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Evaluation of Interleukin-8 −251 T/A Polymorphisms in Visceral Leishmaniasis

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ABSTRACT

Background: Interleukin (IL)-8 plays important roles in the recruitment and activation of immune cells during visceral leishmaniasis (VL). Genetic variations in IL-8 modulate the expression of IL-8 protein and may be associated with VL. This study aimed to evaluate polymorphisms at the IL-8 −251 position in VL patients.

Methods: This cross-sectional study was performed on three groups: *Leishmania*-seropositive patients with clinical symptoms of VL (n=91), seropositive patients without clinical symptoms (n=104), and healthy controls (n=110). Polymorphisms at the IL-8−251 position were analyzed using allele-specific polymerase chain reaction (PCR). Anti-*Leishmania* antibody titers were assessed by immunofluorescence.

Results: IL-*8*−251 polymorphism was significantly associated with VL (*P*<0.002). The IL-8−251 T/T genotype was significantly higher in group 1 than in groups 2 and 3 (*P*<0.002). The validity of the data was analyzed using Hardy-Weinberg equilibrium and one-way analysis of variance (ANOVA), as well as x^2 tests.

Conclusions: IL-8−251 polymorphism was significantly associated with impaired immune responses in VL and might be considered a risk factor for disease development.

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Introduction

Leishmaniasis, also known as kala-azar, is a vector-borne parasitic disease caused by various species of *Leishmania*[1]. Three clinical manifestations of *Leishmania* infections include cutaneous, mucocutaneous, and visceral leishmaniasis (VL)[2,3]. The outcome of leishmaniasis is determined by the quality and quantity of host immune response[4]. Interleukin (IL)-8 is a proinflammatory cytokine that plays key roles in the recruitment and activation of immune cells, especially neutrophils, in leishmaniasis[5]. Therefore, genetic variations in IL-8 (NG-029889.1) that lead to a reduction in IL-8 expression might be associated with impaired immune response against leishmaniasis. Functional polymorphism at the −251 (rs4073) position, located within the promoter region of IL-8, affects IL-8 expression and is associated with several immune-related diseases[6-8].

This study aimed to evaluate the status of IL-8 −251 polymorphism in seropositive VL patients with and without clinical presentation, in comparison with healthy controls. To the best of our knowledge, this is the first study to evaluate the relationship between genetic polymorphisms and pathogenesis of leishmaniasis in Iran.

Methods

Subject

This cross-sectional study was performed during 2004 to 2012 on 91 *Leishmania*-seropositive patients with clinical symptoms of VL (group 1), 104 seropositive patients without clinical symptoms (group 2), and 110 healthy control individuals (group 3) to analyze IL-*8* −251 T/A polymorphism. The sample size required for the study was estimated based on previous studies and the degree of polymorphism observed in this region. An expert infectious diseases specialist diagnosed VL in the patients using their medical histories, clinical presentations, and laboratory findings. The VL patients as well as the participants in groups 2 and 3 belonged to East Azerbaijan, where *L. infantum* is endemic[9-11].

Informed consent was obtained from all the participants, and the protocol of the study was approved by the Ethical Committee of Hamadan University of Medical Sciences, Iran.

DNA extraction

Genomic DNA was extracted from peripheral blood using a commercial kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions.

Detection of polymorphism

IL-8 −251 T/A gene polymorphisms were evaluated by allele-specific PCR. Both regions were amplified in a PCR reaction mixture (volume, 20 µL) containing 0.6 µL MgCl2 (50 mM), 0.4 µL of each dNTP (dATP, dCTP, dGTP, and dTTP at a concentration of 10 mM each), 1 µL of each primer (10 pmol/µL), 0.3 µL of Taq DNA polymerase (5 units), 1 µL of prepared DNA template, 2 µL of Taq DNA polymerase buffer (10×), and sterile double distilled DNase free water. The forward and reverse primers for the IL-8 −251 T/A (114 bp) region are listed in Table 1. The PCR conditions were as follows: 95 °C for 5 min (denaturation), 35 cycles of 95 ºC for 30 s, 53 °C for 30 s, and 72 ºC for 40 s. PCR amplification was performed using a thermal cycler (Bioneer, South Korea). The PCR product was analyzed by electrophoresis on an ethidium bromide-pretreated 2.5% agarose gel, in parallel with a 50-bp ladder (Cinnaclon, Tehran, Iran).

A commercial kit from Qiagen, USA was used to estimate anti-*Leishmania* antibody titers according to the manufacturer's instructions.

Statistical Analysis

The validity of the data was analyzed using Hardy-Weinberg equilibrium and one-way analysis of variance (ANOVA), and $\chi 2$ tests (SPSS software, version 13) were used to evaluate the differences between the groups. *P*-values less than 0.05 were considered significant.

Table1: The primer sequences for analysis of IL-8 -251 T/A polymorphism

	Group 1		Group 2	
Anti-*Leishmania* antibody titration	**Mean**	**SD**	**Mean**	**SD**
IL-8 -251A/A genotype	3.7	0.25	3.5	0.13
IL-8 -251 A/T genotype	2.8	0.32	3.5	0.14
IL-8 -251 T/T genotype	2.4	0.24	3.4	0.09
P value	0.010	0.010	0.569	0.569

Results

Polymorphisms at IL-8 −251 were significantly associated with VL (*P*=0.002). The IL-8 −251 T/T genotype was significantly (*P*<0.002) more common in group 1 than in groups 2 and 3 (Table 2). Statistical analysis revealed that the differences between the groups regarding the −251 T and A alleles were not significant (*P*=0.187; Table 2). VL patients (group 1) carrying the IL-8 −251 A/A genotype had higher anti-*Leishmania* antibody titers than those carrying the IL-8 −251 T/T genotype (*P*< 0.01). There were no differences in anti-*Leishmania* antibody titers among the group-2 participants, who had various polymorphisms at the IL-8 −251 position (Table 3).

The most frequency of genotypes A/A in group 2, A/T in group 3 and T/T in group 1 were observed (Table 2). The most frequency of alleles A and T in groups 2 and 3 were observed, respectively (Table 2). In the PCR gel image, more detail was seen (Figure 1).

Table 2: The status of IL-8 -251 T/A polymorphism in patients with clinical presentation of VL and seropositive for the *Leishmania* (group 1), without clinical presentation but seropositive (group 2) and healthy controls (group 3)

IL-8 -251T/A polymorphism	**Group 1**		**Group 2**		**Group 3**		**P value**
	Number	**Percent**	**Number**	**Percent**	**Number**	**Percent**	
Genotypes							
A/A	21	23.0	27	26.0	11	10.0	0.002
A/T	25	27.5	36	34.5	55	50.0	
T/T	45	49.5	41	39.4	44	40.0	
Alleles							0.187
A	67	36.8	90	43.3	77	35.0	
T	115	63.2	118	56.7	143	65.0	

Table 3: Anti-*Leishmania* antibody titration in group 1 and 2 with various IL-8 -251T/Apolymorphism

IL-8 -251 T/A polymorpism	
F1	5`-CTAGAAATAAAAAAGCATACAT-3`
F2	5`-CTAGAAATAAAAAAGCATACAA-3`
R	5`-AATACGGAGTATGACGAAA-3`

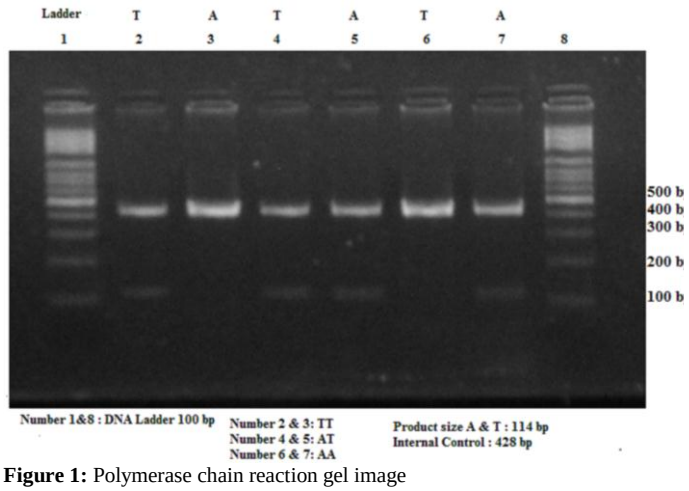

Figure 1: Polymerase chain reaction gel image

Discussion

Various types of cells express high concentrations of IL-8 mRNA and protein rapidly in the presence of stimulants, rather than constitutively. This observation suggests that IL-8 gene expression is tightly controlled to regulate neutrophil migration and activation. Thus, elucidation of the mechanism underlying IL-8 activation might help devise strategies to control leukocyte infiltration, and thereby help alleviate inflammation.

IL-8 plays important roles in immune cell recruitment to infected tissues in a Janus kinase 3-dependent manner[12]. Therefore, it seems likely that IL-8 plays key roles in inducing immune responses against *Leishmania* infections. Our results revealed that polymorphisms in the IL-8 −251 position were significantly associated with VL in Iranian population. Additionally, the current results demonstrated that the IL-8 −251 TT/AA genotypes were significantly more common in group 1 than in groups 2 and 3 (Table 2). Our results suggest that these genotypes may be considered as risk factors for leishmaniasis in Iranian populations. They may play crucial roles in impaired immune responses against leishmaniasis. IL-8 plays key roles in inducing immune responses against *Leishmania* infections[13,14]. Safaiyan and colleagues demonstrated that neutrophils, the main responding cells to IL-8,

were unable to produce inflammatory molecules in patients with cutaneous leishmaniasis[15]. Interestingly, Elshafie et al. demonstrated that neutrophil counts, as well as serum levels of IL-8, were significantly decreased in VL patients than in healthy individuals[5].The mRNA levels of IL-8 were increased in cutaneous leishmaniasis[16]. Collectively, these results suggest that although IL-8 expression is elevated in other forms of leishmaniasis, it is suppressed in VL patients. Moreover, our results demonstrated that the IL-8 −251 T/T genotype is associated with VL. Hence, it may be concluded that this genotype is associated with the down regulation of IL-8 in VL patients.

Frade et al. examined polymorphisms at the IL-8 −251 position in Brazilian VL patients, and their results revealed that the polymorphisms were not associated with leishmaniasis[17]. Our results demonstrated that anti-*Leishmania* antibody titers were significantly decreased in VL patients carrying the IL-8 −251 T/T genotype (Table 3). Interestingly this genotype was significantly more common in VL patients (Table 2). Hence, this genotype is not only responsible for impaired IL-8 production in VL, but also affects anti-*Leishmania* antibody production in this population.

Conclusions

Genetic variations can play important roles in determining the outcome of leishmaniasis, and IL-8 genetic variations, especially IL-8 −251 polymorphism, need to be examined in details in future studies.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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