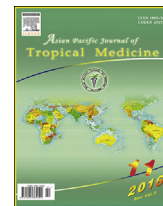




Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Medicine

journal homepage: <http://ees.elsevier.com/apjtm>Original Research <http://dx.doi.org/10.1016/j.apjtm.2016.09.010>

## Evaluation of interleukin 8 +2767 A/T polymorphism in visceral leishmaniasis

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## ARTICLE INFO

## Article history:

Received 4 Jun 2016

Received in revised form 8 Aug 2016

Accepted 16 Aug 2016

Available online 1 Nov 2016

## Keywords:

Interleukin 8

Polymorphism

Visceral leishmaniasis

## ABSTRACT

**Objective:** To evaluate the relationship between the genetic variations at IL-8 +2767 position with VL pathogenesis among Iranian patients.**Methods:** Three groups including patients with VL clinical presentation and leishmania seropositive ( $n = 124$ ), patients seropositive but without clinical presentation ( $n = 82$ ) and healthy controls ( $n = 63$ ) were selected to conduct this cross-sectional study. Polymorphism at +2767 position of IL-8 was investigated using PCR-RFLP techniques. Anti-leishmania antibody titration was evaluated by the immunofluorescence technique.**Results:** We observed higher significant frequencies +2767 A/A and A/T genotypes in Group 1 compared to Group 2 and healthy controls ( $P = 0.001$ ). Also, patients in Group 1 carrying A/A genotype showed higher titer of anti-leishmania antibody than patients with A/T and T/T genotypes ( $P = 0.05$ ). The validity of the data was analyzed using Hardy–Weinberg equilibrium and one way analysis of variance (ANOVA), as well as  $\chi^2$  tests.**Conclusions:** Our findings indicate that the IL-8 +2767 polymorphism is significantly involved in impaired immune responses against VL and it could be considered as a risk factor for the VL progress.

## 1. Introduction

Leishmaniasis, a serious public health problem, is a protozoan disease with multiple clinical features which manifests predominantly in cutaneous, diffuse cutaneous, mucocutaneous and visceral clinical forms [1]. Visceral Leishmaniasis (VL) infections or Kala Azar result in the most severe forms of the disease [2], causing death if left untreated, and are endemic in tropical and sub-tropical regions [3]. It can be undiagnosed or develop into clinical manifestations, including fever, weight loss, pancytopenia, febrile splenomegaly and hepatomegaly [4]. Although VL infection is a protozoan disease, immunologic dysfunctions play a vital role in VL pathogenesis. A main

aspect of the disease is a deficit in immune response to leishmania antigen and lymphokine production [5,6].

Interleukin 8 (IL-8), a proinflammatory cytokine, is produced by leukocytes as well as many types of tissues upon inflammatory conditions and neutrophils are considered to be major specific targets for IL-8 action [7]. Therefore, genetic variations in IL-8 gene resulting in IL-8 expression levels may affect immune responses and leishmaniasis phenotype [8]. It has been implicated that the functional polymorphism at +2767 (rs1126647) position located within 3'UTR of IL-8 gene may influence the level of IL-8 expression [9]. This study aimed to evaluate whether the progress of VL can be associated with IL-8 +2767 polymorphism in the seropositive VL patients with and without clinical presentation.

## 2. Materials and methods

This cross-sectional research was carried out on 124 VL seropositive patients with clinical presentation (Group 1), 82 seropositive patients without clinical presentation (Group 2) and 63 healthy controls (Group 3) to evaluate IL-8 +2767 A/T polymorphism. An expert infectious diseases specialist diagnosed VL in participants based on medical history, clinical presentations and laboratory findings [10]. The VL patients and participants in Groups

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Peer review under responsibility of Hainan Medical University.

Foundation project: This project was supported by a grant from the Tabriz University of Medical Sciences (No. 91-20).

2 and 3 were from East Azerbaijan where the leishmania infantum is endemic [11]. The participants filled out an informed consent form and the ethical approval for this study was obtained from Hamadan University of Medical Sciences.

### 2.1. DNA extraction and polymorphism detection

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

The IL-8 +2767 A/T gene polymorphism was evaluated using RFLP techniques.

To amplify the regions containing this site, PCR was performed in a final volume of 20  $\mu$ L containing 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP [(dATP, dCTP, dGTP, dTTP), 10 pM of each primer (25 ng/ $\mu$ L), 0.5 of Taq DNA polymerase, 100 ng of prepared DNA template and 1 $\times$  PCR buffer. The forward and reverse primers sequencing for amplification of the IL-8 +2767 A/T (222 bp) containing position are F: 5'-CCAGT-TAAATTTTCATTTTCAGGTA-3'; R: 5'-CAACCAGCAA-GAAATTACTAA-3'. The PCR condition was as follows: 1 cycle of 95 °C for 5 min (denaturation) then 35 cycles of 30 s at 95 °C, 53 °C for 30 s and 72 °C for 40 s using a thermal cycler (Bioneer, South Korea). The PCR product was digested with *BstZ171* (Fermentase Company, Finland) in the case of T allele into 198 bp and 24 bp sub-fragments. Thereafter, the PCR product was separated on an ethidium bromide pretreated 2.5% agarose gel in parallel with a 50 bp ladder (Cinnaclon, Iran).

### 2.2. Immunofluorescence assay

Anti-leishmania antibody titration was performed with a commercial kit from Qiagen Company, USA, based on the manufacturer's instructions.

### 2.3. Statistical analysis

The validity of data was evaluated using Hardy–Weinberg equilibrium and the One Way ANOVA as well as  $\chi^2$  tests were used to determine the differences between groups by using the SPSS software version 13. A *P* value less than 0.05 was considered as significant.

## 3. Results

The results from polymorphism analysis showed that the polymorphism at +2767 (*P* = 0.001) was significantly associated with VL. We found that +2767 A/A genotype significantly increased in Group 1 compared to Groups 2 and 3 (Table 1). The

**Table 1**

The status of IL-8 +2767 A/T 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).

Polymorphism		Group 1	Group 2	Group 3	<i>P</i> value
IL-8 +2767 A/T polymorphism	Geno- A/A	25 (20.1)	18 (17.6)	3 (4.8)	0.001
	types A/T	55 (44.4)	44 (43.1)	19 (30.2)	
	T/T	44 (35.5)	40 (39.2)	41 (65.0)	
Alleles	A	105 (42.3)	80 (39.2)	25 (19.8)	0.001
	T	143 (57.7)	124 (60.8)	101 (80.2)	

**Table 2**

Anti-leishmania antibody titration in Groups 1 and 2 with various IL-8 +2767 A/T polymorphism.

Polymorphism	Group 1	Group 2
IL-8 +2767 A/A genotype	3.20 $\pm$ 0.32	3.50 $\pm$ 0.18
IL-8 +2767 A/T genotype	3.00 $\pm$ 0.20	3.80 $\pm$ 0.13
IL-8 +2767 T/T genotype	2.40 $\pm$ 0.24	3.70 $\pm$ 0.12
<i>P</i> value	0.053	0.310

statistical analysis demonstrated a significant difference between groups regarding +2767 A and T alleles (*P* < 0.001, Table 1). Anti-leishmania antibody levels among VL patients with the +2767 A/A genotype was higher than A/T and T/T genotypes (*P* = 0.05, Table 2). As shown in Table 2, the anti-leishmania antibody titration among participants in Group 2 with various genotypes within IL-8 +2767 positions was not statistically different.

## 4. Discussion

Our investigation provides the first evidence that genetic polymorphisms in IL-8 +2767 position among an Iranian population may be involved in impaired immune responses against visceral leishmaniasis. Since IL-8 plays a crucial role in the induction of immune responses against leishmaniasis, genetic variations that influence IL-8 expression or function may contribute to clinically observed leishmaniasis phenotypes and impaired immune response [12]. T cell-mediated immune response predominantly plays significant roles in human leishmaniasis through secretion of cytokines including IL-4 and IL-10, which induce Th1 and Th2 differentiation or IL-12, which promotes Th1 development [12,13]. These observations prompted us to investigate the clinical relevance between IL-8 genetic variation +2767 position and leishmaniasis progress. We found that genotypes and alleles within IL-8 +2767 position might be involved in the VL development in Iranian population. In addition, our results depicted that the IL-8 +2767 A/A genotype was significantly raised in Group 1 compared with Groups 2 and 3 (Table 2). Therefore, it is suggested that this genotype may be associated with VL pathogenesis in Iranian populations. Our hypothesis was verified by several studies focusing on IL-8 relationship with induction of immune responses against leishmania [8,14]. Additionally, it has been shown that contact with Leishmania major results in IL-8 release by monocytes and functions as a neutrophil chemotactic factor [15,16]. It has been reported that Keratinocyte-derived cytokine (also known as CXCL1), the functional murine homologues of human IL-8, is rapidly produced in the skin during L. major infection [17]. On the other hand, Kumar *et al.* found that IL-8 expression levels were high in cutaneous leishmaniasis patients' sera [15]. Since +2767 A/A genotype is associated with VL, it is suggested that this genotype may be involved up-regulation of IL-8 in VL patients and further studies using luciferase assays can be helpful to clarify this issue.

Finally, introducing this IL-8 genetic variation will help us to understand the nature of VL and to design novel chemotherapies or vaccine-based therapies which requires further studies on immune modulations in VL patients [3].

### Conflict of interest statement

The authors declare that they have no conflicts of interest.

## Acknowledgement

This project was supported by a grant from the Tabriz University of Medical Sciences (No. 91-20). The project was implemented in the Hamedan University of Medical Sciences, and approved by the ethics committee of both universities.

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