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Immune receptors and cytokines and their effect on the immune status of leishmaniasis patients

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ABSTRACT
The study samples were collected from Samarra General Hospital and external dermatology clinics. The number of studied samples was 60 samples infected with cutaneous leishmaniasis after clinical confirmation by a specialist physician and 30 samples from healthy individuals. The study period was from October 2022 to March 2023 and the results were as follows: For tumor necrosis factor alpha, the results in patients were (1.1923 + 0.0713) pg/ml, which is high when compared to healthy people (0.4831 + 0.0528) pg/ml. As for the level of interleukin-2, the results in patients were (0.908 + 0.258) pg/ml compared to healthy people (0.4259 + 0.0554) pg/ml. As for the levels of interleukin-4, the results in patients were (0.769 + 0.236) pg/ml compared to healthy people (0.5187 + 0.0950) pg/ml. As for the levels of interleukin-13, the results in patients were (1.132 + 0.173) pg/ml, while in people The healthy people had (0.4748 + 0.0776) pg/ml, while the results of Toll-2 receptors in the infected people were (0.700 + 0.184) pg/ml, which is a high percentage compared to healthy people (0.3660 + 0.0874) pg/ml. As for Toll-4 receptors, the results of the infected people were (0.960 + 0.226) pg/ml compared to healthy people (0.574 + 0.108) pg/ml.

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1-INTRODUCTION

Cutaneous leishmaniasis is one of the most widespread epidemics in the world, as annual reports indicate that more than one million people are infected with leishmaniasis (1). Infection with this parasite results in stimulating the immune response, as the host's resistance to the Leishmania parasite depends on the cellular immunity of the first type of helper T lymphocytes (Th1), while the susceptibility to infection with the parasite depends on the secretions of the second type of helper T lymphocytes (Th2). Some cellular kinetics are secreted by Th1 T cells, including IL-2, INF-γ, and TNF-α, which work to resist infection with the parasite. As for Th2 T cells, they secrete a group of cellular kinetics, including IL-4, IL-13, and IL-6, which work to limit the ability of the first helper cells to repel the parasite (2). There are also receptors that play a role in the innate immune response against the parasite, including TLR2 and TLR4 receptors, as these receptors work as antigen-presenting cells. (APCs) on the surfaces of macrophages, dendritic cells, natural killer cells and neutrophils, thus stimulating the immune system after binding to the parasite to kill it, as neutrophils are stimulated and attracted to the site of the bite by secreting some substances, including chemokines, cytokines and other molecules produced by the body. Natural killer cells are also stimulated mainly by cytokines, especially IL-2, IL-12, TNF-α and IFN

produced by the infected macrophage (3). The role of interleukins in the immune response against leishmaniasis is very important, as IL-4 activates T cells and stimulates B cells to produce antibodies, in addition to its effect in activating other types of cells such as mononuclear cells, epithelial cells, and fibroblasts (2). Interleukin-13 also stimulates the proliferation of B cells and the production of immunoglobulins (4). It is also required to stimulate the synthesis of IgE, especially when the level of IL-4 is low or absent. As for interleukin-2, its immune role is represented by enhancing the immune response of Th1 cells, which are characterized by pre-inflammatory cytokines such as IFN-γ, which enhances the activity of phagocytic cells against parasites (5). It was also indicated (6) in experimental models that this interleukin has a role in the formation of granulomas, which is a distinctive feature of the immune response against leishmaniasis, as these granules accumulate at the site of infection and stimulate the local immune response to kill The parasite, as well as tumor necrosis factor plays important roles in leishmaniasis infection, as it plays a crucial role in eliminating leishmania by increasing the activity of macrophages and the production of nitric oxide (NO), and has the ability to enhance Th1/IFN-γ responses against leishmania (7). As for toll-like receptors, they play a role in the body's defense against cutaneous leishmaniasis, as the link between TLR2 receptors and lipophosphoglycan (LPG) antigens occurs, through which alerts are sent to the immune system to stimulate specialized cells to eliminate the parasite. Studies conducted by researchers also indicate that TLRs receptors are linked to Leishmania molecules, and through them cytokines are stimulated that begin to stimulate and regulate the inflammatory response necessary to control the spread of the parasite and generate an adaptive immune response (8,9).

2-MATERIALS AND METHODS

(60) samples were collected from people infected with cutaneous leishmaniasis after being examined by a dermatologist at the consulting clinic of Samarra General Hospital and outpatient clinics for the period from October 2022 to March 2023, with (30) samples from healthy people. After confirming their infection, 5 ml of blood was drawn to conduct immune tests that include (IgG, IgM, IL-2, IL-4, IL-13, TNF-α, TLR2, TLR4 (and the samples were placed in blood separation tubes Gel tube and centrifuged at a speed of 3000 rpm for 3 minutes to obtain the serum. Then the serum was transferred to new white tubes and placed at a temperature of (20-) and used to conduct immune test.

ELISA assays:

This ELISA kit uses the Sandwich-ELISA principle. The ELISA microplate provided in this kit is pre-coated with an antibody specific for each test. Samples are added to the wells of the ELISA microplate and combined with the specific antibody, then the rest of the solutions are added according to the worksheet and the measurement is performed at a wavelength of ±450 nm.

3-RESULTS AND DISCUSSION

1-Tumor necrosis factor-α levels in people with cutaneous leishmaniasis:

The results of the current study to detect tumor necrosis factor alpha in patients with cutaneous leishmaniasis showed significant differences at the probability level ($P<0.05$), as the percentage in infected persons reached (1.1923+0.0713) pg/ml, and these numbers are statistically significant when compared with the percentage in healthy persons (0.4831+0.0528) pg/ml. Table (1) shows the results:

Table (1): Levels of the TNF-α in infected and healthy individuals in leishmaniasis

	TNF-α St.Dev± M	P-value
Infected people	0.0713± 1.1923	0.018
healthy people	0.0528± 0.4831	0.013

The results of our current study are consistent with many previous studies to detect the level of tumor necrosis factor-α, in which the level of this factor was high compared to healthy people, as (11) recorded an increase in the rate of tumor necrosis factor-α in people infected with cutaneous leishmaniasis compared to the control group, where the rate of infected people reached (0.103±0.201) pg/ml, and these results are consistent with (12), and also consistent with the results of researchers (10) where they recorded an increase in the level of tumor necrosis factor for people infected with cutaneous leishmaniasis compared to healthy people, and these results are consistent with what was indicated by (13) that infection with cutaneous leishmaniasis leads to an increase in the production of α-TNF, as its production increases by macrophages to get rid of the parasite, as they produce nitric oxide NO, which helps them in Removal of leishmaniasis, and tumor necrosis factor plays a role in enhancing the INF-γ\Th1 response, especially in L. major.

2- Interleukin-2 levels in people with cutaneous leishmaniasis:

The results of the current study of patients with cutaneous leishmaniasis showed significant differences at the probability level (P<0.05), as the percentage among infected persons reached (0.908 + 0.258) pg/ml, and these numbers are statistically significant when compared with the percentage among healthy persons (0.4259 + 0.0554) pg/ml, Table (2) shows the results:

Table (2): Levels of the IL-2 in infected and healthy individuals in leishmaniasis

	IL-2 St.Dev ± M	P-value
Infected people	0.258 ±0.908	0.064
healthy people	0.0554± 0.4259	0.014

The results of the study agree with what was reached by (14) when they indicated a significant increase in the level of Interleukin -2 in the case of infection with cutaneous leishmaniasis. The results also agree with what was reached by (15) who indicated an increase in this cytokines in those infected in endemic areas. The role played by this Interleukin in the case of parasitic infection is very important as its increase plays a strong role in the immune response by T cells Th1 that protect the body from parasitic infection. Also, low levels of it lead to the exacerbation of the disease, as it was found that some patients who had low levels of IL-2 and IFN-γ suffered from the onset and exacerbation of the disease (16). Also, the mechanism of protection in which this Interleukin participate is through activating phagocytic cells and improving their ability to swallow the parasite. Also, this Interleukins play a role with other cytokines such as TNF-α and IL-12 in creating an effective immune response, as high concentrations of this Interleukin give better clinical results in the case of infection with leishmaniasis (17).

3- Interleukin-4 levels in people with cutaneous leishmaniasis:

The results of the current study of patients with cutaneous leishmaniasis showed significant differences at the probability level (P<0.05) as the ratio in infected people reached (0.769 + 0.236) pg/ml and these numbers are statistically significant when compared with the ratio in healthy people (0.5187 + 0.0950) pg/ml, Table (3) shows the results:

Table (3): Levels of the IL-4 in infected and healthy individuals in leishmaniasis

	IL-4 St.Dev± M	P-value
Infected people	0.236 ± 0.769	0.059
healthy people	0.0950± 0.5187	0.024

The results of our study were consistent with the findings of (18), (19) and (20), where researchers indicated an increase in the concentration of (IL-4) when infected with the cutaneous leishmaniasis parasite, as the response of the helper T cells Th2 is stimulated, thus stimulating

the production of IL-4, where the humoral immune response is stimulated and stimulates B cells to differentiate and produce antibodies, and with it a decrease in the response of the helper T cells Th1 occurs (21). A study conducted by (22) confirms that IL-4 increases in the case of infection with the leishmania parasite and that it leaves an indirect effect in destroying the parasite as it stimulates the production of antibodies that in turn work to destroy the parasite. However, the results of the study differed from what was reached by (23) when they indicated a decrease in the level of cytokines-4 when infected with the cutaneous leishmaniasis parasite, as the occurrence of a cellular immune response, the production of IFN-y, an increase in the level of cytokines-12, and the occurrence of a response by helper T cells Th1 will inhibit the production of cytokines-4 and thus reduce the work of Th2.

4- Interleukin-13 levels in people with cutaneous leishmaniasis:

The results of the current study of patients with cutaneous leishmaniasis showed significant differences at the probability level (P<0.05), as the percentage among infected persons reached (1.132 + 0.173) pg/ml, and these numbers are statistically significant when compared with the percentage among healthy persons (0.4748 + 0.0776) pg/ml, Table (4) shows the results:

Table (4): Levels of the IL-13 in infected and healthy individuals in leishmaniasis

	IL-13 St.Dev± M	P-value
Infected people	0.173 ± 1.132	0.043
healthy people	0.0776± 0.4748	0.019

Interleukin -13 is elevated in cutaneous leishmaniasis infection due to stimulation of Th2 helper cells to produce Interleukin -13, which in turn stimulates the humoral immune response and stimulates B-cells to differentiate and produce antibodies (21). The results are consistent with what was reached by (24), (25), and (26), where the results of the tests conducted indicated an increase in the concentration of Interleukin in people infected with leishmaniasis, and are also consistent with the study of researchers (27) who indicated an increase in the level of interleukin-4 and 13 as a result of the cellular immune response against infection with the leishmaniasis parasite.

5- Toll-like receptor 2 (TLR2) levels in people with cutaneous leishmaniasis:

The results of the current study of patients with cutaneous leishmaniasis showed significant differences at the probability level (P<0.05), as the percentage among infected persons reached (0.700 + 0.184) pg/ml, and these numbers are statistically significant when compared with the percentage among healthy persons (0.3660 + 0.0874) pg/ml. Table (5) shows the results:

Table (5): Levels of the TLR2 in infected and healthy individuals in leishmaniasis

	TLR2 St.Dev± M	P-value
Infected people	0.184 ± 0.700	0.046
healthy people	0.0874± 0.3660	0.022

The results of the current study are consistent with what was reached by (28), who indicated an increase in TLR-2 receptors in people infected with cutaneous leishmaniasis, as this type of TLR receptors is the most elevated in infection and is secreted mainly by Macrophage. (29) also indicated that a decrease in the level of TLR-2 leads to an exacerbation of the disease due to the lack of association of LPG antigens on the surface of the parasite with TLR-2 receptors on the surface of NK cells and stimulating Th1 to differentiate and produce TNF-α and IFN-γ. (30) also indicated in a study he conducted that a deficiency in these receptors leads to an increase in the parasite and an exacerbation of the infection by activating the immunity of Th2 cells.

6- Toll-like receptor 4 (TLR4) levels in people with cutaneous leishmaniasis:

The results of the current study of patients with cutaneous leishmaniasis showed significant differences at the probability level (P<0.05), as the percentage among infected persons reached

(0.960 + 0.226) pg/ml, and these numbers are statistically significant when compared with the percentage among healthy persons (0.574 + 0.108) pg/ml. Table (6) shows the results:

Table (6): Levels of the TLR4 in infected and healthy individuals in leishmaniasis

	TLR4 St.Dev± M	P-value
Infected people	0.226 ± 0.960	0.056
healthy people	0.108± 0.574	0.027

A study conducted by (31) indicated an increase in TLR4 receptors in people infected with cutaneous leishmaniasis. The reason for the increase is due to the possibility of interference between TLR receptors and antigens on the surface of the parasite such as lipophosphoglycan (LPG), or the possibility of interference of signaling pathways within cells that lead to stimulating the production of receptors. (32) also explained that TLR4 and TLR2 receptors clearly interfere in the production of tumor necrosis factor, and thus neutralizing these receptors leads to a decrease in the production of these cytokines and affects the immune response and kills the parasite. (28) also indicated the possibility of linking the importance of TLR4 to alternative activation through the elastase enzyme in neutrophil cells in the early steps of cutaneous leishmaniasis.

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