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Original Article

The level of IL-12, IL-10, IL-4, and MMP-13 in cutaneous leishmaniasis patients

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Abstract

Background: Cutaneous leishmaniasis (CL) is a dermatological condition that arises due to the presence of protozoan parasites. The disease disproportionately impacts those residing in economically disadvantaged regions and is closely linked to factors such as inadequate nutrition, population displacement, substandard living conditions, and compromised immune systems. **Objectives:** Investigating the most important immunological changes associated with infection in patients with CL by measuring the levels of some cellular kinetics interleukins-12 (IL-12), IL-10, IL-4, and human matrix metalloproteinase-13 (MMP-13). **Materials and Methods:** This study involved a sample that included 90 individuals, comprising 20 control subjects and 70 patients diagnosed with CL. The age distribution of the participants in the study spanned from 3 months to 65 years, encompassing individuals of both genders. The sample consisted of 50 females and 40 males. The amounts of IL-12, IL-10, IL-4, and MMP-13 were quantified via an enzyme-linked immunosorbent assay (ELISA) test. **Results:** The current study showed a significant increase in the level of IL-12 in patients with CL (461.5 ± 14.8) compared to the control group (321.6 ± 57.9) at probability 0.05, whereas non-significant differences in levels of IL-10 and MMP-13 in patients with CL (143.5 ± 6.8) (5.7 ± 0.2) compared with the control group (198.0 ± 35.2) (6.9 ± 0.6) at $P \leq 0.05$, respectively. As well as a highly significant decrease in the level of IL-4 in patients with CL (15.5 ± 1.5) compared to the control group (46.3 ± 5.7) at $P \leq 0.05$. **Conclusion:** There was an increasing serum of IL-12 and decreased IL-4 in patients with CL compared with control, while non-significant decreases in the level of MMP-13 and IL-10 between patients with CL and control.

Keywords: cutaneous leishmaniasis, IL-10, IL-12, IL-4, human matrix metalloproteinase-13 (MMP-13)

Introduction

Leishmaniasis (CL) is a major health issue in many regions of the world, with 0.7–1.2 million cases reported each year.[1] Human leishmaniasis is more frequent as cutaneous. This single-celled parasite causes a cutaneous illness when phlebotomine sand flies bite. About three Leishmania species can cause cutaneous leishmaniasis.[2] Cutaneous leishmaniasis in Iraq is caused by two distinct kinds, namely *Leishmania tropica* and *Leishmania major*. They have a similar life cycle but different clinical symptoms and hosts. The eastern wart, Baghdad seed, and Aleppo seed are all names for cutaneous leishmaniasis, which varies with the host's response and interaction.[2,3] The skin is a vital component of the immune system, serving as an organ that comprises many types of immune cells, including macrophages and T cells. Keratinocytes play a role in innate immune responses through the secretion of cytokines and chemokines, as well as their involvement in antigen presentation.[4] The immune response to the Leishmania parasite is carried out through cellular immunity mediated by T helper lymphocyte CD4+, which can be divided into two types according to the cellular kinetics that are secreted into T-helper cell type 1 and T-helper cell type 2, where the host body's resistance to the parasite depends on the cellular immunity of the first type helper T lymphocytes, while the susceptibility

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to infection depends on the secretions of the second type T cells.[5] IL-12 is a major interleukin in regulating T-cell responses, and lack of IL-12 production increases vulnerability to *L. major* infection. IL-10 suppresses the cellular immune response and produces inflammatory cytokines (TNF-α, IFN) that allow the parasite to survive at the infection site. IL-4 is a crucial factor in the process of Th0 cell development into Th2 cells. Its production is mostly attributed to Th2 cells, mast cells, and basophils. Additionally, it has been observed that it diminishes the synthesis of chemical substances that attract Th1 cells to the location of the infection.[6-9] Human matrix metalloproteinases (MMPs) are a group of enzymes that rely on zinc for their catalytic activity. These enzymes are responsible for the degradation of proteins in the extracellular matrix. The production of MMP is found to be elevated in patients suffering from mucosal leishmaniasis, which is the more severe form of *Leishmania braziliensis* infection.[7,8,10]

Materials and Methods

Study period and sample

The research period began to collect samples from September 2022 to March 2023. Ninety samples were collected from people of different ages and genders, males and females, and ages ranging from 3 months to 65 years. The parasite was diagnosed clinically by a dermatologist. A sample was taken from each person for a special questionnaire that included several information, including the location of the pustule, the number of pustules, and their size, shape, and texture, where 70 samples were taken from patients and 20 healthy groups.

Collecting blood samples

Five milliliters of venous blood were taken using a sterile and dry medical syringe from every person infected with cutaneous leishmaniasis from some hospitals and in Salah Al-Din Governorate, where the blood sample was placed in a gel tube, and the tubes were left at room temperature for 15–20 min to allow the blood to coagulate and then placed in a centrifuge at 3000 revolutions per minute for 10 min to extract the serum, then transfer the acquired serum into Eppendorf tubes, to perform different tests, and store it at 20°C until the immunological test was performed. Examine all samples at one time to avoid repeated freezing and thawing, which may affect the quality of the results.

Clinical diagnosis

Clinical diagnosis was performed by examining ulcers of 90 samples of patients with fresh skin lesions. The ulcer was diagnosed by a dermatologist at the consulting clinic of Sam Salah al-Din Hospitals, where the clinical symptoms and the stages of ulcer development and some epidemiological criteria were observed.

Microscopic examination by direct smear

The infection was diagnosed using a direct smear according to the method described,[11] whereby a sample was obtained from the edge of an ulcer for detecting amastigote stages of the parasite in fixed phagocytic cells in skin tissue. The smear method was carried out as follows:

1. The skin ulcer was cleaned with ethyl alcohol 70%. The dermal fluid sample was drawn from the edge of the ulcer with a medical syringe with a capacity of 1 mL, then it was placed on the tip of a clean glass slide for the purpose of making a thin swab, and the swab was left to dry at room temperature.
2. The dermal fluid smear was stained using Giemsa stain, as the slides were immersed in 70% methyl alcohol for 3 min, left to dry at room temperature, then dipped in Giemsa dye and left for 30 min.
3. The slides were transferred to tap water to wash off the excess layer and shorten it, with the latter being replaced three to four times. Slides were left to dry at room temperature.
4. The slides were examined by a light microscope and by using an oil lens at a magnification of 1000 to detect the amastigote stage of the parasite, and the sample was considered negative if no such stage was found. It must be pointed out that he had done three replicates for one sample.

Immunological test

Multiple tests were incorporated, with a particular focus on immunological assays encompassing IL-12, IL-10, IL-4, and MMP-13. A total of 70 blood samples were collected from patients diagnosed with CL, while an additional 20 blood samples were obtained from healthy individuals serving as controls.

Human IL-12 ELISA test

The ELISA method was employed to evaluate the serum concentrations. The measurement of IL-12 levels was conducted in accordance by using ELISA technique. The plate was pre-coated with an antibody specific to human IL-12. The sample is supplemented with IL-12 content. The concentration of human IL-12 exhibited a positive correlation with the emergence of color in the substrate solution. The cessation of the process is achieved by the introduction of an acidic stop solution, followed by the quantification of absorbance at a wavelength of 450 nm.

Human IL-10 ELISA test

The serum concentrations were evaluated by ELISA. The plate was pre-coated with an antibody specific to human IL-10. The IL-10 concentration of the sample is introduced. The concentration of human IL-10 exhibited a positive correlation with the emergence of color in the substrate solution. The process is halted by the introduction

of an acidic stop solution, after which the absorbance is quantified at a wavelength of 450 nm.

Human IL-4 ELISA test

The concentration of IL-14 was determined using ELISA. The plate has been pre-coated with a human anti-IL-4 antibody. Upon the introduction of IL-4 into the sample, it forms a binding interaction with the antibodies that have been immobilized on the wells. Next, the immune complex is treated with a human biotinylated conjugated IL-4 antibody, which is subsequently introduced to the substrate. The process is terminated by the introduction of an acidic stop solution, after which the absorbance is quantified at a wavelength of 450 nm.

Human matrix metalloproteinase-13 (MMP-13) ELISA test

The serum concentrations were measured using ELISA. The plate was pre-coated with human MMP-13 antibody. Sample MMP-13 is added. The substrate solution's color coincided with human MMP-13 levels. An acidic stop solution stops the process and measures absorbance at 450 nm.

Analytical statistics

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS, IBM Company, Chicago, IL 60606, USA). The continuous variable format was mean ± SE. The link between categorical factors was examined. *P*-value ≤ 0.05 were significant.

Ethical approval

This study was conducted based on the ethical standards stipulated in the Declaration of Helsinki. Before taking the sample, The patient's informed written and verbal agreement was obtained after the review and approval of the study protocol and subject's information by the local ethics committee according to document number 12,948 (including the number and the date on September 6, 2022.

Results

Microscopic examination

The investigation found Amastigotes, small, spherical entities (2–4 µm in diameter) with indistinct cytoplasm, nucleus, and rod-shaped kinetoplast, as shown in Figure 1.

Serum IL-12 level in patients with CL and control groups

The study observed a significant increase in the average concentration of IL-12 in infected patients with CL (461.5) compared to the control group (321.6), as shown in Table 1 and Figure 2.

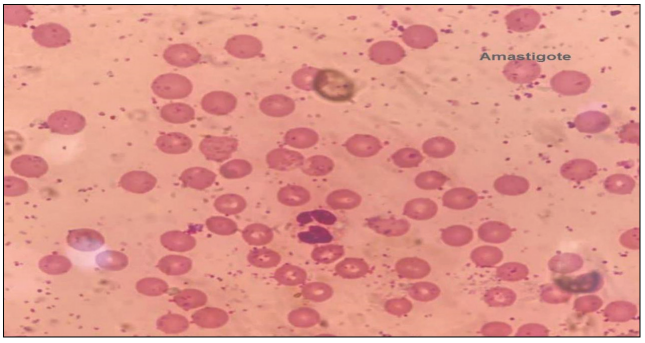

Figure 1: Amastigote stage stained by Giemsa stain 40x

Table 1: Serum IL-12 level in patients with CL and control groups

IL-12 Pg/mL	Patients *N* = 70	Control *N* = 20	*P* value
Mean ± S.E	461.5	321.6	**0.029****

** *P* value is significant at *P* ≤ 0.05

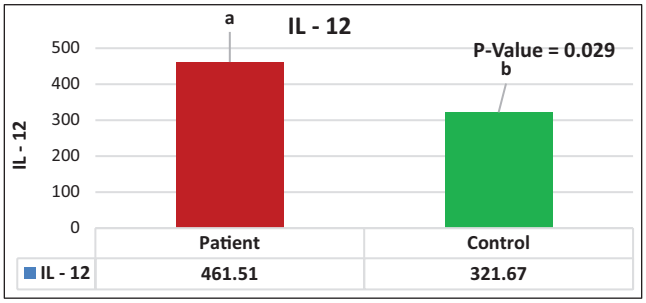

Figure 2: Serum IL-12 level in patients with CL and control groups

Table 2: Serum IL-10 level in patients with *L. cutaneous* and control group

IL-10 Pg/mL	Patients *N* = 70	Control *N* = 20	*P* value
Mean ± S.E	143.5 ± 6.8	198.0 ± 35.2	**0.144****

** *P* value is non-significant at *P* ≤ 0.05

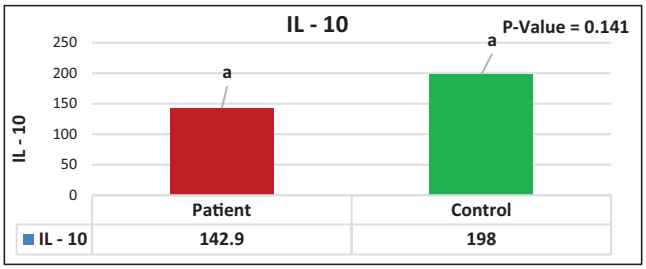

Figure 3: Serum IL-10 level in patients with CL and control groups

Serum IL-10 level in patients with CL and control groups

The study observed non-significant differences in the average concentration of IL-10 in infected patients with CL (143.5 ± 6.8 Pg/mL) compared to the control group (198.0 ± 35.2 Pg/mL), as shown in Table 2 and Figure 3.

Serum IL-4 level in patients with CL and control groups

The study observed a highly significant decrease in the average concentration of IL-4 in infected patients with CL (15.5 ± 1.5 Pg/mL) compared to the control group (46.3 ± 5.7 Pg/mL), as shown in Table 3 and Figure 4.

4-Serum MMP-13 level in patients with CL and control groups

The study observed no differences in the average concentration of MMP- 13 in infected patients with CL (5.6 ± 0.2 Pg/mL) compared to the control group (6.9 ± 0.6 Pg/mL), as shown in Table 4 and Figure 5.

Table 3: Serum IL-4 level in patients with CL and control groups

IL-4 Pg/mL	Patients *N* = 70	Control *N* = 20	*P* value
Mean ± S.E	15.5 ± 1.5	46.3 ± 5.7	**0.003****

**P* value is highly significant at $P \leq 0.05$

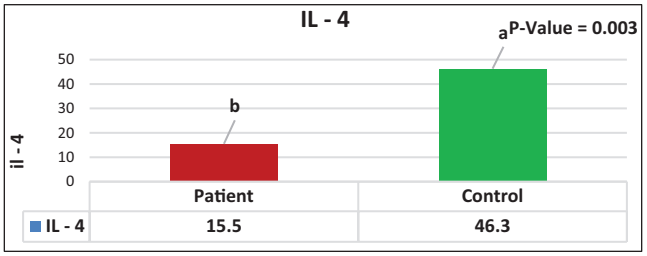

Figure 4: Serum IL-4 level in patients with CL and control groups

Table 4: Serum MMP-13 level in patients with CL and control groups

MMP-13 (Pg/mL)	Patients *N* = 70	Control *N* = 20	*P* value
Mean ± S.E	5.7 ± 0.2	6.9 ± 0.6	**0.111****

**P* value non-significant at $P \leq 0.05$

Discussion

During the initial stages of Leishmania parasite invasion, T cells and the cytokines play a pivotal role in determining the nature of the immune response and deciding the infection's outcome. The result of the current study was compatible with,[12] but it was incompatible with.[13]

IL-12 plays a crucial role as a cytokine in the process of Th1 cell development during leishmaniasis. The involvement of IL-12 in the establishment of the Th1 response is mediated through the production of IFN by natural killer cells and T cells, additionally inducing the development of naive T cells into Th1 effectors while concurrently suppressing the death of T cells that play a crucial role in their survival. The resistance to initial infection is influenced by the involvement of IL-12 in the differentiation of T cells into Th1 or Th2 immunological responses in CL.[6]

The result of the current study is not inconsistent with Dakheel.[14] IL-10 is an allergic and immunosuppressive factor in CL. It produces IL-10 and IL-4 in response to the parasite antigen, which plays an anti-IFN-γ production role, thus enhancing the susceptibility to infection.[15]

The parasite is killed by macrophages through the ability of these cells to produce (NO) with the help and stimulation of the enzyme (iNOS) and in addition to the activity of nitrogen oxide (NO), other oxidizing media are also produced by macrophages activated, as it enhances the ability to kill the parasite, macrophages cells are stimulated and activated to produce nitric oxide by IFN-γ. TNF-α then activates the ability of IL-4, IL-10 cells to produce macrophages and thus reduces and weakens the ability of those cells to kill and exterminate the parasite.[16]

The elevated level in patients with CL is due to inhibition of the T cell response.[17] CL activates the internal secretion of IL-10 as a mechanism to enhance the process of parasitism, as it inhibits the production of IFN-γ and thus facilitates the survival of the parasite inside the cells as well

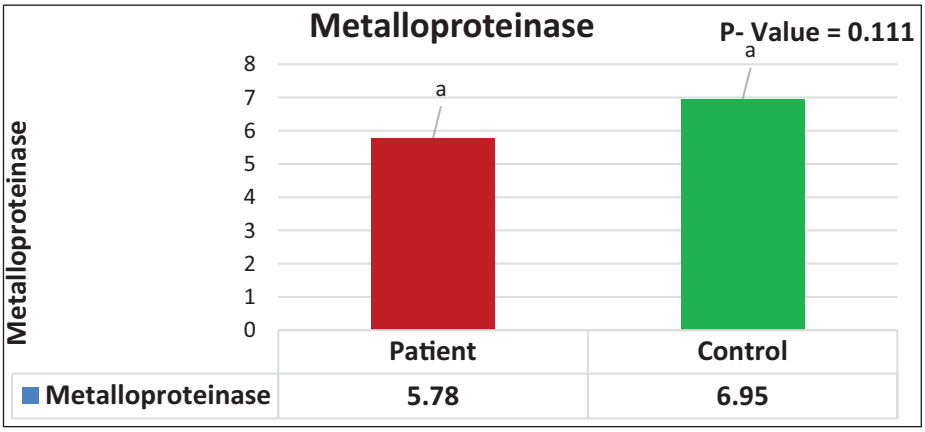

Figure 5: Serum MMP-13 level in patients with CL and control groups

as inhibits the oxidative and inflammatory response.[18] The increase in the level of IL-10 is closely associated with the severity and virulence of the Leishmania parasite, so it is possible to use specific antibodies that block the activity of IL-10 or block its receptors, which may be effective in the treatment of cutaneous leishmaniasis.[19] A lot of studies and research on the immune response in cutaneous leishmaniasis indicate the main role that plays a balance of response of Th1 and Th2 in the speed of healing or self-healing of ulcers or the survival and persistence of ulcers for a longer period, the prevalence of response TH2 in patients was associated with a high level of IL-10, IL-4, that the low-level IL-10 follows the treatment period while the high IFN-γ indicates the acute state of the disease.[20]

The result of the current study was incompatible with.[14] It is a potent cytokine and has a vital role in the host immune response in Leishmania infection. It also has a role in the adverse response observed after Leishmania infection by downregulating the expression of associated cytokines.[21] IL-4 plays a significant role as a cytokine in determining the vulnerability to Leishmania infection.[6]

The result of the current study on MMP was inconsistent with those of Campos *et al.*[10] The enzymes degrade the extracellular matrix, and high levels of this enzyme may cause tissue damage and lead to excessive deterioration of the basement membrane and the transfer of inflammatory cells to the site of infection and the development of ulcers. It has been shown that when infected with Leishmaniasis, human macrophages increase the secretion of the enzyme because it is a type IV component of collagen. The main basement membrane in the skin increases and causes excessive tissue damage through the migration of inflammatory cells to the site of injury.[10]

The increased and unregulated activity of these enzymes contributes to chronic inflammation and increased tissue destruction and may help pathogens enter the bloodstream and invade different tissues. The increase in MMP activity results from both increased enzyme secretion and decreased tissue inhibition of gastric protein secretion (TIMP), which leads to damage. Severe tissue and the spread of microbes to the host, leading to death. The enzyme is also a bad biomarker, which indicates a high risk of parasite spread. It has been documented that leukocytes are the main source of the enzyme.[22,23]

Conclusion

There was increasing serum of IL-12 and decreased IL-4 in patients with CL compared with control, while non-significant decreases in the level of MM-13 and IL-10 between patients with CL and control.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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