The Association of -308 TNFα Polymorphism and multiple sclerosis in Iranian Patients

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ABSTRACT
Multiple Sclerosis is a chronic neuro-immune disorder of the central CNS. MS belongs to the large group of multifactorial and multi-genic disease. The TNFα gene encodes pro-inflammatory cytokine that takes an ambiguous part in the development of various disorders especially autoimmune disease such as MS. In this study we investigated the association of -308A/G TNFα polymorphism and multiple sclerosis in Iranian patients. One hundred MS patients and one hundred ethnically, age and sex matched control individuals were selected. A (-308) TNFα polymorphism was analyzed based on the polymerase chain reaction with sequence-specific primers (developed during the 13IHWC and supplied by Heidelberg University (Heidelberg, Germany)). The frequency of the G allele and G/G genotype at the -308 TNFα position was significantly higher in MS patients in comparison to control subjects (OR: 1.685, 95% CI: 1.046–2.715, P: 0.041; OR: 1.933, 95% CI: 1.095–3.411, P: 0.032, respectively). It indicated that G allele and G/G genotype had susceptibility effect on MS among Iranian patients. But more studies with large sample size and specially investigation of different TNFα alleles in relation to other genes and haplotypes are needed to explain exact effect of TNFα polymorphisms in the MS.

Key words: Multiple Sclerosis, -308 TNFα polymorphism, Gen disease, Iranian patients

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1. INTRODUCTION
Multiple Sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease. MS is a heterogenic and multifactorial disease. According to twin studies, around 30% concordance rates were calculated in monozygotic twins in comparing with dizygotic twins (about 5%) (1). these findings suggested a strong genetic component. Human leukocyte antigen (HLA) genes were the first genes which was studied and showed the strongest associations to MS. HLA region includes 20-60% of genetic susceptibility to MS (2). Recently, genome-wide association (GWA) studies demonstrated that other loci had association to MS (3). Like most other autoimmune disorders, MS is associated to the factors which are involved in immune responses, such as cytokines. Cytokines are one of the most important factors in the regulation of inflammatory immune response. Therefore they can effect on the pathogenesis which are the autoimmune diseases such as MS (4, 5). Tumor necrosis factor alpha (TNFα) is a potent inflammatory cytokine which stimulates cytokine and has crucial effect of on the immune responses. Because of Th1 cytokines, like TNFα increase the inflammation, they can influence on the pathogeneses of the MS (6-11). The position of TNFα is on the chromosome 6 in the HLA class III region.
Some single nucleotide polymorphisms (SNPs) were found in the promoter of TNFα gene. Among these SNPs, -308 G/A TNFα polymorphism has been studied in several diseases such as ankylosing spondylitis (AS), asthma, MS (12-15). Substitution of Guanine (G) nucleotide in -308 positions creates G allele which is the common allele while substitution of Adenosine (A) nucleotide in this position makes the rare allele. The rare allele with unknown exact mechanisms was found in higher expression of TNFα (16, 17). But in contrast to these studies, some research reported the other effect of -308 A TNFα allele on the expression or production of TNFα gene (14, 15). Among different studies on the -308 TNFα position, only two studies demonstrated significant association of this position with MS, whereas their results were inconsistent together (18-24). The aim of our study was investigation of the association of -308 TNFα polymorphism with MS.

2. MATERIALS AND METHODS

2.1. Patients and controls
One hundred Iranian patients (mean± SD age of 32.95 ±6.51 years, range of 20-42 years) with MS from medical genetics department of Sarem Women hospitals were selected. The diagnosis of MS was made according to the McDonald criteria (25). Besides, one hundred ethnically, age and sex matched healthy individuals (mean± SD age of 29.8±7.8 years, range of 20-52 years) without personal or family backgrounds of autoimmune disease were enrolled as control group. Demographic and clinical data of MS patient and control group were shown in Table1. All individuals were made to sign the written form Consent.

2.2. DNA extraction and -308 TNFα genotyping
Genomic DNA was extracted from peripheral venous blood samples by applying salting out method (26). (-308) TNFα polymorphism was analyzed based on the polymerase chain reaction with sequence-specific primers, developed during the 13IHWC and supplied by Heidelberg University (Heidelberg, Germany). Amplification was carried out and the amplified products were made to run on the agarose gels.

2.3. Statistical analysis
To examine the susceptible -308 TNFα polymorphism on MS disease, Fisher’s exact test was accomplished. P value <0.05 was regarded statistically significant. All the analyses were analyzed using SPSS 18.0 for windows software.

3. RESULTS AND DISCUSSION
Demographic and clinical data of MS patient and control group were shown in Table 1. The clinical characteristics revealed that all patients (100%) had relapsing-remitting MS, the mean age of onset, duration and EDSS were 32.95 ±6.51, 4.86 ± 5.535 and 3.775 ± 2.226 years, respectively (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical profiles of MS patients and controls</th>
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<td><strong>Variables</strong></td>
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<tr>
<td>Female/Male No. (%)</td>
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<tr>
<td>Age (mean ± SD, year)</td>
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<tr>
<td>Age Range (years)</td>
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<tr>
<td>Age at onset (mean ± SD, year)</td>
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<tr>
<td>Relapsing-Remitting Course [No. (%)]</td>
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<td>Duration (mean ± SD, year)</td>
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<td>EDSS(a) (mean ± SD)</td>
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\(a\): Expanded Disability Status Scale of Kurtzke

The frequency of the G allele at the -308 TNFα position was significantly higher in MS patients than controls (82% vs. 73%, OR: 1.685, 95%CI: 1.046-2.715, P: 0.041). Moreover, the G/G genotype was significantly more frequent in patients than in control (65% vs. 46%, OR: 1.933, 95%CI: 1.095–3.411, P: 0.032) (Table 2). The frequencies of A/G heterozygosity in patients (34%) were decreased in compare to controls (48%), but this difference was not significant (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Frequencies of -330 IL2 alleles and genotypes in MS patients and healthy controls</th>
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<tr>
<td><strong>Allele</strong></td>
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<td>A</td>
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<td>G</td>
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<td>G/G</td>
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\(+:\) value of Fisher’s exact test. N: number of individuals, n: number of chromosomes.
Multiple Sclerosis is a chronic neuro immune disorder of the central CNS. MS belongs to the large group of multifactorial and multi genic diseases. The TNFα gene encodes pro-inflammatory cytokine that takes an ambiguous part in the development of various disorders especially autoimmune disease such as MS. The aim of the present study was to assess the role of IL2 gene polymorphisms that are known to influence MS susceptibility or progression. According to our results, the frequency of -308G TNFα allele and G/G genotype were higher in MS in comparing to control subjects. It indicated that G allele and G/G genotype had susceptibility effect on MS among Iranian patients. In 2007, Kamali et al. found -308 TNFα polymorphism was not different between MS patients and controls and this polymorphism had no susceptibility effect on MS in Iranian patients (22). In contrast, in 2008, Serial et al. showed that -308G TNFα alleles and G/G genotype decrease significantly in Iranian MS group than controls (23). Inconvenience to two before studies on Iranian MS patients and similar to our results, in 2011, Shahbazi et al. reported that -308G TNFα allele and G/G genotype were significantly more frequent in MS patients versus control individuals (24). Investigation of Sweden MS patients showed no significant difference regarding the -308 G/A TNFα promoter polymorphism between MS patients and controls group (20). Study of the MS patients revealed no association between -308G/A TNFα polymorphism and MS (27). Also after sequencing of TNFα gene, it was shown that the genetic variation in the TNFα gene did not effect on the course or outcome of MS disease, significantly (28). A study in Turkish children with MS reported no susceptibility effect of -308 TNFα polymorphism on MS (29). One other -308 TNFα polymorphism study in Turkish MS patients and also a population based case control study and one study in Netherlands revealed no association between this position and susceptibility to MS (30-32). One study in Australia demonstrated -308A TNFα allele had two time higher levels of transcription than -308G TNFα allele. But they suggested that this altered TNFα expression might be affected by some type of HLA haplotype (33). TNFα production was higher in MS patients but this difference could not be attributed to the -308G/A TNFα polymorphism (15). Maurer et al. reported that although the -308A TNFα allele was correlated with higher TNFα mRNA levels, but this allele had not association with susceptibility to MS (34). One study, in 2000, demonstrated -308G TNFα alleles had different activity in U937 monocytes and Jurkat T cell, and not in the Raji (B cell line), HeLa (epithelial carcinoma cell line), HepG2 (hepatoma cell line) and THP-1 (monocyte). Also physiological stimulators had no effect on promoter activity. They found other different stimulus which had various effects on promoter activity. So they suggested stimulus and cell type influence the -308 TNFα promoter polymorphism expressions (35).

4. CONCLUSION
In conclusion our study revealed that the -308G TNFα promoter polymorphism had significant positive association to MS disease. But more studies with large sample size and specially investigation of different TNFα alleles in relation to other genes and haplotypes are needed to explain exact effect of TNFα polymorphisms in the MS.

Funding/ Support
Not mentioned any Funding/ Support by authors.

ACKNOWLEDGMENT
We gratefully acknowledge Sarem Women Hospital for their valuable collaborations. The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION
This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST
The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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