Original Article

Interleukin-4 receptor alpha T1432C and A1652G polymorphisms are associated with risk of visceral leishmaniasis

Alireza Ahmadi, Mehrdad Hajilooi[1], Ghasem Solgi[1], Mohammad Abasi, Ahad Bazmani[2], Mohammad Matini[3], Khosro Sardarian[3]

Departments of Internal Medicine, [1]Immunology, [3]Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, [2]Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Background: Immune responses play significant roles in protection against leishmaniasis. Polymorphisms within the interleukin 4 receptor alpha chain (IL-4Rα) gene affect the production of cytokines, which is important for the clearance of many pathogens. The aim of the current study was to identify the relationship between visceral leishmaniasis (VL) infection and polymorphisms at positions T1432C and A1652G of IL-4Rα in an Iranian population.

Materials and Methods: This cross-sectional study was performed during 2004–2012 and included three groups of participants: VL patients (Group 1, $n = 124$), seropositive healthy controls (Group 2, $n = 101$), and seronegative healthy controls (Group 3, $n = 55$). The IL-4Rα T1432C and A1652G polymorphisms were evaluated using a polymerase chain reaction-restriction fragment length polymorphism technique, and anti-*Leishmania* antibody titers were determined by using immunofluorescence technique. Alleles and genotypes were compared between groups of the study as well as Groups 1 and 2 based on the titer of antibodies. The validity of the data was analyzed using Hardy–Weinberg equilibrium and one-way analysis of variance, as well as χ^2 tests.

Results: The polymorphisms at IL-4Rα positions T1432C and A1652G were significantly associated with active VL infection. These results demonstrated that the IL-4Rα T1432C and A1652G polymorphisms were not associated with anti-*Leishmania* antibody production.

Conclusion: Our results indicate that these IL-4Rα polymorphisms may be risk factors for the development of VL.

Key Words: Genetic variations, interleukin-4, visceral leishmaniasis

Address for correspondence:
Dr. Khosro Sardarian, Department of Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
E-mail: sardarian@umsha.ac.ir

Received: 26.04.2015, **Accepted:** 13.07.2015

INTRODUCTION

Infection with *Leishmania* species can lead to various clinical presentations, such as cutaneous, mucocutaneous, and visceral leishmaniasis (VL).[1] The VL form, the most and lethal, is also known as Kala Azar.[2] Previous research has shown that VL patients suffer from an impaired immune response against *Leishmania* parasites; hence, the parasite cannot be cleared completely.[3] Interleukin 4 (IL-4) is a cytokine that is, produced by Th2 lymphocytes and plays key

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.166131

How to cite this article: Ahmadi A, Hajilooi M, Solgi G, Abasi M, Bazmani A, Matini M, *et al*. Interleukin-4 receptor alpha T1432C and A1652G polymorphisms are associated with risk of visceral leishmaniasis. Adv Biomed Res 2015;4:195.

roles in the induction of a humoral immune response, including isotype switching to IgE.[4] Additionally, IL-4, as a Th2 cytokine, suppresses the Th1 responses that are crucial for the elimination of intracellular pathogens such as *Leishmania*.[5,6] Therefore, sufficient, but not excess, production of IL-4 leads to appropriate immune responses to parasites.[7,8] Previous studies have shown that IL-4 is significantly upregulated in leishmaniasis,[9,10] indicating that this cytokine plays a key role in the induction of immune responses against *Leishmania*. IL-4 performs its function by attaching to its corresponding receptor (IL-4R) through a JAK/STAT signaling pathway, suggesting that polymorphisms altering IL-4R expression can alter the outcome of *Leishmania* infection. Single nucleotide polymorphisms at positions 1432 (T1432C) and 1652 (A1652G) of the IL-4Rα gene have been associated with altered IL-4Rα expression.[11] Therefore, these polymorphisms may be associated with impaired immune responses against *Leishmania* in VL. Thus, this study was designed to evaluate the IL-4Rα T1432C and A1652G polymorphisms in seropositive VL patients compared to healthy controls with and without anti-*Leishmania* antibodies in a selected Iranian population.

MATERIALS AND METHODS

Subjects

This cross-sectional study was performed during 2004–2012. IL-4Rα T1432C and A1652G genotyping were evaluated in 124 VL patients (Group 1) with mean age of 19.1 ± 14.2 years, 101 seropositive healthy controls (Group 2) with mean age of 25.7 ± 18.03 years, and 55 seronegative healthy controls (Group 3) with mean age of 21.5 ± 14.0 years. The control subjects were matched regarding age, nationality, and race. All study subjects were from three close cities (Kelybar, Ahar and Mianeh) in East-Azerbaijan province, North-West of Iran, which is an endemic region for *Leishmania* infantum.[12-14] Also, the sex ratios (female/male) were 58/66 in Group 1, 48/53 in Group 2 and 29/26 in healthy controls, respectively.

VL was diagnosed in participants by a specialist, based on clinical presentations, laboratory findings, and medical history. The signs and symptoms of VL were fever, severe anemia, weakness, cough, diarrhea, vomiting, fatigue, and appetite loss. The Ethical Committee of the Hamadan University of Medical Sciences approved the study protocol and written informed consent was obtained from all participants prior to sample collection.

DNA extraction

Ten milliliters of venous blood were collected from each subject into tubes containing 50 mmol/L ethylenediaminetetraacetic acid, and genomic DNA was isolated from anti-coagulated peripheral blood buffy coat using Miller's salting-out method. All samples from cases and controls were handled in identical fashion as previously described.[15]

Polymorphism detection

IL-4Rα polymorphisms T1432C and A1652G were evaluated using a polymerase chain reaction (PCR)-restriction fragment length polymorphism technique that has been described elsewhere.[11] Briefly, the amplification reactions were performed in a volume of 20 microliters containing 50 ng of genomic DNA, ×1 PCR buffer, 1.5 mM $MgCl_2$, 0.5U Taq DNA polymerase (Roche, Germany) and allele-specific primers,[11] using thermocycler (PEQLab, Germany). However, the restriction enzymes for genotyping were used as follows; BseRI and AvaI for IL-4Rα T1432C and AvaI for A1652G genotypes, respectively.

Immunoflorescence assay

Anti-*Leishmania* antibody was detected and titred using a IFA *Leishmania* commercial kit (Euroimmun, Germany) according to the manufacturer's guidelines. In brief, double fold dilutions of patients' sera (from 1/10 to 1/320) were tested against commercial parasite coated slides in the presence of negative and positive controls. The observation of fluorescence in samples with dilution over 1/160 was considered as positive.

Statistical analysis

Hardy–Weinberg equilibrium analysis was used to validate the raw data. The χ^2 and one-way tests were performed using SPSS (SPSS Inc., Chicago, IL, USA) to determine the differences in polymorphism prevalence between groups. $P < 0.05$ was considered significant.

RESULTS

Participant groups showed a significant difference in IL-4Rα genotypes at the two positions so that, high frequencies of IL-4Rα 1432 TT and 1652 AA genotypes were observed among VL patients compared to healthy controls ($P = 0.026$ and $P < 0.001$, respectively), [Table 1]. Also, higher significant frequency of IL-4Rα 1652 A allele was found among VL patients versus controls ($P = 0.003$), [Table 1].

These results also revealed that anti-*Leishmania* antibody titers did not differ significantly between participants in Group 1 ($P = 0.251$) and Group 2 ($P = 0.769$) with various IL-4Rα T1432C genotypes, or between participants in Group 1 ($P = 0.077$) and Group 2 ($P = 0.116$) with various IL-4Rα A1652G genotypes [Table 2].

Table 1: The prevalence of IL-4Rα T1432C and A1652G genotypes and alleles among seropositive VL patients (group 1) and seropositive and seronegative healthy controls (group 2 and 3)

	VL patients (group 1) (%)	Seropositive healthy (group 2) (%)	Seronegative healthy (group 3) (%)	*P*
The IL-4Rα T1432C polymorphism				
Genotypes				
T/T	91 (73.4)	77 (76.2)	35 (63.6)	0.026
T/C	25 (20.2)	17 (16.9)	20 (36.4)	
C/C	8 (6.4)	7 (6.9)	0 (0)	
Alleles				
T	207 (83.5)	171 (84.6)	90 (81.8)	0.810
C	41 (16.5)	31 (15.4)	20 (18.2)	
The IL-4Rα A1652G polymorphism				
Genotypes				
A/A	107 (86.3)	79 (78.3)	34 (61.8)	<0.001
A/G	13 (10.5)	22 (21.7)	21 (38.2)	
G/G	4 (3.2)	0 (0)	0 (0)	
Alleles				
A	227 (91.5)	180 (89.1)	89 (81.0)	0.003
G	21 (8.5)	22 (10.9)	21 (19.0)	

IL-4Rα: Interleukin 4 receptor alpha, VL: Visceral leishmaniasis

Table 2: The titration of anti-leishmania antibody in group 1 and 2 carrying various IL-4Rα T1432C and A1652G genotypes

IL-4Rα genotypes	Anti-leishmania antibody titration	
	VL patients (group 1)	Seropositive healthy (group 2)
1432 T/T	2.84±0.17	3.68±0.08
1432 T/C	3.36±0.28	3.61±0.16
1432 C/C	3.5±0.73	3.85±0.34
P	0.251	0.769
1652 A/A	2.39±0.17	3.7±0.07
1652 A/G	3.58±0.48	3.42±0.13
1652 G/G	2±1	3.6±0.07
P	0.077	0.116

IL-4Rα: Interleukin 4 receptor alpha, VL: Visceral leishmaniasis

DISCUSSION

IL-4 is released by many immune cells, including Th2 lymphocytes and mast cells. Up-regulation of IL-4 during parasitic infections leads to IgE production by B lymphocytes and also the development of mast cells, eosinophils, and basophils that are important in combating parasites such as leishmaniasis.[11] Conversely, ectopic expression of IL-4 results in immune responses toward Th2 profile which has been reported in VL cases.[3,16] Because IL-4 functions via interaction with a corresponding receptor (IL-4R),[11] genetic and epigenetic changes that alter IL-4R expression can lead to alterations in the immune response to *Leishmania*. Previous studies have shown that expression of IL-4R is modulated by polymorphisms within the IL-4Rα gene.[11] Our results revealed that the IL-4Rα 1432 T/T genotype and the IL-4Rα 1652 A/A genotypes were significantly associated with VL in these Iranian patients. VL patients are unable to eradicate the parasite from the body.[3] So, it may be concluded that IL-4Rα 1652 A/A genotypes, A allele and also IL-4Rα 1432 T/T genotype, which were more prevalent in Group 1, may be associated with an impaired immune responses against *Leishmania*.[3] Additionally, it has been reported that serum levels of IL-4 are significantly elevated in VL patients in comparison to healthy controls.[17,18] Therefore, it is speculated that although IL-4 production is necessary in VL patients, impaired expression of IL-4R, possibly related to the polymorphisms evaluated in this study, results in reduced IL-4 function against VL. To our knowledge, this is the first study that has shown as the association between IL-4Rα polymorphisms at positions T1432C and A1652G and VL disease. However, previous studies have examined the association of distinct polymorphisms within the IL-4 gene and VL disease. For instance, polymorphisms within intron 2 and 3 of IL-4 (IL4RP1 and 2) have been associated with VL.[19] Interestingly, polymorphisms within the IL-4 gene have also been found to be associated with other parasitic infections, such as *Plasmodium falciparum*.[20,21] and *Trypanosoma cruzi*.[22] Our results support the view that IL-4 polymorphisms are an important genetic risk factor for the development of VL. Our results also revealed that VL patients and seropositive healthy controls with various statuses of IL-4Rα T1432C and A1652G genotype and alleles did not differ in the titers of anti-*Leishmania* antibody. Therefore, it may be concluded that the genotypes we examined did not alter humoral immunity against leishmaniasis.

CONCLUSION

Our findings indicate that the IL-4Rα gene polymorphisms at two positions T1432C and A1652G could be considered as possible genetic risk factors in the immunopathogenesis of VL disease in Iranian patients. Although, further studies on larger groups of patients and evaluation of IL-4R at protein levels are needed to clarify the exact role of this molecule in susceptibility and/or immunopathogenesis of visceral leishmaniasis.

Financial support and sponsorship

This project was supported by a grant from the Tabriz University of Medical Sciences (No. 91-20). The project was implemented in the Hamadan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Singh S. New developments in diagnosis of leishmaniasis. Indian J Med Res 2006;123:311-30.
2. Newlove T, Robinson M, Meehan SA, Pomerantz R. Old World cutaneous leishmaniasis. Dermatol Online J 2012;18:32.
3. Ali N, Mekuria AH, Requena JM, Engwerda C. Immunity to visceral leishmaniasis. J Trop Med 2012;2012:780809.
4. Jabara HH, Ahern DJ, Vercelli D, Geha RS. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. J Immunol 1991;147:1557-60.
5. Kazemi Arababadi M. Interleukin-4 gene polymorphisms in type 2 diabetic patients with nephropathy. Iran J Kidney Dis 2010;4:302-6.
6. Biedermann T, Zimmermann S, Himmelrich H, Gumy A, Egeter O, Sakrauski AK, *et al.* IL-4 instructs TH1 responses and resistance to *Leishmania major* in susceptible BALB/c mice. Nat Immunol 2001;2:1054-60.
7. Noben-Trauth N. Susceptibility to *Leishmania major* infection in the absence of IL-4. Immunol Lett 2000;75:41-4.
8. Noben-Trauth N, Kropf P, Müller I. Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. Science 1996;271:987-90.
9. Gama ME, Costa JM, Pereira JC, Gomes CM, Corbett CE. Serum cytokine profile in the subclinical form of visceral leishmaniasis. Braz J Med Biol Res 2004;37:129-36.
10. Habibi GR, Khamesipour A, McMaster WR, Mahboudi F. Cytokine gene expression in healing and non-healing cases of cutaneous leishmaniasis in response to *in vitro* stimulation with recombinant gp63 using semi-quantitative RT-PCR. Scand J Immunol 2001;54:414-20.
11. Woitsch B, Carr D, Stachel D, Schmid I, Weiland SK, Fritzsch C, *et al.* A comprehensive analysis of interleukin-4 receptor polymorphisms and their association with atopy and IgE regulation in childhood. Int Arch Allergy Immunol 2004;135:319-24.
12. Mohebali M, Javadian E, Yaghoobi-Ershadi MR, Akhavan AA, Hajjaran H, Abaei MR. Characterization of *Leishmania* infection in rodents from endemic areas of the Islamic Republic of Iran. East Mediterr Health J 2004;10:591-9.
13. Oshaghi MA, Ravasan NM, Hide M, Javadian EA, Rassi Y, Sadraei J, *et al*. Phlebotomus perfiliewi transcaucasicus is circulating both *Leishmania donovani* and L. infantum in northwest Iran. Exp Parasitol 2009;123:218-25.
14. Oshaghi MA, Ravasan NM, Javadian EA, Mohebali M, Hajjaran H, Zare Z, *et al.* Vector incrimination of sand flies in the most important visceral leishmaniasis focus in Iran. Am J Trop Med Hyg 2009;81:572-7.
15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
16. Bhattacharya P, Ali N. Involvement and interactions of different immune cells and their cytokines in human visceral leishmaniasis. Rev Soc Bras Med Trop 2013;46:128-34.
17. Costa AS, Costa GC, Aquino DM, Mendonça VR, Barral A, Barral-Netto M, *et al.* Cytokines and visceral leishmaniasis: A comparison of plasma cytokine profiles between the clinical forms of visceral leishmaniasis. Mem Inst Oswaldo Cruz 2012;107:735-9.
18. Rai AK, Thakur CP, Singh A, Seth T, Srivastava SK, Singh P, *et al.* Regulatory T cells suppress T cell activation at the pathologic site of human visceral leishmaniasis. PLoS One 2012;7:e31551.
19. Mohamed HS, Ibrahim ME, Miller EN, Peacock CS, Khalil EA, Cordell HJ, *et al.* Genetic susceptibility to visceral leishmaniasis in The Sudan: Linkage and association with IL4 and IFNGR1. Genes Immun 2003;4:351-5.
20. Cabantous S, Poudiougou B, Oumar AA, Traore A, Barry A, Vitte J, *et al.* Genetic evidence for the aggravation of *Plasmodium falciparum* malaria by interleukin 4. J Infect Dis 2009;200:1530-9.
21. Vafa M, Maiga B, Berzins K, Hayano M, Bereczky S, Dolo A, *et al.* Associations between the IL-4 -590 T allele and *Plasmodium falciparum* infection prevalence in asymptomatic Fulani of Mali. Microbes Infect 2007;9:1043-8.
22. Flórez O, Martín J, González CI. Interleukin 4, interleukin 4 receptor-a and interleukin 10 gene polymorphisms in Chagas disease. Parasite Immunol 2011;33:506-11.