Interleukin 18 Polymorphisms and its serum level in Patients with Multiple Sclerosis

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Abstract

Background: Multiple sclerosis (MS) is a chronic demyelinating disorder of central nervous system. Although the definite pathogenesis of MS has not been understood, crucial role of environmental and genetic risk factors has been proposed. **Propose:** To determine the serum level of interleukin-18 (IL-18) as well as gene polymorphisms of IL-18 (rs1946518, rs360719, and rs187238). **Methods:** In this case–control study, 110 MS patients diagnosed according to the McDonald criteria and 110 healthy individuals were recruited. IL-18 gene polymorphisms were genotyped by polymerase chain reaction high-resolution melt test, and IL-18 serum level was determined by enzyme-linked immunosorbent assay technique. **Results:** The mean age of the MS patients (89 females and 21 males) and the control group (89 females and 21 males) was 30.3 ± 9.25 and 30.28 ± 9.13 years, respectively. The mean serum levels of IL-18 in MS patients and healthy individuals were 341.56 ± 39.22 Pg/Ml and 146.52 ± 29.30 Pg/Ml, respectively ($P < 0.001$). The genotype of rs1946518 (but not rs360719 and rs187238) was significantly different between groups ($P = 0.037$ and $P = 0.069$, respectively). **Conclusion:** In this study, we showed the significant higher IL-18 serum level and significant different frequencies of two polymorphisms of IL-18 in MS patients. These results show the important roles of IL-18 in MS pathogenesis. However, more studies are needed to verify our results in larger sample size.

Keywords: Multiple sclerosis, interleukin 18, serum level, single-nucleotide polymorphism

Introduction

Multiple sclerosis (MS) is the most common chronic demyelinating disorder of central nervous system. The etiology of MS is still elusive though environmental and genetic factors have been proposed as its triggers.[1-3] In this regard, major histocompatibility complex class II genes have been shown as the most important alleles, and many other single-nucleotide polymorphisms (SNPs) have been suggested as risk factors for MS development.[4-6] Cytokines and mediators have crucial roles in pathogenesis of MS.[7,8] Interleukin-18 (IL-18), also known as interferon gamma (IFN-γ)-inducing factor, is a proinflammatory cytokine and is secreted by macrophages, dendritic cells, and other microglial and antigen-presenting cells. IL-18, similar to IL-12, induces IFN-γ secretion by T helper and natural killer cells.[9]

In the literature about the pathogenesis of other autoimmune disorders such as systemic lupus erythematous, rheumatoid arthritis, inflammatory bowel diseases, and systemic juvenile idiopathic arthritis, important roles for serum IL-18 have been suggested.[10-13] To the best of our knowledge, there are limited studies to investigate IL-18 serum level and SNPs in MS patients.[14-20] In the current study, we aimed to evaluate the frequency of three IL-18 SNPs and IL-18 serum level in Iranian MS patients.

Methods

In the current study, 110 relapsing–remitting MS (RRMS) patients and 110 age- and sex-matched healthy individuals were included. All of the MS patients were diagnosed according to the McDonald criteria[21] by an expert neurologist. Before blood sampling, all of the participants submitted written informed consent form. IL-18 evaluation was performed by enzyme-linked immunosorbent assay technique using available commercial kit (Boster, Pleasanton, California, USA). Genotyping of rs1946518, rs360719, and rs187238 SNPs was done using polymerase chain reaction high-resolution melt test. Details of our technique was similar to what described elsewhere.[14,22] SPSS software (Version 18, SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results and Discussion

The mean age of the MS patients (89 females and 21 males) and the control group (89 females and 21 males) was 30.3 ± 9.25 and 30.28 ± 9.13 years, respectively. The mean duration of disease and expanded disability status scale was 4.1 ± 3.5 years and 1.00 ± 0.27, respectively. The mean

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serum levels of IL-18 in MS patients and healthy individuals were 341.56 ± 39.22 Pg/Ml and 146.52 ± 29.30 Pg/Ml, respectively ($P < 0.001$). Allele and genotype frequencies of IL-18 polymorphisms are presented in Table 1.

As shown in Table 1, frequency of genotypes of rs360719 and rs187238 was not different between patients and healthy individuals, although different frequencies of rs1946518 genotypes were detected. Furthermore, different frequencies of alleles in three SNPs were not found [Table 1]. In addition, IL-18 serum level was not significantly different among patients with varied SNPs ($P > 0.05$).

In the current study, we detected significantly higher serum level of IL-18 in MS patients ($P < 0.001$). However, we did not find significant different frequencies of IL-18 alleles between MS patients and the control group though higher frequency of AC genotype of rs1946518 SNP was demonstrated ($P = 0.037$). In line with our results, Chen *et al*. found significantly higher level of IL-18 in MS patients than healthy individuals;[14] Nicoletti *et al.* reached the same results and found significantly higher level of IL-18 in RRMS patients in the acute phase of disease comparing RRMS patients in the stable phase ($P = 0.002$). Moreover, significantly higher level of IL-18 was detected in patients with secondary progressive MS comparing with RRMS patients in either acute or stable phase of disease ($P < 0.05$ and $P < 0.001$, respectively).[18] Losy and Niezgoda similarly found significantly higher levels of IL-18 in RRMS patients and showed that IL-18 is elevated in MS patients with active MRI lesions comparing with those patients without such lesions.[17] It is to be noted that some studies did not show elevated serum level of IL-18 in Bulgarian RRMS patients.[19,20] Interestingly, cerebrospinal fluid (CSF) levels of IL-18 have been reported significantly higher in MS patients comparing with healthy individuals.[17] In another study, detectable levels of IL-18 in RRMS patients and chronic progressive MS patients were reported; however, they did not find detectable CSF levels of IL-18 in the control group.[18]

Our genotyping showed significant difference in distribution of AC genotype of rs1946518 SNP between MS patients and healthy individuals ($P = 0.037$). Furthermore, we found odds ratio (OR) of 1.88 and 2.17 for MS development in AC and CC genotypes of rs1946518, respectively. In a study by Karakas Celik *et al*., rs187238/rs1946518 genotyping on Turkish MS patients showed significantly higher frequency of CC genotype for IL-18-137G/C polymorphism in MS patients comparing with healthy individuals ($P = 0.03$). Furthermore, they proposed C allele in IL-18-137 polymorphism as a risk factor of MS (OR = 1.909).[23] In the present study, we did not find a significant difference of genotypes of IL-18 rs360719 polymorphism between RRMS patients and control group. In addition, neither allele C nor allele G of this polymorphism was demonstrated as a risk factor of MS development ($P > 0.05$). In agreement with our study, Orhan *et al.* showed nonsignificant difference in frequency of GC, CC genotypes of IL-18-137 polymorphism and significant difference in frequency of CA genotype of IL-18-607 polymorphism ($P = 0.067$, $P = 0.571$, and $P = 0.462$, respectively).[19]

Conclusion

The present study showed that serum level of IL-18 is significantly higher in MS patients comparing to healthy

Table 1: Comparison of IL-18 polymorphisms among MS patients and healthy subjects

Loci		Case (n=110)	Control (n=110)	OR (95%CI)	P
Rs360719	Genotype				
	GG	75 (68)	80 (73)	Reference	>0.05
	GC	29 (26)	27 (24)	0.469 (0.113-1.942)	
	CC	6 (6)	3 (3)	0.537 (0.122-2.363)	
	Allele				
	G	179 (81)	187 (85)		>0.05
	C	41 (19)	33 (15)		>0.05
Rs1946518	Genotype				
	AA	27 (25)	23 (21)	Reference	0.037
	AC	53 (48)	39 (35)	1.878 (0.915-3.856)	
	CC	30 (27)	48 (44)	2.174 (1.175-4.025)	
	Allele				
	A	107 (49)	85 (39)		>0.05
	C	113 (51)	135 (61)		>0.05
Rs187238	Genotype				
	AA	49 (45)	66 (60)	Reference	0.069
	AG	50 (45)	37 (34)	0.472 (0.171-1.306)	
	GG	11 (10)	7 (6)	0.860 (0.304-2.430)	
	Allele				
	A	148 (67)	169 (77)		>0.05
	G	72 (33)	51 (23)		>0.05

individuals. Furthermore, we found a significant frequency difference of IL-18-137 genotype between MS patients and control group with OR of 2.17 for CC genotype of this SNP. Further studies on frequency of IL-18 SNPs and its serum level in RRMS patients are recommended to clarify the roles of these SNPs in the MS course.

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Conflicts of interest

There are no conflicts of interest.

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