

Original Article

Evaluation of interleukin 17: A polymorphism in patients with visceral leishmaniasis

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Abstract

Introduction: Visceral leishmaniasis (VL) is an infectious disease that it has spread in more than 60 countries, in Iran it is caused by *Leishmania infantum*. The infection is transmitted by sand flies, often affects children under 10 years of age, and it can be fatal if no treatment is undertaken. The progression of leishmaniasis infection in the host depends on cellular immunity. Studies have associated immune responses against leishmaniasis to host genotype, resistance due to Th1 and T CD8+ cells, and responses leading to macrophage activation and parasite killing. The parasite can stimulate the production of interleukin 17A (IL-17A) by Th17. It was shown that IL-17A is strongly and independently associated with protection against VL. Moreover, it seems that IL-17A has a complementary role in human protection against VL.

Methods: Blood samples were collected from 259 people among whom 88 were patients with history and clinical symptoms of leishmaniasis and 171 were healthy controls with no signs of infection. All participants were residents in an endemic area of VL in east Azerbaijan province, Iran. DNA extraction was performed using salting out method. Then, the control group was divided into two groups of seropositive and seronegative by IFA (indirect fluorescent antibody) test, and for detecting polymorphism of IL-17A (197A/G) ARMS-PCR (Amplification refractory mutation system-Polymerase chain reaction) was used.

Results: The result showed that the G allele was more frequent than the A allele among the groups, but this difference was not statistically significant ($P = 0.8$). In addition, the GG genotype was more frequent than genotype A/G and A/A among the groups, but this difference was not statistically significant ($P = 0.7$).

Conclusion: On the basis of the results, there was no significant association between VL and polymorphism of IL-17A (197A/G).

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Introduction

The interleukin 17 (IL-17) family is the most newly described subclass of cytokines.¹ Until now, the IL-17 family has six members including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F, and there are five IL-17 receptors including IL-17RA, IL-17RB/

IL-25R, IL-17RC, IL-17RD/SEF, and IL-17RE.² Originally, IL-17A (from now on, referred to as IL-17) was thought to be produced totally by T cells, but it is now known to be secreted by a range of innate cells including dendritic cells (DCs), macrophages, natural killer (NK), natural killer T (NKT), and $\gamma\delta$ -T cells.^{3,4} A key

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progress in this field occurred with the recognition that IL-17-producing CD4⁺ T cells arise as a population, distinct from the classic T helper (Th) type 1 and Th2 cells.⁵⁻⁷ While it was known for decades that IL-12 induces Th1 cells [interferon (IFN)- γ producers] and IL-4 induces Th2 cells (IL-4, IL-5, and IL-13 producers), recently it was confirmed that Th17 cells differentiate upon exposure to combinations of IL-1, IL-6, and transforming growth factor (TGF)- β .⁸

Visceral leishmaniasis (VL) is an infectious disease caused by *Leishmania infantum* (*L. infantum*) in Iran and it has become endemic in some regions in Iran such as east Azerbaijan province.^{9,10} These protozoan parasites evade the immune system, penetrate the macrophages, proliferate in phagolysosomes, and spread infection in hosts.¹¹ The infection is transmitted by sand flies, often affects children under 10 years of age, and can be fatal in children with no treatment.^{12,13} Infected children die due to the multiplication of leishmania in liver and spleen, associated with splenomegaly, pancytopenia, anemia.¹⁴

Studies have shown that immune responses against leishmaniasis are associated with host genotype and resistance due to TH1 and T CD8⁺ cells.¹⁵ Pitta et al. reported that *L. donovani* highly stimulated production of IL-22 and IL-17 and these cytokines play a complementary role in protection against *L. donovani*.¹⁶ They also showed that *L. donovani* stimulated the proliferation of TH17, and TH17 produced IL-17 and IL-22.¹⁶ Another study has showed that IL-17 plays an important role in intracellular pathogens; for example, pulmonary infection in mice with the intracellular bacterium *Francisella* (*F.*) *tularensis* induced production of IL-17 by Th17 cells.¹⁷ Accordingly, the absence of IL-17 pathway in mice resulted in increased susceptibility to pulmonary tularemia and correlated with decreased Th1 responses.¹⁸

The mechanism by which IL-17 regulates the Th1 pathway appears to be via induction

of IL-12 and IFN γ in APCs (Antigen Presenting Cells). Following IL-17 stimulation, both DCs and macrophages produced IL-12 and IFN γ , and regulated downstream immune responses.¹⁷ For example, IL-17-dependent-DC-derived IL-12 was able to drive the differentiation of naive T cells into Th1 cells, while IL-17-dependent macrophage-derived IFN γ was able to activate macrophages for control of intracellular *Francisella*.¹⁸

IL-22 and IL-17, accompanied with immune system cells, can influence other tissues and are associated with T cell-related chronic inflammatory diseases, such as psoriasis, Crohn's disease, and rheumatoid arthritis.¹⁹⁻²¹

According to recent studies, genetic variations may have an important role in people's sensitivity to and resistance against *L. infantum*, which is the most common cause of VL in northwestern Iran, because most people in endemic regions of leishmaniasis did not suffer from infections.²²

The human IL-17A gene is composed of three exons (two introns) and located on chromosome 6p12 which is composed of 4252 and rs1974226 SNP (single nucleotide polymorphism) is located on 3 exon in the 3'UTR region. Since the 3'UTR is involved in regulation of gene expression, such as mRNA stability and/or degradation, and translation efficiency, a potential mechanism of the rs1974226 effect is alteration of gene regulation.^{23,24} Alternatively, other SNPs in high linkage disequilibrium with this SNP may have a biological function.

Methods

The study population consisted of 259 participants, 88 of whom had history and symptoms of leishmaniasis and 171 were healthy subjects. By using the IFA test (indirect fluorescent antibody) and due to presence of anti-leishmania antibodies, the controls were divided into two groups of seropositive and seronegative consisting of 88 and 83 participants, respectively. All the participants were residences of the endemic region of VL.

From each subject, 2 ml of blood sample was taken and dropped in a sterile Falcon containing 2 mg/ml EDTA (Ethylenediaminetetraacetic acid).

DNA was extracted by salting out method; then ARMS-PCR (Amplification refractory mutation system-Polymerase chain reaction) method used, which is a suitable method for detecting mutations in single nucleotides, the subject's genotype was detected in 197A\G (SNP). The IL-17 reverse common primer (TCCAAAGCAAGAGCATC), IL-17 forward G (TTCCCATTTCCT TCAGAAGG), and IL-17 forward A (TTCCCATTTCCTTCAGAAGA) were used. The sequence of forward control was ACACAACTGTGTTT ACTAGC and of reverse control was CAACTTCATCCACGTTCCACC. The primers used in this study were obtained from Bioneer Inc., Daejeon, Republic of Korea and Taq polymerase enzyme was obtained from PAK GENE YAKHTEH Co. Ltd., Islamic Republic of Iran. PCR products were electrophoresed on 2% agarose gel, and then presence or absence of allele-related bands were assessed in comparison with controls by transilluminator.

Allele and genotype frequencies were calculated on patient and control subjects by direct gene counting. The statistical analysis of the differences between groups was performed by χ^2 test using SPSS for Windows (version 16;

SPSS Inc., Chicago, IL, USA). All P values less than 0.05 were considered significant.

Results

The results showed that the G allele was more frequent than the A allele among the study groups (83.0%), but this difference was not statistically significant ($P = 0.8$). In the patient group, 76 individuals (33.7%) bore the G allele. Furthermore, G allele frequency in the seropositive and seronegative groups was 34.8% (76) of individuals and 31.5% (71) of individuals, respectively (Figure 1). The AG genotype was more frequent (44.4%) than genotype G/G and A/A in the study groups, but this difference was not statistically significant ($P = 0.7$). In the patients group, 43 individuals (37.0%) bore the A/G heterozygote form. In addition, in this group, the frequency of A/A and G/G forms were 35.0% (12) and 30.0% (34), respectively. In the seropositive group, 38 individuals (33.0 %) bore the A/G heterozygote form, and 10 (30.0%) and 40 (36.5 %) bore A/A and G/G forms, respectively. In the seronegative group, the frequency of A/G heterozygote form, and A/A and G/G homozygote forms were 30.0% (34), 35.0% (12), and 33.5% (37), respectively. Figure 2 shows the frequency of alleles among the study groups (patients, seropositive, and seronegative group).

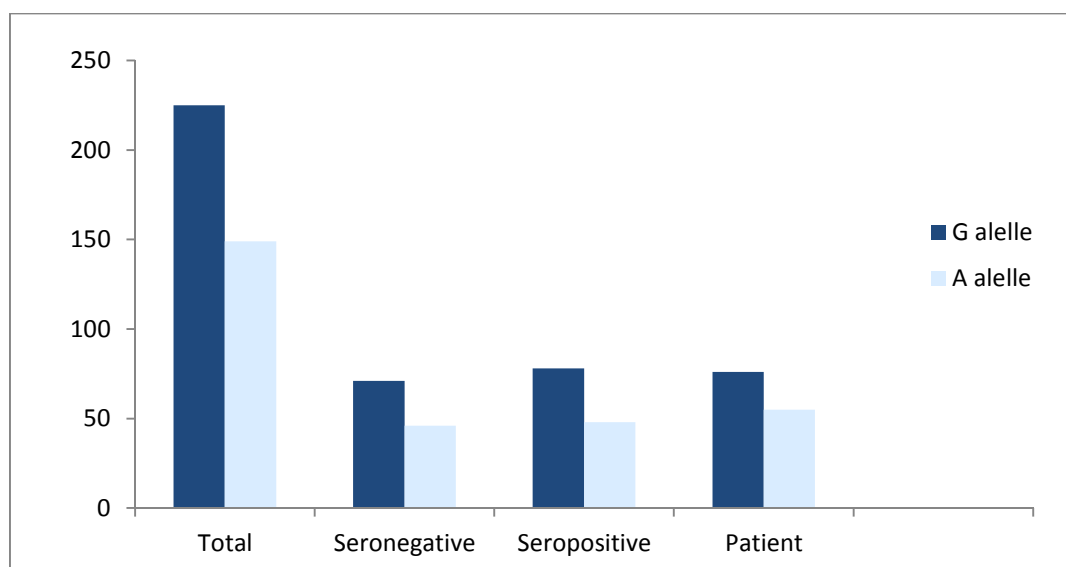


Figure 1. Frequency of alleles among groups

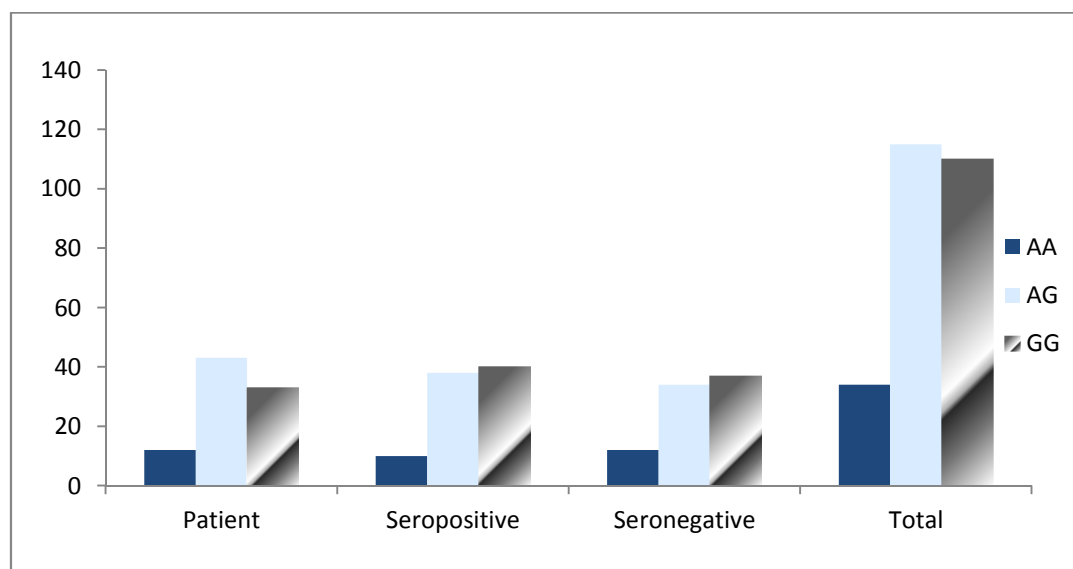


Figure 2. Frequency of genotypes among groups

Discussion

VL is an infectious disease caused by *L. infantum*. These protozoan parasites evade the immune system, penetrate the macrophages, proliferate in phagolysosomes, and spread infection in hosts.¹¹ Since the parasite causes an intracellular infection, the effective immune response against it is mediated by cell-mediated immunity with a focus on macrophage activation by IFN- γ production. On the subject of the leishmaniasis, it seems that Th1/Th2 cytokine balance may be involved in the resistance or susceptibility to leishmaniasis infection. In this respect, Th1 cytokines confer resistance, while Th2 cytokines predispose leishmaniasis.²⁵ Therefore, each cytokine influencing this balance can affect the outcome of the infection with leishmaniasis. One of these cytokines is IL-17, representing an important link between the innate and adaptive immune responses. It was found that murine $\gamma\delta$ T cells are the major producers of IL-17 during the infection with leishmaniasis. It has also been clarified that IL-17 is required for the induction of IFN- γ and IL-12 in macrophages and DCs. Thus, it seems that IL-17 can affect the induction of Th1 immunity which is necessary for controlling leishmaniasis.

Pitta et al. reported that *L. donovani*

highly stimulated the production of IL-22 and IL-17 and these cytokines play a complementary role in protection against *L. donovani*.¹⁶ They also showed that *L. donovani* stimulated proliferation of TH17 and TH17 produced IL-17 and IL-22.¹⁶

We could not find any study on IL-17 gene polymorphisms and leishmaniasis. It was impossible to compare our results with that of other studies, but there are several studies on IL-17 gene variants and some diseases that the mechanism of the immune response to them as *Leishmania* is mediated through Th1 pathway. Rasouli et al. examined the association between polymorphisms of IL-17 and disease brucellosis. In their study, 176 patients with brucellosis and 84 healthy animals, who consumed contaminated raw milk and dairy products from animals with brucellosis, were included.²⁶ All individuals were genotyped for 9 SNPs (rs4711998AG, rs8193036CT, rs3819024AG, rs2275913AG, rs3819025AG, rs8193038AG, rs3804513AT) selected using NCBI SNP database and literature using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The distribution of IL-17 rs4711998, rs8193038, and rs3748067 AA genotypes, and AAGAA haplotype was significantly more frequent in the patients than in the controls. However, IL-17

genotypes rs3819024GG and rs3819025AA were more frequent in the controls than the patients.

Based on the results, IL-17 rs4711998, rs8193038, and rs3748067 AA genotypes and AAGAA haplotype could be considered as susceptibility factors for brucellosis while the inheritance of IL-17 rs3819024GG and rs3819025AA genotypes might be resistance factors against the disease.²⁶ In another study, Ocejó-Vinyals et al. examined the role of IL-17 polymorphisms in region rs1974226, and susceptibility to infection with gram-positive bacteria. In their study, 697 patients and 517 control subjects participated. Their results showed that the frequency of G allele and genotype GG in patients were more than controls, and this difference was statistically significant.²⁷

Wu et al. assessed the association between IL-17A G197A and IL-17F A7488G polymorphisms and risk of gastric cancer. They demonstrated that polymorphism of IL-17A G197A and IL-17F A7488G increased susceptibility to gastric cancer.²⁸

Conclusions

The G allele was more frequent than the A allele in the study groups, but there was no significant difference in units distribution among the different groups. Moreover, the A allele distribution among groups was almost identical. GG genotype was more frequent than genotype A/G and A/A among the groups, but this difference was not statistically significant. According to the results, we conclude that there is no direct correlation between IL-17 polymorphisms and susceptibility to VL.

Conflict of Interests

Authors have no conflict of interest.

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