Interleukin 10 Gene Polymorphism in Iranian Patients with Multiple Sclerosis

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Abstract-

Purpose: IL-10 suppresses several activities of the immune response by inhibition of Th1 and Th2 cells.

Methods: We studied 110 Iranian patients with clinically definite multiple sclerosis (MS) and 100 ethnic and age matched controls. Three single-nucleotide polymorphisms in the proximal region of IL-10 promoter gene (-1082/-819/-592) were analysed by amplification refractory mutation system (ARMS) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods.

Results: The -1082 (G/A), -819 (T/C) and -592 (A/C) genotypes were similarly distributed between MS patients and the controls. There was no statistically significant difference in the allelic and genotype distribution between patients and controls. In addition, gender, course and progression index did not reveal any statistically significant differences in the allele and genotype distribution of IL-10 polymorphisms.

Conclusion: As a non-European patient population, according to our results, IL10 polymorphism is not associated with MS and its subtypes nor influences the disease progression.

Key Words: multiple sclerosis, interleukin 10, polymorphism

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INTRODUCTION

The Interleukin (IL)-10 is a 36 kDa homodimeric anti-inflammatory cytokine. This cytokine is produced primarily by monocytes and to a lesser extent by lymphocytes. It is mainly expressed in monocytes and type 2 T helper cells, mast cells, and CD4(+)/CD25(+)/Foxp3(+) regulatory T cells. This cytokine has pleiotropic effects in immun regulation and inflammation. It is capable of inhibiting the synthesis of proinflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF made by cells including macrophages and the type 1 T helper cells[1,2,3]. IL-10 gene is mapped to the chromosome 1(1q31-1q32). The IL-10 promoter is highly polymorphic containing, two microsatellites and three single nucleotide polymor-

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phisms (SNPs): a G to A substitution at position -1082, a C to T at -819 and a C to A at -592[1,2]. The higher IL-10 production was related to the development of T regulatory cells, producing high levels of IL-10 and TGF-β[3,4].

Multiple sclerosis (MS) is a common inflammatory disease of the central nervous system characterized by myelin loss and progressive neurological dysfunctions. Although The causes of MS are unknown, but both genetic and environmental factors play important roles[5]. In this study, we reported single-nucleotide polymorphisms in the proximal region of IL-10 promoter gene (-1082/-819/-592), as an anti-inflammatory cytokine, in Iranian patients with multiple sclerosis and its association with course and severity of the disease.

METHODS

Patients and Controls

One hundred and ten unrelated Iranian patients with MS (78 women, 22 men, and mean age of 32.2 years) were recruited from the Department of Neurology of the Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. The diagnosis of MS was made according to the Poser criteria[5].

Furthermore, 100 ethnically matched healthy unrelated individuals (51 women, 49 men, mean age of 33.41) were enrolled into the study as the control group. Kurtzke's Expanded Disability Status Scale (EDSS) score was assessed for each patient and progression index (EDSS/ disease duration in year) was calculated for patients with MS duration > 2 years, without acute exacerbations in the previous 3 months. The Ethnic Committee of Shiraz University of Medical Sciences approved the study protocol. A written informed consent was obtained from all subjects for collection, DNA isolation and gene polymorphism determination.

Genetic polymorphisms analysis

Peripheral blood from patients and controls was collected in EDTA tube and DNA was extracted by a commercial kit (DNP plus DNA Extraction Kit, Sinagene, Iran).

IL-10 (-1082) polymorphism was analysed using amplification refractory mutation system (ARMS). The IL-10 (-819, -592) polymorphism was analysed by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) method[6]. Some samples from each genotype were sequenced with a Genetic Analyzer (Applied Biosystems, Foster City, CA) which confirmed the PCR-ARMS results.

Statistical analysis

The analyses were performed with the SPSS software (Statistical Package for the Social Sciences, version 11.5, SSPS Inc, Chicago, Ill, USA). Variables were compared with logistic regression test or Student's *t* test according to the variable types. Results were considered to be significantly different when the value for P was less than 0 .05. The observed numbers of different IL10 genotypes in controls were compared with those expected for a population based on Hardy- Weinberg equilibrium.

RESULTS

IL-10 promoter (-1082 -819 -592) polymorphism: Allele and Genotype Frequency

The observed distribution of genotypes in controls did not show significant difference when compared with those predicted from the Hardy-Weinberg equilibrium (P > 0.05)

Table 1 shows the allele and genotype frequencies of the mentioned polymorphism in 110 patients with MS and 100 matched controls.

There was also no statistically significant difference in the distribution of the allelic (C, T) (1082), and genotypes (CC, CT, TT) among patients and controls. (OR: 2.4, CI 95%: 0.21-1.85, P:0.5) and (OR: 2.9, CI 95%: 0.1-4, P: 0.5).

There was no statistically significant difference in the distribution of the allelic (C, T) (-819), and genotypes (CC, CT, TT) among patients and controls. (OR: 2.19, CI 95%: 0.76-1.95, P: 0.6) and (OR: 0.91, CI 95%: 0.76-2.21, P: 0.7).

According to the locus (-592), there was no statistically significant difference in the distribution of the

allelic (C, A) (-592), and genotypes (CC, CA, AA) among patients and controls: (OR: 0.87, CI 95%: 0.5-4.7, P: 0.72) and (OR: 0.99, CI 95%: 0.1-2.7, P: 0.8) respectively.

When the analysis was performed with respect to the gender of the subjects, it did not reveal any statistically significant differences in IL-10 (-819) genotypes (OR: 2.3, CI 95%: 0.31-2.21, P:0.85); IL-10 (-592) genotypes (OR: 1.1, CI 95%: 0.2-1.2, P: 0.85) and IL-10 (-1082) genotypes (OR: 2.9, CI 95%: 0.11-2.96, P: 0.95) among female patients, as compared to female controls.

Male patients also did not show significant difference in IL10 (-819) genotypes (OR: 5.3, CI 95%: 0.71-2.91, P: 0.4); IL10 (-592) genotypes (OR: 1.1, CI 95%: 0.2-4, P: 0.89) and IL10 (-1082) genotypes (OR: 1.3, CI 95%: 0.11-4, P: 0.43) respectively.

Phenotype frequency of IL10 promoter haplotype in MS patients and controls was compared in Table 2. There was no difference in haplotype frequency between patients and controls. When the analysis was performed with respect to the gender of the subjects, it did not reveal any statistically significant differences (p> 0.05).

There were also no significant difference in allele and genotype distribution of A/G polymorphism between relapsing-remitting and primary progressive courses (P > 0.05).

The mean EDSS of our MS population was 0.56 ± 0.45 at the time of sample collection. As Table 3 pre-

Table 2. *Haplotype frequencies of Interleukin interleukin 10 (1082), (819), and (592) polymorphisms in patients with multiple sclerosis and in control groups*

Haplotype	MS	Control	OR	95%CI	P-value
GCC	81	72	3.14	0. 1-4.1	0.51
ACC	91	75	3.14	0.08-10	0.53
ATA	20	16	0.11	0.04-3	0.19
GTA	16	12	1.47	0.05-10	0.82

Table 1. *Allele and genotype frequencies and effect size (V) of Interleukin 10 (819), (592), (1082) polymorphism in patients with multiple sclerosis and in control groups*

		Allele (- 819) (V=0.04)		Genotype (- 819) (V=0.08)			Allele (- 592) (V=0.01)		Genotype (-592) (V=0.07)			Allele (-1082) (V=0.01)		Genotype (-1082) (V=0.04)		
Group	n	C (%)	T(%)	CC(%)	TC(%)	TT(%)	C(%)	A(%)	CC(%)	AC(%)	AA(%)	A(%)	G(%)	AA(%)	AG(%)	GG(%)
MS	110	159(72)	61(28)	63(57)	33(30)	14(13)	171(78)	49(22)	69(63)	33(30)	8(7)	125(59)	95(41)	21(19)	83(75)	6(6)
Male	26	39(75)	13(25)	16(62)	7(27)	3(11)	44(85)	8(15)	19(73)	6(23)	1(4)	27(52)	25(48)	4(15)	19(73)	3(12)
Female	84	120(71)	48(29)	47(56)	26(31)	11(13)	127(76)	41(24)	50(60)	27(32)	7(8)	98(58)	70(42)	17(20)	64(76)	3(4)
Control	100	151(76)	49(24)	64(64)	23(23)	13(13)	151(76)	49(24)	62(62)	27(27)	11(11)	111(56)	89(44)	16(16)	79(79)	5(5)
Male	49	73(73)	25(27)	34(69)	5(11)	10(20)	77(79)	21(21)	33(67)	11(22)	5(11)	55(56)	43(44)	10(20)	35(71)	4(9)
Female	51	78(76)	24(24)	30(59)	18(35)	3(6)	74(73)	28(27)	29(57)	16(31)	6(12)	56(55)	46(45)	6(12)	44(86)	1(2)

Table 3. *Distribution of different genotypes and haplotypes of interleukin 10 (IL-10) according to progression index (PI) in multiple sclerosis patients*

	Genotype (-819) (%)			Genotype (-592) (%)			Genotype (-1082) (%)			Haplotype GCC	Haplotype ACC	Haplotype ATA	Haplotype GTA
	CC	TC	TT	CC	AC	AA	AA	AG	GG	21(36)	29(32)	3(15)	3(19)
PI ≤1	22(36)	8(25)	6(43)	25(37)	10(31)	1(13)	10(48)	26(32)	0				
PI >1	39(64)	25(75)	8(57)	44(63)	23(69)	7(87)	11(52)	57(68)	6(100)	60(74)	62(68)	17(85)	13(81)

sents, the patients with high and low progression index did not reveal any statistically significant difference according to the IL-10 genotypes (_819: P=0.12, -592: P=0.51, -1082: P=0.66). The patients with high progression index and low progression index had no significant difference in IL-10 genotypes too. [GCC: P=0.74, ACC: P=0.66, ATA=P:0.62, GTA=P:0.62]

DISCUSSION

The current study conducted in MS patients from a homogenous Iranian population revealed a lack of association of the allelic and genotype distribution of IL-10 and presentation of multiple sclerosis. In addition, there was no significant association between allelic and genotype distribution of IL-10 was found with either course or progression index.

Interleukin-10 is an important Th1 inhibitory cytokine. The inherited factors seem to play an important role in the regulation of IL-10 production[3]. The IL-10 promoter is highly polymorphic containing several polymorphisms which have been described in the promoter region of IL-10 that might be related with transcriptional regulation of this gene[1,2].

There is controversy about the association between allelic and genotype distribution of IL-10 and evolution and progression of multiple sclerosis. Luomala et al.[7], Wergeland et al.[8] and Almeras et al.[9] found associations between IL-10 polymorphisms and the severity or response to treatment in MS patients. Other studies suggested that IL-10 polymorphisms do not appear to be associated with MS or to influence disease progression 10-19. This is the first report in Iran on MS susceptibility and IL-10 polymorphisms. Our results confirm those previous reports 10-19 that no outstanding association exists between any of the IL-10 alleles and susceptibility to MS.

As a summary, our results from a non-European cohort showed no significant associations between IL-10 polymorphisms and neither MS nor any of its subtypes. In our previous studies with same population, Iranian patients with MS did not have significant difference with controls according to intercellular adhesion molecule-1 (ICAM-1) and cytotoxic T lymphocyte associated antigen-4 gene polymorphisms.[20,21] Further genetic studies for finding susceptibility genes of Iranian MS patients should be conducted.

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