Research Article

Role of ICAM1 Gene in the improvement or Spreading Wounds Caused by *Leishmania major*

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Abstract

Background: Leishmaniasis is a common infectious zoonosis disease, which is caused by different species of Leishmania genus. The disease is transmitted by sandflies, which infects mononuclear phagocytes in the human host of Leishmania as an intracellular parasite. Depending on the type of parasite and host immune response, the disease will have different clinical manifestations so that the symptoms subclinical infections and self-healing cutaneous occlusion, diffuse and progressive skin occlusion, and mucous membranes, and the entire reticuloendothelial system vary. The main response of the body to resist this infection is cell-mediated immunity. **Materials and methods**: The study was performed on 44 patients who had previously been diagnosed with Leishmania Major by sampling from the wound and carrying out PCR test. In Addition, 10cc blood was taken from 25 health people. The samples were isolated and cultured using the Ficclean gradient method. Then, RNA was isolated from them in two steps before and after induction with PHA, and converted to cDNA. The ICAM1 gene expression rate of RTPCR was measured and compared in both patient and control groups. **Results**: The 44 patients were evaluated, 64% of which were males and 36% were females. The average age of these individuals was 37.6 years old. The highest wound rates were observed in people's hands. The difference in expression of ICAM1 gene before and after induction with PHA was significant in patients with cutaneous leishmaniasis compared to the control group by Mann-Whitney test. **Conclusion**: The current study expresses that immunity in leishmaniasis is dependent on induction of the immune system and the presence of multiple wounds in the body causes faster and more severe immune responses and stronger immunity to the disease. This study also describes the role of Th1 in dermatological leishmaniasis. Accordingly, the expression of the ICAM1 gene in people with cutaneous leishmaniasis would indicate an increase in the activity of neutrophils in the infection site and an increase in inflammation, which can also contribute to the spread of Th1 immune response, and can also lead to parasite limitation, by increasing inflammation.

Keywords: *Leishmaniasis,* ICAM1, PHA induction

Introduction

Leishmaniasis is one of the most important infectious and parasitic diseases in the world, which is transmitted to the vertebrate host through sandflies (Jorjani et al., 1987). Leishmaniasis is a major health problem around the world, with two million new cases of global disease occurring every year, and about one in ten of the world's population are at risk of infection (Khan & Muneeb 2005; Roberts et al., 2000). According to the World Health Organization (2014), about 3.1 to 7 million new cases of cutaneous leishmaniasis occur in the world. More than 95% of American, Mediterranean, the Middle East, and Central Asia countries are infected with leishmaniasis. More than two-thirds of the cases occur in six countries including Afghanistan, Algeria, Brazil, Colombia, Iran, and Syria (Leishmaniases ECotCot, 2014). In Iran, visceral and cutaneous leishmaniasis (dry and wet) is one of the major diseases. According to the reports of the Center for Disease Management, the number of patients with different types of leishmaniasis in our country is 20,000 people per year, which is likely to be more than this number. Cutaneous leishmaniasis is one of the major health problems in 15 provinces of 31 provinces in the country (Ershadi et al., 2005). The history of this disease in Iran is

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long seen in most parts of Iran. Since dry occlusions are recovered after about one year without treatment, they are referred to as Salak in Persian (Saibi, 2009). The life cycle of Leishmania consists of two stages and two different hosts, namely, vertebral and invertebrate. Invertebrate host, which is the carrier of the disease, is sandfly. The female sandfly needs to alternately be blooded for laying eggs and growing their eggs. They swallow some of the parasite-infected macrophages that are present in the skin and blood when sucking patients' blood. These cells are torn in the midgut of the sandfly and the Leishman bodies are released. These bodies change slightly and become long and spindle shaped in the form of Proma Stegot. The parasite grows up in the sandfly's midgut and clings to its mucosa by its flagellum. The leptomonads then migrate to the anterior part of the stomach and then esophagus, pharynx, and appendages of the insect, and then stay there until the next bloodstream to enter the host's skin (skin histocytes). They occasionally cause midgut and throat obstruction in the sandfly's nest. This period takes about 7 to 10 days in the sandfly. If such an infected sandfly bites a person, it injects some of these flagellate forms into the host's skin combined with its saliva. These promosiguts are phagocytosed by macromammag cells and lose their flagellum within the cytoplasm and become round and small in the form of amastigotes. Thus, transmitting and evolving the parasite is done. They are enclosed in the alienated cells amastigotes within the parasitophorus vacuoles and then, they fix their position. Amasitguts prevent the activity of the lysosomal enzyme that attaches to the vacuole. They resist the lysosomal enzymes and begin to grow inside vacuoles. Then they are divided in non-sexual form. As a result of the proliferation and spread of the parasite, the infected macrophage is torn and releases a large number of amphibians. Then again, each amastigote invades another macrophage (Belding, 1965; Macdonald et al., 1995). The disease carrier is the phlebotomus species in the ancient world and Lutzomyia in the modern world, which vary according to the type of leishmaniasis of the reservoir or reservoirs. For example, the reservoir Leishmania tropica or urban type leishmaniasis is a human and rarely a dog. The reservoir in the rural type Leishmania Major is large rodents. Gerbil rats and the species of Rumombis opium are the reservoirs in Iran.

The main response of the body to resist infection with leishmaniasis is cell mediated immunity. In this type of immunity, the cells of TH1 and the resulting cytokines (especially IFN-γ) play a major role in activating macrophages and enhancing their destructive ability (Lieke et al., 2011). To the same extent, the cooperation of macrophage cells and NK cell (essential immunity) is important in preventing the onset of leishmaniasis (Carvalho et al., 2012; Farah et al., 1975). Acquired immunity plays a very important role in the pathology of intracellular microorganisms. This type of immunity in leishmaniasis is important due to the involvement of T cells and their subgroups (Rouse, 2005). Immunity against leishmania is associated with the TH1 response, and if these cells are down-regulated, the leishmaniasis appears. In other words, the disease is characterized by destructive cutaneous occlusions, which are mediated by the immune system, which may be due to inadequate immune regulation (Khattri et al., 2003). Typically, T helper cells are divided into TH1, TH2, and TH17 groups according to the cytokines they produce (Liyanage et al., 2002). Among various cytokines, the ICAM1 (Intercellular Adhesion Molecule 1) genes are involved in the activation of Th1 cells in preventing the spread of leishmaniasis (De Trez et al., 2009). In this study, the extent of the expression of this gene and its relationship with the disease were examined.

Materials and methods

In this study, 44 patients with suspected cutaneous leishmaniasis who were referred to a Salak lab of the Shohadaye Valfajar in Shiraz in the second half of 2014 were sampled by bistoury from under the wound and extracting tissue containing macrophages. After staining with Giemsa, the lambs were examined with a magnification of 100 to see the parasite. If bodies with leishmania were seen inside and outside the macrophages, patients were included in the study. Then, 8 to 10ml of blood was taken from the patient and transferred to EDTA tubes. Microscopic examination of the lambs was confirmed by extraction of the DNA of the secretions on the lam using the Ge Net Bio kit, and the PCR test was performed to detect Leishmania Major.

The Buffy Coat layer containing lymphocytes was slowly removed from blood samples taken from patients with Ficus gradient method. The 250µl of lymphocytes were extracted in order to extract RNA in 750 µl of Tripure solution and in a freezer at -70 °C. 250 µl of other tubular lymphocytes were used to culture stimulate cells.

Cell culture materials were transferred to a cell plate under fully sterile conditions under the hood and as much as intake (with a ratio of: Newborn Blood serum 1500 Landa, antibiotic 150 Landa, L-glutamine 150 Landa and RPMI1640 13200 Landa) and 250 Landa of lymphocyte was also added. Then, PH2 µ2 was added to each well in order to stimulate the cells and placed in a co2 incubator (humidity 98%, temperature 37°C, CO2 0.05%) for 6 hours. After 6 hours, the plate was removed from the incubator. 1

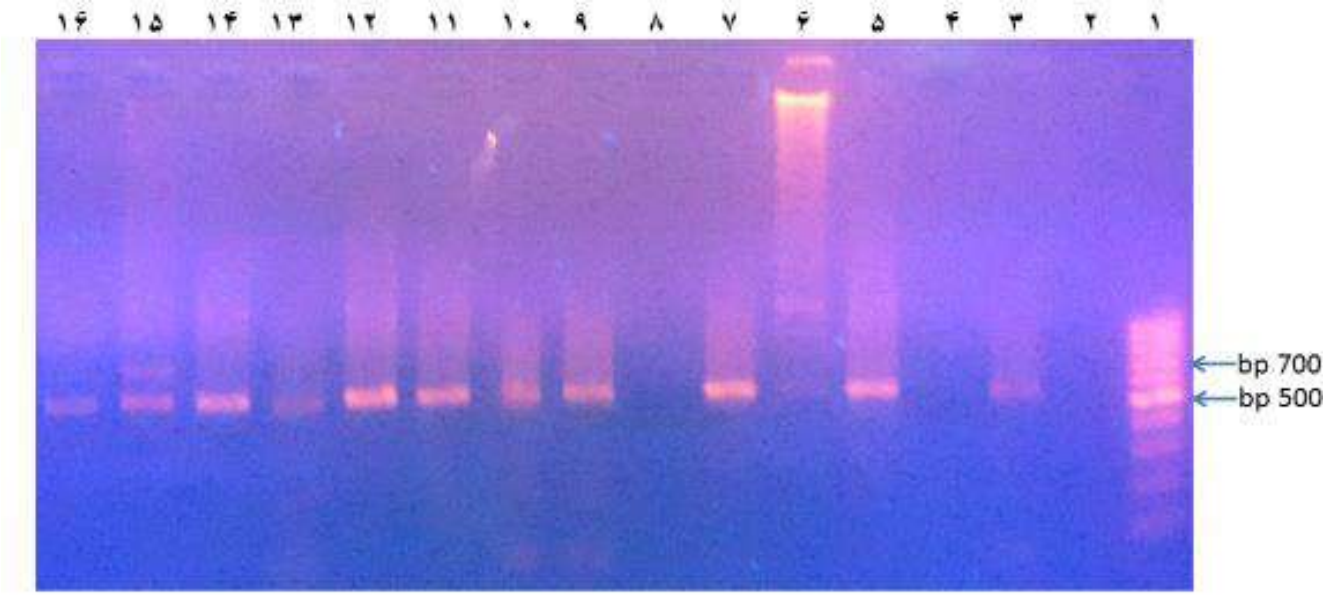

Figure 1. An example of electrophoresis results of the PCR product of the Leishmania kDNA gene related to a number of isolated samples from patients with cutaneous leishmaniasis from endemic areas. The first column in the figure shows that 1kb DNA ladder gel to estimate the size of the PCR product bands. No. 2 Negative Control No. 3 Positive Control of Leishmania Major and Nos. 4 to 16 are Patient Samples, which, apart from the two negative samples (4 and 8) and a sample of contamination of the mix (No. 15), produced the rest of the band of Leishmania Major band.

ml of its contents were transferred to a 1.5 µm microtipoil and centrifuged with a micro-centrifuge machine for 10 minutes at a rotational speed of 2600 rpm. The 500µl was removed from the supernatant and again centrifuged at the same time for another 10 minutes. Then, 250 µl of the supernatant were removed and the remaining 750µl of the Tripure was added and microtips were slowly shaken and placed in a freezer at -70°C.

The extraction of RNA by Tripure was carried out according to the instructions of the German company of Roche. Then, a BIONEER Accu Power Cycle Scrript PreMix (dN6) cDNA (dN6) was used in South Korea according to its protocol for the synthesis of cDNA. 11 µl of RNA and 9µl of distilled DEPC water were poured into the cDNA synthesis microtips and inserted into the ThermoCycler device. After microtiops were introduced from ThermoCycler, the ratio 1 to 2 was diluted with sterile distilled water.

Table 1. Materials used in Real time PCR

Concentration of solutions	Consumed volume for a reaction (in microliter)	Consumed volume for 120 reaction (in microliter)
Forward primer 10 pmol	4 0	48
Revers primer 10 pmol	4 0	48
Master mix	5	600
Sterile distilled water	2 2	264
cDNA 100ng	2	240
Total volume	10	1200

In the present study, TaqMan Real time PCR was used to examine the expression of the ICAM1 gene. Also, beta 2 microglobulins were also used as reference gene for analyzing the results along with them.

The 0.1 ml of the device, 8µl of the reaction mixture and 2µl of the cDNA of the sample were added inside each micro machine. Using the comparison method of two standard curves (dividing the values obtained from the ICAM1 curve as the studied genes on the values of the beta-DMG gene as normalizer), the real mean values of target genes in each cell were obtained.

Results and discussion

Totally, 44 patients were studied, of which 64% were males and 36% were females. The mean age of these subjects was 37.6 years old. In the study, the Mann-Whitney test showed that there was no significant difference in sex ratio between patient and control groups (P = 0.976).

Table 2. ICAM1 gene expression before PHA induction in the group of patients with cutaneous leishmaniasis and control group in the study "The role of ICAM1 gene in the process of improving or spreading wounds caused by leishmania major"

Groups	Number	Mean	Standard deviation	Minimum	Maximum	Test Result
ICAM1 Patient	44	3.41	3.08	00 0	11.36	P=0.013
ICAM1 Healthy	25	1.3	1.53	00 0	5.27	

According to the data in table 2, there is a significant difference between the expression level of ICAM1 before induction with PHA in patients with cutaneous leishmaniasis compared with the control group using Mann-Whitney test (P = 0.013).

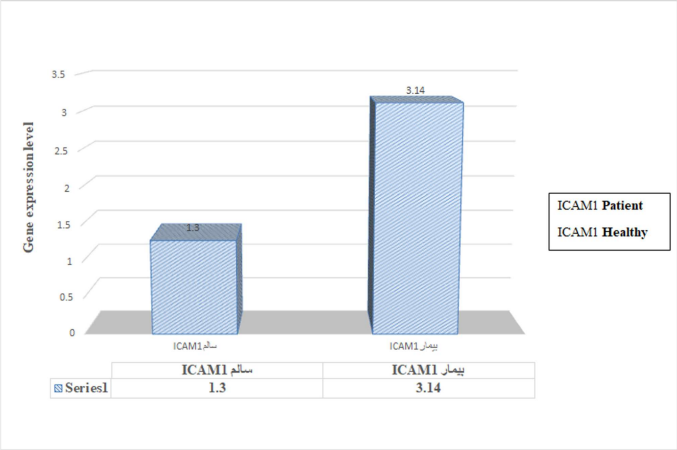

Figure 2. ICAM1 gene expression before PHA induction in the group of patients with cutaneous leishmaniasis and control group in the study "The role of ICAM1 gene in the process of improving or spreading wounds caused by leishmania major"

According to the data in Table 3, there is a significant difference between the expression level of ICAM1 after induction with PHA in patients with cutaneous leishmaniasis compared to the control group by Mann-Whitney test (P = 0.002).

Table 3. ICAM1 gene expression after PHA induction in the group of patients with cutaneous leishmaniasis and control group in the study "The role of ICAM1 gene in the process of improving or spreading wounds caused by leishmania major"

Groups	Number	Mean	Standard deviation	Minimum	Maximum	Test Result
ICAM1 Patient	44	30.6	51.5	0.03	251.1	P=0.002
ICAM1 Healthy	25	1.50	2.2	0.01	8.76	

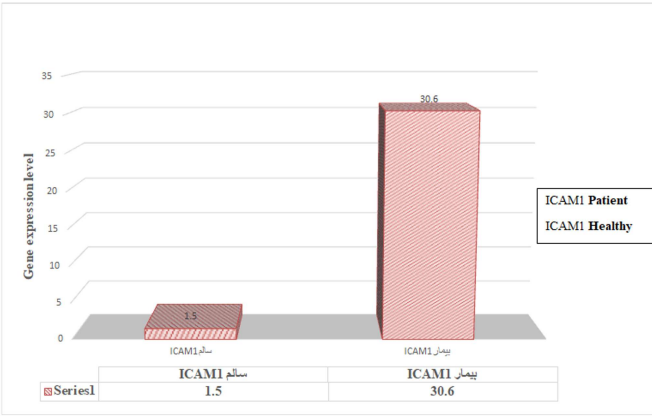

Figure 3. ICAM1 gene expression after PHA induction in the group of patients with cutaneous leishmaniasis and control group in the study "The role of ICAM1 gene in the process of improving or spreading wounds caused by leishmania major"

According to the calculations, the comparison of the ICAM1 gene expression level after induction with PHA was significant based on the occlusion site by Wilcoxon test (P = 0.001).

Table 4. Comparison of ICAM1 gene expression level based on the occlusion site in the study "The role of ICAM1 gene in the process of improving or spreading wounds caused by leishmania major"

Groups	Number	Mean	Standard deviation	Minimum	Maximum	Test Result
ICAM1 Patient	44	30.6	51.5	0.03	251.1	P=0.001
Wound	85	1.9	0.9	1	5	

According to the calculations, the comparison of the ICAM1 gene expression level before induction with PHA was not significant based on the occlusion site by Wilcoxon test (P = 0.352).

The highest wound rates were observed in people's hands, but according to the calculations and analyzing the two groups with Mann-Withny test, it was concluded that there was no significant difference between the different groups in terms of the wound site (P > 0.05).

Conclusion

Comparing the level of ICAM1 gene expression in the body of people with leishmaniasis and healthy subjects in this study revealed that the mean of ICAM1 gene expression in patients with major Leishmaniasis is significantly higher than healthy subjects. According to Mann-Whitney test, this increase was significant. Comparison of the level of ICAM1 gene expression according to the occlusion site indicated that ICAM1 gene expression level was correlated with the occlusion site and this correlation was significant, but by further analysis (pairwise) with the Mann-Whitney test, this correlation was not attributed to a specific group. ICAM1 gene expression in people with cutaneous leishmaniasis suggests an increase in the activity of Th1 cells and the release of pro-inflammatory cytokines, resulting in the absorption of neutrophils to the occlusion site in response to the disease. The immune system of people who have multiple wounds in their bodies responds more quickly and responsibly than single wounds. Following severe occlusion, the wounds improve sooner because immunity to leishmaniasis depends on the induction of the immune system, and the presence of multiple wounds in the body causes a faster and more severe immune response and a stronger immunity to the disease. In general, it can be concluded that ICAM1 gene expression

would be indicative of an increase in the activity of neutrophils in the site of infection and an increase in inflammation, which could also contribute to the expansion of the Th1 immune response, and it can also lead to parasite limitation by increasing inflammation.

Conflicts of interest: Nil

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