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Evaluation of IL-22 polymorphism in patients with visceral leishmaniasis

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ABSTRACT

Objective: To examine the IL-22 gene polymorphism in visceral leishmaniasis (VL) patients in comparison to healthy people as the control group.**Methods:** The blood samples were collected from 236 people, among them 74 were patients with a history and clinical symptoms of leishmaniasis and 162 were healthy with no signs of infection and all of them were residents in endemic area of VL in East Azerbaijan Province. DNA extraction was performed by using salting out method and amplification refractory mutation system-polymerase chain reaction was used for detecting the polymorphism of IL-22 promoter (*rs2227473A/G*).**Results:** In patients group, 9 (12.16%) individuals showed A/A genotype and 29 (39.18%) showed G/G genotype. The heterozygote form (A/G) was 36 (48.64%) among this group. In the control group, the proportions of genotypes were 8.02%, 46.09% and 45.06%, respectively.**Conclusions:** According to our results, there was no significant association between VL and polymorphism of IL-22 gene promoter (*rs2227473A/G*).

1. Introduction

Visceral leishmaniasis (VL) is an infectious disease that caused by *Leishmania infantum* in Iran and it is endemic in some regions in Iran such as East Azerbaijan Province[1,2]. Leishmaniasis, a vector-borne disease that is caused by obligate intra-macrophage protozoa, is endemic in large areas of the tropics, subtropics and the Mediterranean basin. It is caused by more than 20 leishmanial species and is transmitted to humans by ~30 different species of Phlebotomine sandflies[3]. VL is a zoonosis of worldwide distribution and mainly affects populations in developing countries. VL is a systemic disease caused by the *Leishmania donovani* (*L. donovani*) complex and it will be fatal if left untreated[3].

VL is a chronic systemic disease that is characterized by persisting fever, weight loss, asthenia, adynamia and anemia, among other clinical manifestations. The disease is fatal when untreated, with death generally occurring one to two years after the onset of clinical manifestations[4]. VL (kala azar) is common in 62 countries with an estimated annual incidence of 500 000[5]. There is an estimated of 500 000 new cases of VL and more than 50 000 deaths from the disease each year[6]. The majority (>90%) of cases occurs in just six countries: Bangladesh, India, Nepal, Sudan, Ethiopia and Brazil. Migration, lack of control measures and HIV-VL co-infection are the three main factors driving the increased incidence of VL[7,8]. The parasites disseminate through the lymphatic and vascular systems and infect other monocytes and macrophages in the reticulo-endothelial system, resulting in infiltration of the bone marrow, hepato-splenomegaly and sometimes enlarged lymph nodes[6]. These protozoan

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parasites evaded from immune system, penetrated the macrophages, proliferated in phagolysosomes and caused the spread of infection in hosts[9]. The host specific cell-mediated immune response has an important role in controlling the infection. In VL patients, the inability to control *L. donovani* infection is associated with a profound T cell unresponsiveness to *L. donovani* antigens and the production of interleukin[10,11].

The infection is transmitted by sandflies and it often affects children under 10 years old, and it can be fatal in children without treatment[12,13]. Infected children die due to the multiplication of *Leishmania* in liver and spleen, associated with splenomegaly, pancytopenia, anemia, disseminated hemorrhages[14]. Studies have shown that immune responses against leishmaniasis is associated with host genotype and resistance occurred by TH1 and T CD8⁺ cell[15]. IL-22 and IL-17, accompanied with immune system cell, can influence other tissues and are associated with T cell related chronic inflammatory diseases such as: psoriasis, Chron's disease and rheumatoid arthritis[16–18].

Recent studies suggested that IL-22 that produced by TH17 and NK cell has an important role in protection against mycobacterial infections, in which pathogenicity is similar to that of leishmaniasis[19,20].

In vivo studies have shown that IL-22 can directly affect human macrophages and enhanced immune cell functions against mycobacterial infections[21].

In another study, it has been suggested that IL-22 could prevent the intracellular growth of mycobacterial by facilitating phagolysosomal adherence, the mechanism that has an important role in defense of NK cell against *Mycobacterium tuberculosis* (*M. tuberculosis*)[20,21]. Association of *rs2227473A/G* polymorphism in IL-22 gene-promoter with resistance against tuberculosis has been reported in Chinese populations[22]. Pitta *et al.* reported that *L. donovani* highly stimulated production of IL-22 and IL-17 and these cytokines have complementary role in protection against *L. donovani*[23]. They also showed that *L. donovani* stimulated proliferation of TH17 and TH17 produced IL-17 and IL-22[23]. In another study, the association of single nucleotide polymorphism (SNP) in IL-22 gene with the risk of colon cancer has been shown[24].

According to the last studies, genetic variations may have an important role in people's sensitivity and resistance against *Leishmania infantum*, the main cause of VL in northwestern of Iran, because most of the people in endemic regions of leishmaniasis didn't suffer from

infections[24].

Since previous studies have shown that *rs2227473A/G* (SNP) polymorphism can influence the IL-22 gene expression and people's genetic variations influence the type and severity of immune responses against infections with pathogenicity similar to leishmaniasis such as tuberculosis[22], possibly these variations may have important role in leishmaniasis. So we conducted this study to evaluate the association of *rs2227473A/G* polymorphism and VL.

2. Materials and methods

2.1. Study population and sampling

The study population consisted of 236 cases, among them 74 were patients with a history and symptom of leishmaniasis and 162 people were healthy as the control group recruited between July 210 and March 2011. Each participant gave a blood sample that was placed into a tube containing 2 mg/mL anticoagulant ethylene diamine tetraacetic acid for immune-fluorescence assay (IFA) test and genetic analysis.

2.2. IFA

Cases suspected of being infected by *Leishmania* spp. were selected by the IFA technique, with those having IFA titers greater than 1:40 considered seropositive.

2.3. DNA extraction

DNA was extracted by using the salting out method. The DNA was eluted in polymerase chain reaction-grade (PCR-grade) water and adjusted to a final concentration of 10 ng/ μ L with NanoDrop ND-1000 spectrophotometer. For further PCR reactions, 5 μ L of each DNA sample was used. DNA extraction was successfully got in 100% of both cases and controls. Samples were genotyped using amplification refractory mutation system-polymerase chain reaction by reverse common primer (GAA GTA GGG ATG TAA ATA ATT GT) and IL-22 Forward *rs2227473G* (CAT ATA TGT TTT GAT ATA CGC ATG) and IL-22 Forward *rs2227473A* (CAT ATA ATG TTT TGA TAT ACG CAT A). The sequences of forward control were ACA CAA CTG TGT TCA CTA GC and for reverse control were CAA CTT CAT CCA CGT TCA CC. The used primers in this study

were from Bioneer (Korea) and *Taq* polymerase enzyme was from PAK GEN YAKHTEH Co. Ltd. (Iran). PCR products were electrophoresed on 2% agarose gel, and the alleles related bands were assessed in comparison with control by transilluminator (Uvitec, Germany).

2.4. Statistical analysis

For comparison between groups, the parametric data was done by ANOVA. The results on the A/G ratio were compared between groups using the Kruskal–Wallis test and Dunn's test ($P < 0.05$).

3. Results

Our results showed that IL–22 polymorphism was not significantly associated with VL. From our positive samples, 39.8% of them were females and 60.2% were males and there was a significant difference between sexes ($P < 0.05$).

Our results showed that in patient group 36 (48.64%) individuals showed A/G heterozygote form, and in this group the frequency of A/A and G/G were 9 (12.16%) and 29 (39.18%), respectively. In seropositive group 38 (42.69%) individuals showed A/G heterozygote form and 8 (8.98%) and 43 (48.13%) showed A/A and G/G forms. In the seronegative group, it was showed that the frequency of A/G, A/A and G/G homozygote forms was 35 (47.94%), 5 (6.75%) and 33 (44.59%), respectively. In control group, the frequency of A/G heterozygote, G/G and A/A forms was 45.06%, 46.91% and 8.02% respectively. These results showed that there was no significant association between IL–22 polymorphism and VL.

4. Discussion

IL–22 is a member of the IL–10 family with known roles in prompting production of IFN– γ and differentiation of type I T cells that has been associated with response to microbial infection and suggested as a potential link between inflammation and immune response and chronic diseases development^[11,12]. Many researchers have been reported that IL–10 plays an important role in *Leishmania*^[8]. Increased serum levels of IL–10 in Leishmaniasis patients were also reported by Neuber^[9].

There are not any formal studies about the role of IL–22 on different kinds of leishmaniasis. To the best of our knowledge, this is a unique study which examined IL–22 gene polymorphism in VL patients in comparison to healthy people.

IL–22 has shown ability as an important cytokine in defense against pathogens that have similar pathogenicity to leishmaniasis. The association of IL–22 polymorphism with these diseases has been assessed. In this study, we assessed the association of *rs2227473A/G* SNP with leishmaniasis. According to the results there is no significant association between leishmaniasis and *rs2227473A/G* polymorphism.

In patient group, the A/G heterozygote form was 48.64%, G/G and A/A forms were 39.18% and 12.16%, respectively. There is no acceptable data currently available to suggest why the IL–22 A/G genotype may be responsible for up–regulation of this cytokine or why patients with the A/G and G/G genotype would express more IL–22 than patients with A/A.

Zhang *et al.* showed the association of SNP in IL–22 promoter with sensitivity to *M. tuberculosis*^[22]. They studied SNPs that had functional role in IL–22 gene promoter and influenced on transcription factor in ligand– receptor attachment site^[14]. The relevance between IL–22 polymorphism and resistance to *M. tuberculosis* has been demonstrated in Chinese populations. They demonstrated that an allele in this polymorphism led to sever expression of IL–22 and host resistance to this infection^[14]. But in our study, there are not any significant differences between A allele and VL.

Interestingly, the public National Center of Biotechnology Information SNP database includes several polymorphisms in the gene for the IL–22 receptor, making this gene an attractive candidate for further studies on the susceptibility to chronic diseases. Further studies are necessary to conclude potential interactions between genes involved in the etiology of this disease.

Conflict of interest statement

We declare that we have no conflict of interest.

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