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Cytokine Polymorphism in Patients with Migraine: Some Suggestive Clues of Migraine and Inflammation

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Abstract

Objective. There are contrasting results obtained in migraineurs concerning the levels and the role of both pro-inflammatory and anti-inflammatory cytokines. In this study, the association of the occurrence and clinical characteristics of migraine with the polymorphisms of tumor necrosis factor α (TNF-α) –308 G/A (rs1800629), interleukin-1α (IL-1α) +4845 G/T (rs17561), IL-1β +3953 C/T (rs1143634) and interleukin-1 receptor antagonist variable number tandem repeat (IL-1RA VNTR) genes were studied. We also investigated the genetic linkage between these genes.

Design, Setting, Patients. Sixty-seven patients with migraine without aura (MwoA) and 96 unrelated, age- and sex-matched migraine-free, healthy control subjects from the same geographic area were investigated.

Results. We observed significant differences in the genotypic distribution of the TNF-α –308 G/A and IL-1β +3953 C/T polymorphism for migraineurs compared with controls ($P = 0.004$). Frequency of the TNF-α –308 GG genotype was higher in the control group than MwoA group (82.1% vs 55.2%). Differences in the distribution of the allele frequencies were also observed, being the TNF-α –308 G allele overrepresented in control group and TNF-α –308 A allele in MwoA group. In addition, there was a significant increase of the IL-1β +3953 T allele in MwoA cases compared with controls ($P = 0.004$).

Conclusions. In conclusion, the present results indicate the possible contribution of TNF-α and IL-1β gene polymorphisms to migraine headache generation in MwoA patients.

Key Words. Migraine Without Aura; Polymorphisms of TNF-α, IL-1α, IL-1β; Interleukin-1 Receptor Antagonist

Introduction

Migraine is a paroxysmal neurological disorder affecting up to 12% of males and 24% of females in the general population, with highest prevalence between ages 25 and 55 years [1,2]. The disease is characterized by recurrent attacks of disabling, mostly unilateral headache, associated with nausea, vomiting, photo and phonophobia, and malaise (migraine without aura [MwoA]). In about one-third of the patients, the attacks are preceded or accompanied by transient focal neurological symptoms (*MwoA*). Genetic factors modulate individual thresholds for migraine, including internal and environmental factors, such as hormonal fluctuations, fatigue, relaxation after stress, meteorological changes, and substance misuse [3].

Although consistent genetic basis has been established only for familial hemiplegic migraine (FHM), migraine has a strong genetic component with 40–50% of the susceptibility being ascribed to multiple genetic factors [4,5]. Approximately 50% of migraineurs have an affected first-degree relative [6]. Family and twin studies have provided conflicting results with respect to the mode of inheritance of migraine. The study of genetic determinants of migraine with aura (MWA) is difficult because it is a complex, probably multifactorial and polygenic disease. Also, there is no clinical difference between sporadic and familial cases.

FHM is a rare type of migraine with aura. It appears to be transmitted by an autosomal dominant mode of inheritance. FHM has been linked to mutations in the calcium channel gene CACNA1A on chromosome 19 and the sodium/potassium pump gene ATP1A2 on chromosome 1.

The pathogenesis of migraine is still unclear, but much evidence suggests a role of inflammation in pain generation. Calcitonin gene related peptide, nitric oxide, and cytokines are all molecules shown to be involved both in animal and human studies [7]. Cytokines are important mediators of the inflammatory pathway and have been

recently linked to migraine pathogenesis. In many studies focusing on peripheral and central levels of cytokines (tumor necrosis factor [TNF]-α, IL-1α, IL-1β, IL-4, and IL-5), conflicting results are found [8–10]. But all these studies have indicated the role of inflammation in migraine headache.

TNF-α and IL-1 can promote powerful hyperalgesia by causing protanoid release, increasing the expression of nerve growth factor (NGF) and bradykinin receptors, or by modulation of activity within sympathetic fibers. IL-1 is a potent pro-inflammatory cytokine and mediates the response to acute and chronic inflammatory central nervous system diseases. It has two structurally different forms, IL-1α and IL-1β, encoded by two separate genes located on the long arm of chromosome 2. TNF-α gene is located on the short arm of chromosome 6. Polymorphisms that have functional significance have been described for all these genes. Polymorphisms may cause inter-individual variations in the levels of cytokine production. As cytokines are considered to be pain mediators in neurovascular inflammation, these polymorphisms may have an effect on the generation of migraine pain [11].

In this study polymorphisms of TNF-α –308 G/A (rs1800629), IL-1α +4845 G/T (rs17561), IL-1β +3953 C/T (rs1143634) and interleukin-1 receptor antagonist variable number tandem repeat (IL-1RA VNTR) genes were studied. As migraine is a multifactorial disease with various environmental and genetic etiologies, we attempted to evaluate the role of the above-mentioned gene polymorphisms in pathogenesis of MwoA. MwoA patients were selected because in previous studies a relation has been found among TNF-α, IL-1α polymorphisms, and MwoA. But such a relationship does not exist in migraine with aura patients [12–14].

Material and Methods

In this study, patients with MwoA were randomly selected from the database of Mersin University Hospital Headache Unit. We evaluated 67 migraine patients (57 women, 10 men; mean age 39.6 years). Ninety-six (82 women, 14 men; mean age 37.9) unrelated, age- and sex-matched, migraine-free, healthy controls were selected from the same geographic area. The healthy volunteer control subjects were recruited from hospital workers, students of the University, and family members of our patients. The control subjects without headache were generally normal and received no medication. Exclusion criteria were having known inflammatory disorders, infectious, or immune diseases. Patients having migraine with aura or drug overuse headache were also excluded.

Migraine diagnosis was made according to International Classification of Headache Disorders-II criteria [15]. The study was performed in accordance with the Declaration of Helsinki and under the principles of Good Clinical Practice. The trial was approved by ethics committee of Mersin University, and all patients gave written informed consent. Once enrolled, a neurologist administered questionnaires and blood samplings were drawn at the same visit. In the questionnaire the patients were asked to report the onset time, character, location, duration of the pain, associated symptoms, histories, and medications.

Venous blood samples were collected in ethylenediaminetetra acidic acid containing tubes. DNA was extracted from whole blood by salting out procedure [16]. Polymerase chain reaction (PCR)-restriction fragment length polymorphism assays TNF-α –308 G/A (rs1800629), IL-1β +3953 C/T (rs1143634), and IL-1α +4845 G/T (rs17561). Also, IL-1RA VNTR polymorphism in Intron 2 have determined with PCR. PCR was performed in a 25-μL volume with 100 ng DNA, 100 μm deoxynucleotide triphosphates (dNTPs), 20 pmol of each primer, 1.5 mM $MgCl_2$, 1 × PCR buffer with $(NH_4)_2SO_4$ (Fermentas, Vilnius, Lithuania), 10% dimethyle sulfoxide (DMSO) and 2 U *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). The genotyping of the TNF-α, IL-1β, IL-1α, and IL-1RA gene polymorphisms were determined by fragment separation at 120 V for 40–50 minutes on a 3.5% Agarose gel containing 0.5 μg/mL ethidium bromide. A 100-bp DNA Ladder (Fermentas Vilnius, Lithuania) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, Deutschland).

Genotypic Analysis of the TNF-α –308 G/A (rs1800629) Polymorphism

The oligonucleotide primers used to determine the –308 G/A (rs1800629) polymorphism within the TNF-α gene were described previously [17]. The primers, forward 5′-AGGCAATAGGTTTTGAGGGCCAT-3′; reverse 5′-TCC TCCCTGCTCCGATTCCG-3′ were used to amplify of the TNF-α gene. PCR conditions were 2 minutes for initial denaturation at 95°C; 35 cycles at 95°C for 45 seconds for denaturation, 1 minute at 60°C for annealing and 90 seconds at 72°C for extension, followed by 7 minutes at 72°C for final extension. After amplification PCR products were digested by restriction endonuclease 10 U *Nco*I (Fermentas, Vilnius, Lithuania) for 14 hours at 37°C. The *Nco*I restricted products of TNF-α –308 G/A; GG, GA; and AA genotypes had band sizes of 87 bp/20 bp, 107 bp/87 bp/20 bp, and 107 bp, respectively.

Genotypic Analysis of the IL-1RA VNTR Polymorphism

The oligonucleotide primers used to determine the VNTR in Intron 2 polymorphism within the IL-1RA gene were described previously [18,19]. The primers, forward 5′-CTCAGCAACACTCCTAT-3′; reverse 5′-TCCTGGTCT GCAGGTAA-3′ were used to amplify of the IL-1RA gene. PCR conditions were 2 minutes for initial denaturation at 95°C; 35 cycles at 95°C for 45 seconds for denaturation, 1 minute at 60°C for annealing and 90 seconds at 72°C for extension, followed by 7 minutes at 72°C for final extension. PCR products of the IL-1RA gene were: allele

1: 410 bp (four repeats); allele 2: 240 bp (two repeats); allele 3: 500 bp (five repeats); allele 4: 325 bp (three repeats); and allele 5: 595 bp (six repeats).

Genotypic Analysis of the IL-1β +3953 C/T (rs1143634) Polymorphism

The oligonucleotide primers used to determine the +3953 C/T (rs1143634) polymorphism within the IL-1β gene was described previously [20]. The primers, forward 5′-GTTGTCATCAGACTTTGACC-3′; reverse 5′-TTCA GTTCATATGGACCAGA-3′ were used to amplify of the IL-1β gene. After amplification, PCR products were digested by restriction endonucleases 10 U *Taq*I (Fermentas, Vilnius, Lithuania) for 4 hours at 65°C. The *Taq*I restricted products of IL-1β +3953, allele 1 (CC), and allele 2 (TT) genotypes had band sizes of 135 bp/114 bp and 249 bp, respectively.

Genotypic Analysis of the IL-1α +4845 G/T (rs17561) Polymorphism

The oligonucleotide primers used to determine the +4845 G/T (rs17561) polymorphism within the IL-1α gene were described previously [21]. The primers, forward 5′-ATGGTTTTAGAAATCATCAAGCCTAGGGCA-3′; reverse 5′-AATGAAAGGAGGGGAGGATGACAGAAATGT-3′ were used to amplify of the IL-1α gene. PCR conditions were 2 minutes for initial denaturation at 95°C; 35 cycles at 95°C for 45 seconds for denaturation, 1 minute at 56°C for annealing and 90 seconds at 72°C for extension, followed by 7 minutes at 72°C for final extension. After amplification PCR products were digested by restriction endonucleases 10 U *Sat*I (Fermentas, Vilnius, Lithuania) for 14 hours at 37°C. The *Sat*I restricted products of IL-1α +4845, allele 1 (GG), and allele 2 (TT) genotypes had band sizes of 124 bp/76/29 bp and 153/76 bp, respectively.

The investigators were blinded to the status of the patients' samples being tested. At least 10% of the samples were retested, and the results were 100% concordant. Retested samples were randomly chosen and retesting was performed in order to control the test process.

The Hardy–Weinberg equilibrium was verified using the chi-square test and by estimating the expected genotypic frequencies on the basis of the development of the square of the binomial for the TNF-α, IL-1β, and IL-1α polymorphisms and of the trinomial for IL-1RA polymorphism.

Allelic and genotypic distributions among the different groups were compared using the likelihood-ratio chi-square test or Fisher's exact test. Correction for multiple comparisons was applied when necessary, using Bonferroni method.

Results

DNA samples from 67 MwoA patients and 96 healthy controls were analyzed for TNF-α, IL-1α, IL-1β, and IL-1RN VNTR polymorphisms. The patients' characteristics were listed in Table 1. The genotype and allele distributions for TNF-α –308, IL-1α +4845, IL-1β +3953, and IL-1RN VNTR polymorphisms were shown in Table 2.

Table 1 Demographic and clinical characteristics of migraine patients

	Migraine (n = 67)	
	Mean ± SD	Minimum–Maximum
Age (years)	39.6 ± 10.7	18–67
Female/Male (%)	57 (85%)/10 (15%)	
Duration of headache (hour)	16.1 ± 15.6	6–480
Severity of headache (visual analog scale)	8.1 ± 1.6	5–10
Number of attacks*	8.8 ± 7.1	1–30
Unilateral localizations	33 (48.3%)	
Character of headache		
Pulsating	47 (70.1%)	
Pressing/tightening	20 (29.9%)	
Accompanying symptoms		
Nausea	62 (92.5%)	
Vomiting	34 (50.7%)	
Photophobia	59 (88.1%)	
Phonophobia	53 (79.1%)	
Aggravation of headache by		
Physical activity	50 (74.6%)	
Menstruation	24 (35.8%)	
Emotional stress	61 (91.0%)	

* Average frequency of attacks per month in past years.

We observed significant differences in the genotypic distribution of the TNF-α –308 G/A and IL-1β +3953 C/T polymorphism for migraineurs compared with controls ($P = 0.004$). But no statistical significant difference was ascertained regarding the polymorphisms of the IL-1α +4845 G/T or IL-1RA VNTR between the study and control groups. All the genotype distributions for migraine cases and controls were in Hardy–Weinberg equilibrium ($P > 0.05$).

Frequency of the TNF-α –308 GG genotype was higher in the control group than MwoA group (82.1% vs 55.2%, $P = 0.000$). Differences in the distribution of the allele frequencies were also observed, being the TNF-α –308 G allele overrepresented in control group and TNF-α –308 A allele in MwoA group ($P = 0.012$).

In addition, there was a significant increase of the IL-1β +3953 T allele in MwoA cases compared with controls ($P = 0.004$).

Table 2 TNF-α, IL-1α, IL-1β, IL-1RA; alleles and genotype frequencies in patients with migraine without aura

TNF-α –308	GG	GA	AA
Patients (n = 67)	37 (55.2%)	23 (34.3%)	7 (10.4%)
Controls (n = 96)	79 (82.3%)	16 (16.7%)	1 (1%)
IL-1α +4845	GG	GT	TT
Patients (n = 67)	38 (56.7%)	23 (34.3%)	6 (9.0%)
Controls (n = 96)	50 (52.1%)	37 (38.5%)	9 (9.4%)
IL-1β +3953	CC	CT	TT
Patients (n = 67)	30 (44.8%)	22 (32.8%)	15 (22.4%)
Controls (n = 96)	51 (53.1%)	40 (41.7%)	5 (5.2%)
IL-1RA VNTR	4/4	4/2	2/2
Patients (n = 64)	54 (84.4%)	5 (7.8%)	5 (7.8%)
Controls (n = 96)	79 (82.3%)	13 (13.5%)	4 (4.2%)

TNF-α = tumor necrosis factor α; IL = interleukin; IL-1RA = interleukin-1 receptor antagonist; VNTR = variable number tandem repeat.

Discussion

Cytokines are important mediators of the inflammatory pathway and have been recently linked to migraine pathogenesis. Cytokines and their receptors are widely expressed in the central nervous system (CNS) by all cell types including neurons.

There are contrasting results obtained in migraineurs concerning the levels of both pro-inflammatory (TNF-α and IL-2) and anti-inflammatory cytokines (IL-10 and TGF-α), which have been measured in some cases even peripheral and jugular blood samples ictally, in others interictally, supporting the hypothesis of sterile inflammation in the dura mater during migraine attacks [22–29].

Pro-inflammatory cytokines such as IL-1 [30], IL-6, and TNF-α [31] are predominantly produced by activated immune cells and are involved in amplification of the inflammatory response. Anti-inflammatory cytokines such as IL-1RA, IL-2 [32], and IL-10 are involved in reduction of the inflammatory response. In addition, IL-1β, IL-6, and TNF can indirectly induce hyperalgesia through release of prostaglandins and thromboxanes, modulation of sympathetic fibers, or increasing nerve growth factor and bradykinin receptors.

A genetic predisposition to have an abnormal inflammatory response may be a trigger of headache attacks. Genetic polymorphisms of the genes involved in inflammation like TNF-α and IL-1β may contribute to the generation of migraine headache [33].

Our study shows that the –308 G/A polymorphism of the TNF-α gene and +3953 C/T polymorphism of IL-1β are associated with MwoA. TNF-α –308 A and IL-1β +3953 T alleles are more frequently observed in patients with MwoA than healthy controls. Recently Rainero et al. showed an association between the TNF-α –308 G/A gene polymorphism and migraine [12]. They found that homozygosity for the G allele was associated with an increased risk of migraine. In another similar study from Iran, Mazaheri et al. showed that the frequency of –308 A allele was higher in the MwoA group [13]. Our results were in parallel with the second study. This may be due to the differences of TNF-α polymorphisms among different populations.

T alleles in regulatory regions of the IL-1α and IL-1β genes [34] and A alleles for TNF-α [35] have been found to be a stronger transcriptional activator than C and G alleles, respectively. Chronic overexpression of above-mentioned inflammatory cytokines might stimulate the activation of trigeminal nerves, the release of vasoactive peptides, or other biochemical mediators, such as nitric oxide, and then cause inflammation. Our results for TNF-α and IL-1β are in parallel with this hypothesis. But conflicting results were found in studies focusing on both pro-inflammatory and anti-inflammatory cytokine levels during migraine attacks. This may be caused by the difficulties in sampling and/or the inherent heterogeneity of the disease.

In another recent study, Rainero et al. showed that migraine patients carrying IL-1α T/T genotype had an age at onset of their headache attacks significantly lower than patients with other genotypes [14]. In our study, we could not find such a relation. This may be due to the small number of our study group or relating to localizations of mentioned genes.

In conclusion, the present results indicate the possible contribution of TNF-α and IL-1β gene polymorphisms to migraine headache generation. To our knowledge it is the first time that a relationship between TNF-α and IL-1β gene polymorphisms and MwoA is shown. Additional studies need with a larger number of patients and in different populations aimed to assess the relation of above mentioned gene polymorphisms and migraine.

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