IL-1, IL-1R and TNFα Gene Polymorphisms in Iranian Patients with Multiple Sclerosis

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ABSTRACT

Different research groups have extensively studied the associations of cytokine gene polymorphisms in different diseases. The role of cytokines gene polymorphisms in multiple sclerosis (MS), as a chronic immune-mediated neurodegenerative disease, has been previously reported in the various populations.

For determining pro-inflammatory cytokine gene polymorphisms, 100 relapsing remitting multiple sclerosis (RRMS) Iranian patients and 140 normal individuals as control enrolled in this study. DNA of each sample was extracted by a modified salting out method. Cytokine single gene nucleotide polymorphisms including IL-1α -889, IL-1β (-511 and +3962), IL-1R pst1 1970, IL-1RA mspa1 11100, and TNF-α (-308 and -238) were determined by using the PCR-SSP method.

The results of our data indicate the decrease in frequency of IL-1α TC-889 genotype (p=0.002), IL-1β TC +3962 genotype (p=0.004), IL-1R T pst1 1970 allele (p= 0.0001), IL-1RA TC Mspa1 11100 genotype (p=0.009), TNF-α A-308 allele (p=0.0002) and AG genotype (p=0.00001) in the patients group versus normal subjects. On the other hand the frequency of IL-1α TT -889 genotype (p=0.028), IL-1R C pst1 1970 allele (p=0.0001) and CC genotype (p=0.00006), TNFα G -308 allele (p=0.0002) and GG genotype (p=0.000001) decreased significantly in the patients versus normal subjects.

These results suggest that polymorphic variations of these pro-inflammatory cytokines may play an important role in susceptibility of Iranian multiple sclerosis patients.

Key words: Cytokines; IL-1; TNFα; Gene polymorphism; Multiple sclerosis; Single nucleotide polymorphism

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INTRODUCTION

Multiple sclerosis (MS) is a complex autoimmune disease with a vast demyelination in central nervous system (CNS).\(^1\)\(^2\) Demyelination happens because of infiltration immune system cells (B and T) into CNS and producing antibodies and cytokines against myelin antigens.\(^2\) The pro-inflammatory cytokines such as IL-1 and TNF-\(\alpha\) are produced more in the lesions of MS.\(^4\) Genetic polymorphisms of cytokines are considered as risk factors for MS.\(^5\)\(^-\)\(^8\) The polymorphism of IL-1 family genes as well as TNF-\(\alpha\) may contribute to susceptibility and pathogenesis of MS by altering cytokine production and inducing inflammation.\(^6\) In the present study, we investigated single nucleotide polymorphisms (SNPs) of IL-1 family and TNF-\(\alpha\) in Iranian MS patients and normal individuals. Cytokine gene polymorphisms including TNF-\(\alpha\) (-308 G/A and -238 G/A), IL-1\(\alpha\) (-889 T/C), IL-1\(\beta\) (-511 C/T and +3962 C/T), IL-1R (pst1 1970 C/T), IL-1Ra (mspal 11100 T/C) were determined using the PCR-SSP method.

MATERIALS AND METHODS

Subjects

One hundred unrelated relapsing-remitting multiple sclerosis (RRMS) persons (70 females, 30 males) with clinically defined MS were recruited from MS society of Iran during June 2005 and November 2006. Mean age of patients was 35.02±7.00 years. Mean age of the onset of the disease was 38.03 years and mean duration was 7.54 years. All patients had EDSS (Expanded Disability Status Scale) between 2.0 up 4.0. Control group comprised of 140 normal persons that had been selected randomly from Iranian blood transfusion organization in Tehran. After obtaining informed consent from the subjects, 5 ml venous blood was taken. Peripheral blood mononuclear cells (PBMC) were isolated in the sterile conditions from EDTA blood by density gradient centrifuge using ficoll/paque (Sigma, USA) and were frozen in liquid nitrogen. After collecting all specimens, genomic DNA was extracted by using Proteinase K according to Miller method.\(^9\) Optical densities of the extracted DNA were measured in 260 and 280 nm wavelength.

DNA Analysis

Polymerase chain reaction was performed with Cytokines CTS-PCR-SSP Tray Kit (Heidelberg University, Germany). This kit provided sequence specific oligonucleotide primers for PCR amplification of selected cytokine alleles including TNF-\(\alpha\) (-308 G/A and -238 G/A) and IL-1 family [IL-1\(\alpha\) (-889 T/C), IL-1\(\beta\) (-511 C/T and +3962 C/T), IL-1R (pst1 1970 C/T), IL-1Ra (mspal 11100 T/C)].

Statistical Analysis

Allele and genotype frequencies of IL-1 and TNF-\(\alpha\) cytokine gene in patient group were detected and compared with control group. Chi-square with Yates correction and Fisher’s exact test were performed in this case-control study by using EPI Info Software. The level of significance was chosen as p<0.05.

RESULTS

IL-1\(\alpha\) Polymorphism

Allele and genotype frequency of IL-1\(\alpha\)(C/T) at position -889 was detected in 100 MS patients and 136 Iranian normal people. Although there was no significant difference in the single allele distribution between patients and controls, genotype analysis revealed significant differences in TC (p=0.002) and TT (p=0.028) genotypes between these two groups (Table 1).

IL-1\(\beta\) Polymorphism

Two positions including -511 in promoter and +3962 in encoding region, were studied. Analysis on allele and genotype frequency of -511 positions revealed no significant statistical difference in patient and normal group. In +3962 position, CC and CT genotypes revealed significant differences (p=0.043 and p=0.004) (Table 1).

IL-1Receptor Polymorphism

IL-1R position pst1 1970 showed significant differences between MS patients and normal group in alleles C and T allele frequency (p=0.0001). In addition, significant differences between MS patients and normal people in CC and TC genotype were found (p=0.00006 and p=0.046) (Table 1).

IL-1Receptor Antagonist Polymorphism

In the IL-1R antagonist at mspal 11100 C/T position, no statistically significant differences were found in alleles between total MS patients and normal controls. However, in TC and TT genotypes, Significant differences were shown (p=0.009 and p=0.013) (Table 1).
Cytokine gene polymorphism in Iranian MS Patients

Table 1. Significant allele and genotype frequencies of cytokine SNPs in Iranian MS patients.

<table>
<thead>
<tr>
<th>Allele/ Genotype</th>
<th>Patients [F (%)]</th>
<th>Control [F (%)]</th>
<th>OR (95%CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 alpha(-889)</td>
<td>N=100</td>
<td>N=136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>66(66)</td>
<td>62(45.6)</td>
<td>2.32(1.31-4.10)</td>
<td>0.002</td>
</tr>
<tr>
<td>TT</td>
<td>1(1)</td>
<td>12(8.8)</td>
<td>0.11(0.00-0.79)</td>
<td>0.028</td>
</tr>
<tr>
<td>IL-1β(+3962)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>36(36)</td>
<td>70(50)</td>
<td>0.56(0.32-0.98)</td>
<td>0.043</td>
</tr>
<tr>
<td>TC</td>
<td>61(61)</td>
<td>58(41.4)</td>
<td>2.21(1.27-3.87)</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-1 R pst1 1970</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>88(44)</td>
<td>174(62.1)</td>
<td>0.48(0.33-0.70)</td>
<td>0.0001</td>
</tr>
<tr>
<td>T</td>
<td>112(56)</td>
<td>106(37.9)</td>
<td>2.09(1.42-3.08)</td>
<td>0.0001</td>
</tr>
<tr>
<td>CC</td>
<td>14(14)</td>
<td>54(38.6)</td>
<td>0.26(0.13-0.52)</td>
<td>0.00006</td>
</tr>
<tr>
<td>IL-1RA Mspal11100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>58(58)</td>
<td>56(40)</td>
<td>2.07(1.19-3.86)</td>
<td>0.009</td>
</tr>
<tr>
<td>TT</td>
<td>40(40)</td>
<td>80(57.1)</td>
<td>0.5(0.29-0.87)</td>
<td>0.013</td>
</tr>
<tr>
<td>TNFα(-308)</td>
<td>N=99</td>
<td>N=137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>141(71)</td>
<td>235(85.8)</td>
<td>0.41(0.25-0.66)</td>
<td>0.0002</td>
</tr>
<tr>
<td>A</td>
<td>57(29)</td>
<td>39(14.2)</td>
<td>2.44(1.50-3.95)</td>
<td>0.0002</td>
</tr>
<tr>
<td>GG</td>
<td>42(22.4)</td>
<td>98(71.5)</td>
<td>0.29(0.16-0.52)</td>
<td>0.00001</td>
</tr>
<tr>
<td>AG</td>
<td>57(57.6)</td>
<td>39(28.5)</td>
<td>3.41(1.91-6.11)</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

N: Number; F: Frequency; OR: Odds Ratio; CI: Confident Interval; * Yates corrected

TNF-α Polymorphism

Two positions including -308G/A and -238G/A were studied in both MS patients and normal people. No allele and genotype was significant in -238 position and alleles were significant in -308 position (p=0.0002). Also in -308, significant differences were shown in GG and GA genotypes (p=0.00001) (Table 1).

DISCUSSION

In recent years, it has been demonstrated that polymorphisms of many cytokine genes affect the transcriptional activities, resulting in individual variation in cytokine production.10 Individual variations in the cytokine production, might affect on many autoimmune diseases such as MS. It is assumed that different expression (high; intermediate or low) of pro-inflammatory cytokines (IL-1 and TNF-α) in MS may affect inflammation in CNS.

In this study, we have investigated the genetic polymorphism of the pro-inflammatory cytokines including IL-1 and TNF-α in Iranian MS patients versus normal control group. In accordance with Mann’s study (2002) in UK population, we did not find any significant differences in IL-1α -889 T/C allele frequency between MS patients and normal controls.11 However, significant differences were seen between MS and control groups in TC genotype (66% vs. 45.6%, p=0.002) and TT genotype frequencies (1% vs. 8.8%, p=0.028) (Table 1). In our study on IL-1β -511 T/C SNP, there was not any significant difference between the patients and controls; whereas Mann et al showed a trend with severe and mild disease.11 In +3962 IL-1 T/C SNP, significant difference was shown in CC genotype (36% vs. 50%, p=0.043) and TC genotype (61 % vs. 41.4%, p=0.004) between MS patients and control group.

In our result, differences in frequency of IL1R pst1 1970 C/T, C allele in MS patients (44% vs. 62.1%, p=0.0001) as well as T allele (56% vs. 37.9%, p=0.0001) were significant between both groups (Table 1). In addition, significant difference was shown in CC genotype (14% vs. 38.6%, p=0.00006) and TC genotype (61% vs. 47.1%, p=0.046) between MS patients and control group (Table 1).

In contrast with many studies in other countries,12-14 the IL-1RA mpsal11100 T/C allele frequency did not show significant difference between MS patients and normal control groups but in genotype frequency, TC genotype (58% vs. 40%, p=0.009) and TT genotype (40% vs. 57.1%, p=0.013) were significant in MS patients compared with normal people (Table 1).
Although, in Japanese population reported by Niino et al., no association was observed between MS patients and normal people in IL-1RA.

In TNF-α -308G/A SNPs, our findings showed higher frequencies of A allele (29% in patients vs. 14.2% in controls, p=0.0002) and AG genotype (57.6% in patients vs. 28.5% in controls, p=0.00001) in MS patients and a decreased prevalence of GG genotype (42.4% vs. 71.5%, p=0.00001) (Table 1). In contrast with our result, Maurer et al showed no difference in TNFα-308 position between groups. In -238 A/G position, allele and genotype frequencies did not show any difference between our patients and controls.

Finally, as SNPs have correlation with gene pools in each population, it is suggestive that differences between our results and the data reported in other populations might be due to racial and ethnicity differences. Thus, we need to study these SNP in a large sample size in Iranian MS patients and normal control, in order to confirm genetic susceptibility of MS patients compared with normal subjects.

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REFERENCES