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Association of Pro-inflammatory Cytokine Gene Polymorphism with Meniere's Disease in an Iranian Sample

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ABSTRACT

Meniere's disease (MD) is known as a rare chronic disorder of the inner ear with elevated serum levels of pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, Interleukin (IL)-1, and IL-6. This study aims to evaluate genes polymorphism in some pro-inflammatory cytokines in a group of Iranian MD patients compared to the healthy controls.

In this case-control study, 25 MD patients and 139 healthy controls were enrolled. DNA was extracted from blood samples, and single nucleotide polymorphisms were detected using polymerase chain reaction with sequence-specific primers assay. MD patients and controls were examined in terms of allele, genotype, and haplotype frequency of pro-inflammatory cytokine genes.

Only the frequencies of alleles A/G at position -238 in the promoter of the *TNF-a* gene differed significantly between MD patients and healthy controls. G to A allele ratio was 23 and 3.6 in MD and controls, respectively. In individuals with MD, genotype GG was found to be significantly more prevalent at position -238 of the *TNF-a* gene promoter sequence. In addition, the heterozygote AG variant of -238 A/G TNF-α gene polymorphism was lower in MD patients than controls. Compared to the control group, the haplotype TNF- (-308, -238) AG was higher in MD patients, although not statistically significant.

This is the first study that we know of that evaluates the frequencies of pro-inflammatory cytokine genes in an Iranian MD sample. This study shows the association between TNF-α and susceptibility to MD.

Keywords: Autoimmunity; Chemokines; Interleukins; Meniere disease; Polymorphism; Tumor necrosis factor-alpha

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INTRODUCTION

Ménière's disease (MD) as a rare chronic inner ear disorder, is defined by vertigo attacks lasting from minutes to hours with ipsilateral cochlear symptoms like the sensation of aural pressure or fullness and tinnitus. Fluctuating medium to low frequencies sensorineural hearing loss (SNHL) is another symptom of the disease.[1] The MD physiopathology is explained by an imbalance in endolymph secretion and reabsorption, leading to endolymph accumulation and overpressure in the cochlear duct. This overpressure causes endolymphatic hydrops (EH) and inner ear membranes and structural damages resulting in hearing loss and vestibular deficits.[2,3] The molecular and cellular mechanisms resulting in EH are unknown.[4]

The prevalence of MD is variable in different regions, for instance, 3.5 per 100,000 people in Japan versus 513 per 100,000 in Finland. It is more prevalent in Europeans than Americans or Asians, not frequently found in the sub-Saharan population. This variety can result from different genetic contributions in different ethnicities.[5–7] A link between MD and autoimmune diseases such as psoriasis, systemic lupus erythematosus (SLE), and rheumatoid arthritis has been discovered in several investigations.[7,8] Based on the outcomes of research conducted in small groups of patients, autoimmunity and genetic factors have been identified as probable causes of MD.[9–11] NF-κB-mediated inflammation, innate immune response, and pro-inflammatory cytokines are autoinflammatory and autoimmunity factors involving in MD pathogenesis.[4] Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-6 are some kinds of pro-inflammatory cytokines, which in previous studies showed elevated blood levels in MD patients.[12]

Based on prior research and the potential role of pro-inflammatory cytokines in MD, this study intends to examine pro-inflammatory mediator genes polymorphism in several MD patients in the Iranian population to determine MD pathophysiology.

PATIENTS AND METHODS

A total of 25 MD patients and 139 healthy controls were included in this case-control study. Cases were recruited from patients referred to Amir Alam hospital, Tehran, Iran, in 2017. Inclusion criteria were definite MD according to revised MD diagnostic criteria 2015.[1] Patients with any possibility of cancer, autoimmune diseases, and Alzheimer's disease were excluded. The control group consisted of healthy, normal people with no history of autoimmune illness, allergies, cancer, or other systemic diseases. All participants were Iranian. Tehran University of medical sciences ethics committee approved the study and signed written informed consent was obtained from all participants (number of the ethics approval letter: 8711215078).

Following the principles of the declaration of Helsinki, a 5 mL blood sample was collected from patients and kept with Ethylene-diamine-tetra-acetic acid (EDTA) at −20°C. As previously disclosed, DNA was isolated from nucleated cells using phenol-chloroform.[13]At 260 nm and 280 nm wavelengths, DNA quality, and quantity were evaluated using an ultraviolet spectrophotometer.

The polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany) was used to detect single nucleotide polymorphisms (SNP). As previously stated, extracted DNA was amplified using a PCR Techne Flexigene apparatus (Rosche, Cambridge, UK).[14] Two percent agarose gel electrophoresis was used to examine PCR results, and a photograph was taken after visualization using a UV trans-illuminator. The following single nucleotide gene polymorphisms were assessed: *IL-6* (G/C −174, G/Ant565), *TNF-α* (G/A −308, G/A −238), *IL-1 α* (C/T -889), *IL-1β* (C/T -511, C/T +3962), *IL-1R* (C/T pst-I 1970) and *IL-1RA* (C/T mspa-I 11100). The researcher in charge of genotyping analysis was blinded about the investigation.

Before starting this study, we calculated the minimum required sample size according to the previous article.[15] To evaluate and compare the frequency of IL-1 allele T in the control group as 9.8% and this rate in MD patients as 25.7 with a standard error of 5% and a power of 80%, the sample size was calculated 7 cases in each group. We could enroll more patients and controls are this study.

Statistical Analysis

Direct gene counting was used to calculate allele, genotype, and haplotype frequencies, and then evaluated using the chi-square test. The odds ratios (OR) and confidence intervals (CI) were calculated. The tests were two-sided, and statistical significance was defined as a *p*-value of less than 0.05. SPSS 20

(SPSS Inc., Chicago, IL, USA) was used to analyze the data.

RESULTS

The mean ages of patients and controls were 34.08±11.54 and 38.58±10.75 years, respectively. In controls, 73 subjects (52.2%) were female. In patients, 17 subjects (68%) were female. Twenty-two (88%) of patients had unilateral MD. The level of hearing loss was mild in 14 (56%), moderate in 9 (36%), and severe in 2 (8%) patients.

Allele, Genotype, and Haplotype Frequencies

With SNP analysis, there were no differences in IL-6 (G/C-174, G/Ant565), TNF-α (G/A-308), IL-1α (C/T-889), IL-1β (C/T-511, C/T+3962), IL-1R (C/T pst-I 1970), and IL-1RA (C/T mspa-I 11100) between MD patients and controls. However, between patients with MD and controls, the frequency of alleles A/G at position 238 in the promoter of the TNF-α gene was significantly different. G to A allele ratio was 23 and 3.6 in MD and controls, respectively. Table 1 displays detailed allele frequencies for both patients and controls.

In individuals with MD, genotype GG was significantly higher at position -238 of the TNF-α gene promoter region. In addition, heterozygotes for allele AG were significantly lower in MD patients compared to controls. Table 2 shows the genotype frequencies in detail.

The haplotype TNF-α (-308, -238) AG in the patients with MD was greater but not statistically significant compared to the control group (18.7% vs. 14.2%, OR: 3.51, *p*-value: 0.05, 95% CI: 0.98-13.06).

Table 1. TNF-α, IL-6, and IL-1 cluster gene allele frequencies in Meniere's disease patients and controls

Cytokine gene	Position	Allele	Control (n=139) N (%)	MD (n=25) N (%)	*p*	OR (95%CI)
TNF-α	-308	A	39 (14.2)	11 (22.9)	0.18	1.79 (0.79-4.02)
		G	235 (85.8)	37 (77.1)	0.18	0.56 (0.25-1.27)
TNF-α	**-238**	**A**	**59 (21.5)**	**2 (4.2)**	**0.008**	**0.16 (0.03-0.69)**
		G	**215 (78.5)**	**46 (95.8)**	**0.008**	**6.31 (1.44-38.74)**
IL-6	-174	C	101 (36.3)	14 (30.4)	0.54	0.77 (0.37-1.57)
		G	177 (63.7)	32 (69.6)	0.54	1.30 (0.64-2.71)
IL-6	Nt565	A	50 (18)	8 (17.4)	0.91	0.96 (0.39-2.31)
		G	228 (82)	38 (82.6)	0.91	1.04 (0.43-2.59)
IL-1α	-889	C	186 (68.4)	29 (58)	0.20	0.64 (0.33-1.24)
		T	86 (31.6)	21 (42)	0.20	1.57 (0.81-3.03)
IL-1β	-511	C	154 (55.4)	31 (63.2)	0.38	1.39 (0.71-2.72)
		T	124 (44.6)	18 (36.7)	0.38	0.72 (0.37-1.41)
IL-1β	+3962	C	198 (70.7)	33 (66)	0.61	0.80 (0.41-1.60)
		T	82 (29.3)	17 (34)	0.61	1.24 (0.62-2.46)
IL-1R	Pst-I 1970	C	174 (62.1)	28 (57.1)	0.61	0.81 (0.42-1.57)
		T	106 (44.2)	21 (42.9)	0.61	1.23(0.64-2.38)
IL-1RA	Mspa-I 11100	C	64 (22.9)	7 (14)	0.22	0.55 (0.21-1.35)
		T	216 (77.1)	43 (86)	0.22	1.82 (0.74-4.67)

In this case-control study, comparing allele frequencies of TNF-α, IL-6, and, IL-1 cluster genes in controls and MD patients, Only the frequency of alleles A/G at position 238 in the promoter of the TNF-α gene differed significantly between the two groups of patients. In MD and controls, the G to A allele ratio was 23 and 3.6, respectively.

Table 2. ***TNF-α*, *IL-6*, and *IL-1* cluster gene genotype frequencies in Meniere's disease patients and controls**

Cytokine	Position	Genotype	Control (n=139) N (%)	MD (n=25) N (%)	*p*	OR (95%CI)
		AA	0 (0)	0 (0)	----	----
TNF-α	-308	AG	39 (28.5)	11 (45.8)	0.14	2.13 (0.80-5.61)
		GG	98 (71.5)	13 (54.2)	0.14	0.47 (0.18-1.24)
		AA	1 (0.7)	0 (0)	1.00	0.00 (0.00-102.54)
TNF-α	**-238**	**AG**	**57 (41.6)**	**2 (8.3)**	**0.003**	**0.13 (0.02-0.60)**
		GG	**79 (57.7)**	**22 (91.7)**	**0.003**	**8.08 (1.73-51.82)**
		CC	4 (2.9)	0 (0)	1.00	0.00 (0.00-9.74)
IL-6	-174	CG	93 (66.9)	14 (60.9)	0.74	0.77 (0.29-2.10)
		GG	42 (30.2)	9 (39.1)	0.54	1.48 (1.54-4.02)
		AA	4 (2.9)	0 (0)	1.00	0.00 (0.00-9.74)
IL-6	Nt565	AG	42 (30.2)	8 (34.8)	0.84	1.23 (0.44-3.40)
		GG	93 (66.9)	15 (65.2)	0.93	0.93 (0.34-2.60)
		CC	62 (45.6)	8 (32)	1.08	0.56 (0.21-1.50)
IL-1α	-889	CT	62 (45.6)	13 (52)	0.14	1.29 (0.51-3.29)
		TT	12 (8.8)	4 (16)	0.27	1.97 (0.48-7.49)
		CC	36 (25.8)	8 (33.3)	0.61	1.43 (0.51-3.94)
IL-1β	-511	CT	82 (59)	14 (58.3)	0.86	0.97 (0.37-2.56)
		TT	21 (15.2)	2 (8.3)	0.53	0.51 (0.08-2.51)
		CC	70 (50)	11 (44)	0.73	0.79 (0.31-2.00)
IL-1β	+3962	CT	58 (41.4)	11 (44)	0.98	1.11 (0.43-2.83)
		TT	12 (8.6)	3 (12)	0.70	1.45 (0.30-6.22)
		CC	54 (38.6)	9 (37.5)	0.89	0.96 (0.36-2.53)
IL-1R	Pst-I 1970	CT	66 (47.1)	9 (37.5)	0.51	0.67 (0.25-1.77)
		TT	20 (14.3)	6 (25)	0.22	2.00 (0.62-6.22)
		CC	4 (2.9)	1 (4)	0.56	1.42 (-)
IL-1RA	Mspa-I 11100	CT	56 (40)	5 (10)	0.90	0.38 (0.12-1.14)
		TT	80 (57.1)	19 (38)	0.12	2.38 (0.83-7.12)

In patients with MD, genotype GG was significantly higher at position -238 of the TNF-α gene promoter region, whereas heterozygotes for alleles AG were significantly lower, according to this case-control study comparing genotype frequencies of TNF-α, IL-6, and IL-1 cluster genes in controls and MD patients.

DISCUSSION

In this study, we found a significant association between MD risk and *TNF-α* (-238) A/G polymorphism; whereas no significant effect of *IL-1α* (-889) C/T, *IL-1β* (-511) C/T, *IL-1β* (+3962) C/T, *IL-1R* (pst-I 1970) C/T, *IL-1RA* (mspa-I 11100) C/T, *IL-6* (-174) C/G, *IL-6* (+565) A/G, and *TNF-α* (-308) A/G polymorphism on MD was observed. *TNF-α* (-238) G allele carriers were potentially more

susceptible to MD than noncarriers. The high odds ratios (ORs) suggest a substantial impact of *TNF-α* on the susceptibility of MD. Also, *TNF-α* (-238) A allele and *TNF-α* (-238) GA genotype seems to have a protective influence against MD.

TNF-α is a proinflammatory cytokine that stimulates the immune response and promotes immunocompetent cells to infiltrate the tissues. In a labyrinthitis model in the lab, in guinea pigs, etanercept, as a TNF-α blocker, could decrease the amount of inflammation.[16] In the infiltrated immunocompetent cells, TNF-α triggers amplification of the response that leads to cochlear pathology, according to a study conducted on mice's inner ear.[16] In another study, it was described that TNF-α could recruit inflammatory cells to the cochlea; however, it probably cannot be directly responsible for the immune-mediated labyrinthitis and hearing loss.[17] In a clinical trial, etanercept was shown to alleviate or stabilize symptoms in half of the patients with immune-mediated inner ear illnesses such as MD.[18] Another case-control study revealed that pro-inflammatory cytokines such as IL-1β, IL-1RA, TNF-α, and IL-6 were increased in 21% of MD patients. TNF-α, IL-1, IL-1RA, and IL-6 were found to be elevated in 21% of MD patients in another case-control research. Moreover, extracts from Penicillium and Aspergillus molds caused TNF-α to be released in these patients, which was not found in the controls. This could mean that these molds cause innate-mediated inflammation to worsen in MD patients.[19]

Several other studies have investigated the effects of different cytokines on MD. Fuse et al, investigated the intracellular cytokines in patients with MD. In MD patients, they observed higher activity of natural killer cells.[20] Also, some patients' serum has been found to have higher circulating immune complexes (CIC),[21] and some allelic variations in the *TLR10*, *MICA*, or *NFκB* genes are associated with the SNHL progression in MD.[22–24]

Moreover, it has been suggested that the *macrophage migration inhibitory factor (MIF)* gene, a regulator in the synthesis of TNF, IL-1, and IL-6, has a role in MD pathogenesis.[25] Also, the IL-1A-889T allele carriers, compared to noncarriers, were found to be more susceptible to MD.[15] In our study, unlike TNF-α (-238) A/G polymorphism, IL-1β (-511) C/T polymorphism was not linked to the disease.

Lately, an approach based on molecular networks for the identification of genes associated with MD, found 11 out of 43 genes to have a contribution to biological processes linked to the immune system like *TLR2*, *IL6*, *IL-1R1*, and *CD4*, and four genes (*NOTCH2*, *GPX4*, *GPX5*, and *PTGS2*) were discovered to be linked to cell proliferation and survival.[26] The allelic variations rs3774937 and rs4648011 of the *NFKB1* gene were discovered as a modulator of hearing outcome in patients with MD, using a high-density genotyping array.[24]

The disparity in results might be due to the ethnic or geographic background or due to differences in phenotypic or clinical manifestations of patients in various studies that need careful interpretation.

This is the first study that we are aware of that compares the frequencies of distinct pro-inflammatory cytokine alleles, genotypes, and haplotypes in an Iranian MD sample. The findings of this investigation show a link between TNF-α and MD susceptibility.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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