis. There were no gender differences with respect to age and serum TNF-α levels during the attack and attack-free periods. In the control group the male and female subjects had similar serum TNF-α levels and age.

The patient group had a TNF-α level of 13.58±2.84 pg/mL during the attack and 12.91±3.39 pg/mL during the attack-free period. The control group had a mean TNF-α level of 13.25±2.72 pg/mL. No significant differences were detected in the separate comparisons of the TNF-α levels of the patient group both during the attack and attack-free periods with the TNF-α level of the control group.

When all patients (n=70) with migraine are considered, the mean TNF-α level during the migraine attack was 13.58±2.84 pg/mL while it was 12.91±3.39 pg/mL during the attack-free period. This difference was statistically significant (p= 0.033) (Figure 1).

Analysis of the correlation of the TNF-α levels of the patients with TNF-α 308 G/A polymorphism based on the migraine attack revealed that the mean TNF-α level of the patients with G/A genotype was 14.37±3.4 pg/mL during the migraine attack and 12.25±2.03 pg/mL during the attack-free period. The TNF-α levels during the attack and attack-free periods differed significantly in patients with G/A genotype (p=0.001) (Figure 2) (Table 2).

Table 1. The TNF-α -308 G/A polymorphism genotype distribution of the study subjects.

	Patient n (%)	Control n (%)	p
Genotype G/G	2 (2.9)	0 (0)	
Genotype G/A	38 (54.3)	52 (80)	0.003
Genotype A/A	30 (42.9)	13 (20)	

The mean TNF-α level of the patients with A/A genotype was 12.79±1.54 pg/mL during the migraine attack and 13.91±4.47 pg/mL during the attack-free period. This difference was not significant. As only 2 patients with G/G genotype existed, no statistical analysis could be performed for this genotype (Table 2).

The serum TNF-α level measured during the migraine attack was 14.37±3.4 pg/mL in the patients (n=38) with G/A genotype and 12.79±1.54 pg/mL in those (n=30) with A/A genotype. There was a significant difference between the patients with G/A and A/A genotypes with respect to mean TNF-α level (p=0.034) (Figure 3). The serum TNF-α level measured during the attack-free period was 12.25±2.03 pg/mL in the patients with G/A genotype and 13.91±4.47 pg/mL in the patients with A/A genotype. The difference was not significant (p=0.130). In the control group, subjects (n=52) with G/A genotype had a TNF-α level of 13.120±2.80 pg/mL and those (n=13) with A/A genotype had a TNF-α level of 13.77±2.36 pg/mL (p=0.159). There was no significant difference between G/A and A/A genotypes with respect to TNF-α levels.

DISCUSSION

It is known that migraine is related to some immunological factors [17]. Cytokines are related to a possible pain mediator in neurovascular inflammation and they form the migraine pain generator [17].In our study, 54.32% of our migraine patients had G/A genotype, 42.9% had A/A genotype, and 2.9% had G/G genotype while 80% of the control group had G/A genotype and 20% had A/A genotype. In our study population, TNF-α-308 G/A genotype was significantly associated with susceptibility to migraine without aura as A/A genotype were significantly increased in patients compared to controls (p=0,003).

Table 2. The TNF-α levels of the patients according to the genotype distribution and the migraine attack status.

	n	TNF-α (pg/mL) (attack) mean±SD	TNF-α (pg/mL) (attack-free) mean±SD	p
G/A Genotype	38	14.37±3.4	12.25±2.03	0.001
A/A Genotype	30	12.79±1.54	13.91±4.47	0.622
Total (G/A, A/A, G/G)	70	13.58±2.84	12.91±3.39	0.033

SD: Standard deviation

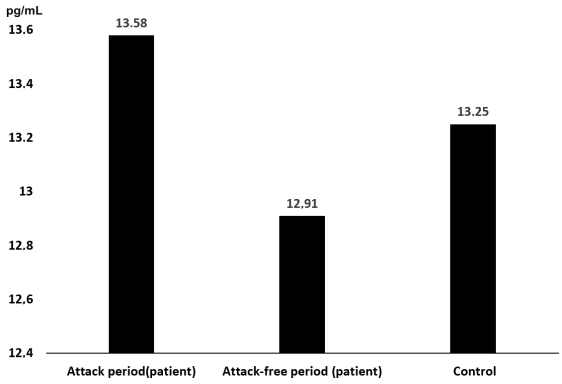

Figure 1. Serum TNF-α levels of the control group and the patients with migraine during the attack and attack-free periods.

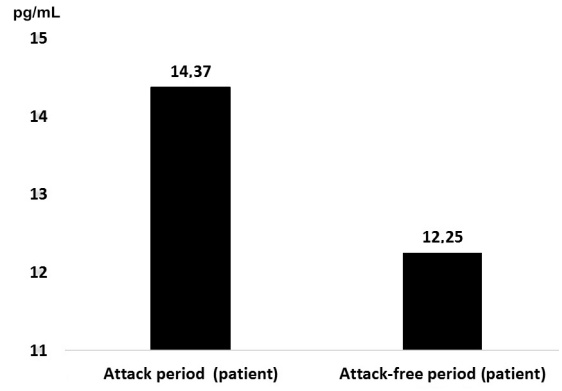

Figure 2. Comparison of the TNF-α levels of the patients with G/A genotype during the attack and attack-free periods.

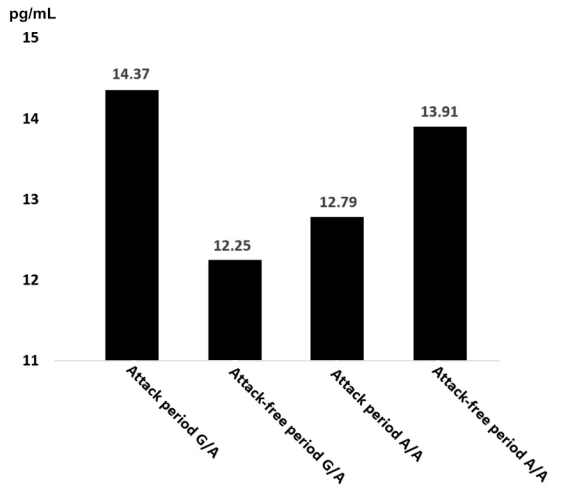

Figure 3. The comparison of the patients with A/A and G/A genotype with respect to TNF-α levels during the attack and attack-free periods.

There are conflicting results in the literature about relationship between genotype distribution and migraine. Trabece et al. reported that there was no significant difference according to genotype distribution between the migraine patients and controls [25]. Whereas association between migraine and TNF-α-308 G/A polymorphism was also shown in different studies [11, 17, 18]. In a recent meta-analysis, they found a susceptibility to migraine without aura in non-Caucasians with A allele, but not in overall study group [21]. Rainero et al. showed that being homozygosity for G allele was a risk factor for migraine and in another study, they found that A allele was higher in patients with migraine without aura [11, 17]. Moreover, in an Egyptian cohort G/A, A/A genotypes and A allele were significantly increased in patients compared to controls and A/A genotype was significantly associated with migraine without aura compatible with our study [18]. The variability of results of the studies made so far may be related to ethnic differences, methodological variations and statistical analyses.

In a previous study that compared TNF-α 308-A allele between a patient population with migraine without aura and a randomly selected control population, a strong positive correlation was found between A allele and women with migraine without aura [26]. Therefore, they concluded that A in the TNF-α 308 position may be a highly productive genotype that is related to increased TNF-α production and transcription [26].

Perini et al. showed that pro- and anti-inflammatory cytokines demonstrated variability in migraine patients [27]. The highest blood levels were observed in those from whom the blood samples were drawn earliest after the onset of a migraine attack [27]. A lower TNF-α level during the attack-free period may be related to down-regulation of the TNF-α metabolism in non-headache period [27]. Gallai reported that this molecule was higher when measured within the first 2 hours after the symptom onset [28]. Sarchielli et al. measured TNF-α level by placing an internal jugular vein catheter in patients with migraine without aura. They reported that TNF-α level reached its apex at the first hour and then normalized gradually and returned to starting level [29]. In our study, the serum TNF-α level during the migraine attack was 14.37±3.4 pg/mL in the migraine patients (n=38) with G/A genotype and 12.79±1.54 pg/mL in the patients (n=30) with A/A genotype (p=0.034). The difference between TNF-α levels of the patients with G/A and A/A genotypes was significantly different during the migraine attack, with TNF-α level being higher in the G/A genotype. In our study, there was a significant difference between TNF-α levels during the attack and attack-free periods in patients with G/A genotype (p=0.001), with the former being higher. In patients with A/A genotype there was no significant difference between the attack and attack-free periods with respect to TNF-α level. Contrast to our results, in the

literature it has been reported that A allele is a stronger transcriptional activator and leads to higher TNF-α levels [18]. As a limitation of our study, the time of blood samples that drawn in attack period was not uniform in all patients. And that variability caused such differences between TNF-α levels and genotype distribution that we found in our study.

Additionally, gene–gene, and gene–environment interactions are likely, since migraine does not fit a simple Mendelian pattern, but is a "multifactorial disease" [30]. Migraine might be associated with inflammation and TNF-α-308 A allele may be one of the many genetic factors for migraine susceptibility.

Perini et al. found no correlation between TNF-α level and age and sex [27]. Also, we found no significant difference between men and women with respect to TNF-α levels during the attack and attack-free periods and age ($p>0.005$).

Our results indicated that, among all study population, there was a significant difference between TNF-α levels during the attack and attack-free periods ($p=0.033$). An excessive spontaneous TNF-α release is present in patients with migraine without aura [5, 18, 26]. An increased TNF-α level at the onset of migraine attack indicates the role of this cytokine in headache development. A genetic susceptibility to TNF-α production may worsen these effects [26]. Perini reported that TNF-α increased during the attack period and decreased during the attack-free period; Gallai reported that TNF-α increased during the attack period; which compatible with our results, on the other hand Emple and Muller reported that TNF-α level did not change during the attack or attack-free periods [27, 31].

Although the pathophysiology of migraine is yet to be elucidated, it is well known that genetic and environmental factors are operational in its development. When TNF-α levels during the attack and attack-free periods were compared in the whole study population, the TNF-α level was higher in the attack period. TNF-α level was significantly higher during the attack compared to the attack-free period in the patients with G/A genotype. There was no difference between the control and patient groups with respect to the alleles. Comparison of the patient and the control groups revealed no significant differences with respect to age, sex, and TNF-α levels both during the attack and attack-free periods.

In conclusion, the result of this study revealed that TNF-α 308 AA genotype associated with risk of migraine without aura. To further understand the relationship between TNF-α 308 G/A polymorphism and TNF-α level, the plasma TNF-α profile should be correlated with time after the onset of symptoms. TNF-α 308 G/A show inter-ethnic differences. Therefore, larger studies in different ethnic groups may aid understanding of the migraine pathogenesis.

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