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Original Research Article

A Study the Immunological Effects Infects with the *Leishmania tropica* Parasite in the Najaf Governorate

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Abstract: Blood samples were collected before treatment and after recovery from infection (healthy individuals and patients with cutaneous leishmaniasis). The research was then carried out at the Al-Amin Center for Advanced Biotechnology Research at the Al-Ataba Al-Alawiyya - Najaf. The aim was to evaluate some of the immune effects in people infected with *Leishmania tropica* parasites that cause cutaneous leishmaniasis. The results showed changes in the immunological markers of interleukin-8 (IL-8) and interleukin-6 (IL-6), and a significant increase in tumor necrosis factor kappa B (TNF-κB) levels in patients with cutaneous leishmaniasis. These levels were significantly higher in patients before treatment compared to the control group. Conversely, a significant decrease in IL-8, IL-6, and IFN-κB levels was observed after recovery, while no immunological changes in interferon gamma (IFN-κB) were recorded in the patients. The current study showed that immune changes are of great importance in controlling parasitic diseases, and that pentostam treatment has a significant effect on the humoral and cellular immune response to control individuals infected with cutaneous leishmaniasis.

Keywords: Leishmaniasis, Interleukin, Pentostam, Immune, Iraq.

INTRODUCTION

Leishmaniasis is a disease that poses a risk to humans, and the sandfly is the intermediate host that transmits this disease.. There are three main types: cutaneous leishmaniasis (*Leishmania tropica*), mucocutaneous leishmaniasis, and visceral leishmaniasis (Karimi and Abipour, 2015; Alemayehu and Alemayehu, 2017). *Leishmania major* and *Leishmania tropic* Leishmaniasis the most widespread parasite causing skin infections, leads to ulceration of exposed parts of the body at the sites of insect bites. The immunological and clinical effects differ between the two types of cutaneous leishmaniasis. It is estimated that 95% of infections are distributed across most regions of the world, including the Americas, the Mediterranean basin, Central Asia, and the Middle East (WHO, 2020). Human migration and deforestation have led to adaptations of this parasite to new vectors and other vertebrate hosts, causing an increase in leishmaniasis in recent years. (Ferro *et al*., 2011; WHO, 2020). Studies have shown that modern treatments with chemical compounds against leishmaniasis are not highly effective (Sundar *et al*., 2014). Pentostam is one of the most commonly used drugs against leishmaniasis and has a high cure rate. However, its use is associated with negative effects such as toxicity, increased failure rates, and long treatment periods. Additionally, it can sometimes lead to side effects in infected individuals, such as kidney or heart failure. Despite these risks, pentostam remains the preferred drug for treating leishmaniasis. (Mendonsa Filho *et al*., 2004). Other additives, such as amphotericin B, miltefosine, and pentamidine, also exist, but they produce side effects; however, they have a longer lifespan (Markle and Makhoul, 2004). The immune response of individuals infected with leishmaniasis is one of the main problems their bodies face due to the parasite's ability to evade and disrupt the immune response. This ability allows the parasite to persist for extended periods within the body, causing chronic inflammation. Studies have shown that the innate immune response is crucial, providing a rapid response against invasive pathogens and enhancing the development of adaptive immunity. Pathogen recognition receptors, which are activated during infection in cutaneous leishmaniasis, are also a key component. (Menberework, 2017; Gurung & Kanneganti, 2015).

MATERIALS AND METHODS

Five milliliters of blood were taken from each individual via venous blood sample. This sample consisted of individuals infected with cutaneous leishmaniasis before treatment, after treatment, and after complete recovery, as well as a control group of uninfected individuals. The blood was placed in sterile test tubes at 37°C for half an hour to coagulate. The tubes were then centrifuged at 250 revolutions per minute for 10 minutes. The serum was collected and placed in serological test tubes and stored at -20°C. Finally, blood cytokine levels (IL-6, IL-8, TNF-κB, and TINF-κB) were measured using an enzyme-linked immunosorbent assay (ELISA) sandwich method.

Table 1: Levels of cellular kinetics in study groups

Cellular Kinetics	Concentration of cellular kinetics pg/mL			p-value
	Control group N=20	N=60 Patient Group		
		Before treatment	post healing	
IL-6 pg/ml	0.55 ±0.03	0.68±0.31	0.44±0.22	0.012
IL-8 pg/ml	0.26±0.06	0.73±0.24	0.25±0.04	0.032
IFN-ϒ pg/ml	4.29±0.29	5.19±0.34	4.88±0.21	0.054
TNF-α pg/ml	0.47±0.23	0.73±0.4	0.55±0.38	0.03

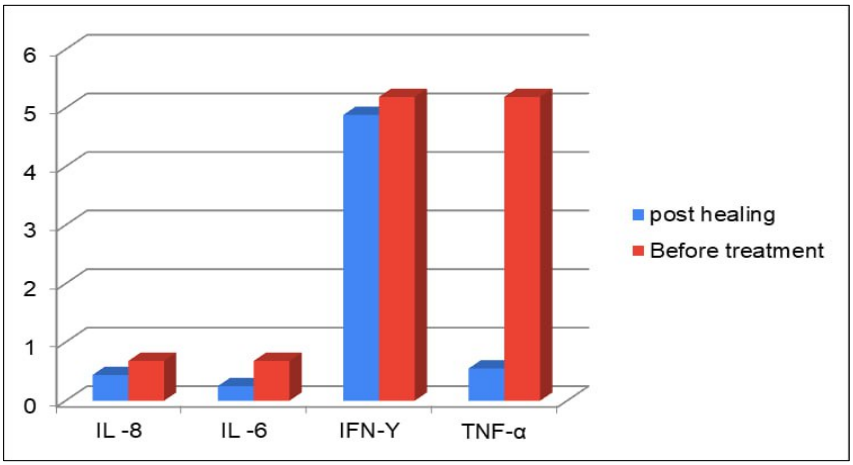

Figure 1: The form of immune standards for healthy and people with skin leishmania and after recovery

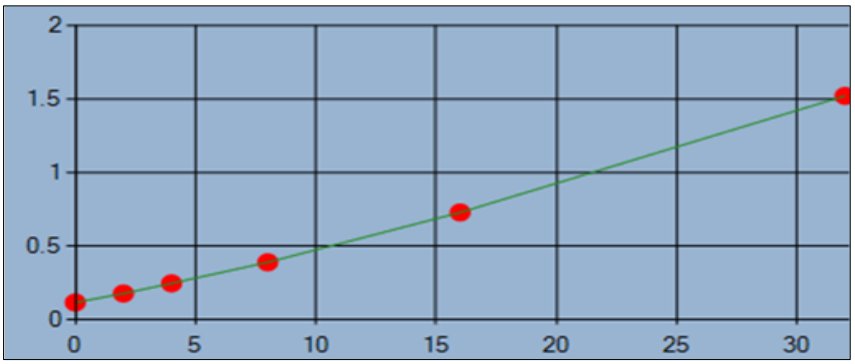

Figure 2: Showing the standard curve in immunostandards (Absorption 450nm)

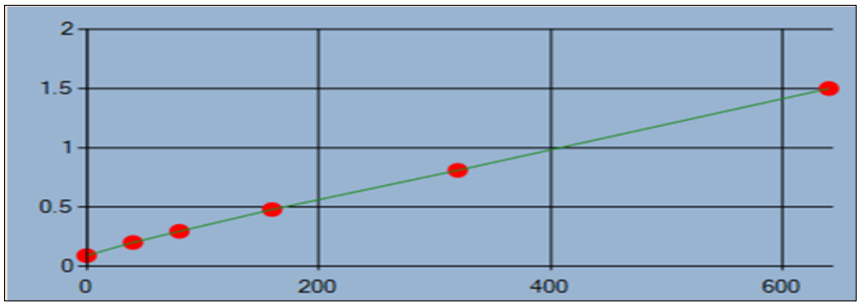

Figure 2: Showing the standard curve in immunostandards (Absorption 450nm)

RESULTS AND DISCUSSION

1. Interleukin-8 (IL-8)

As a result, we observed positive results for interleukin-8 in patients before treatment, showing a significant increase compared to the control group. Conversely, we observed a significant decrease in interleukin-8 levels after recovery. The pre-treatment group showed a significant increase of 0.68 ± 0.31 pg/ml compared to the control group, while we observed a significant decrease in interleukin-8 levels after recovery of 0.44 ± 0.22 pg/ml. These results are corroborated by Kumar *et al*., (2010), who demonstrated that pre-treatment interleukin levels in patients showed that IFN-γ and IL-10 mRNAs were expressed in 100% of the minutes, and that there was a significant correlation between them. It has paradoxical cytokine effects on the main network against intracellular pathogens, and it binds to IL-10, interfering with both TNF-α and IL-8 while also signaling TNF-α and IL-8. IL-8, also known as monocyte-derived neutrophil clearing factor, is a potent neutrophil chemokine and cytokine-activating factor. The levels of cytokines IFN-γ, TNF-α, IL-1, IL-8, IL-10, and IL-4 are rapidly dependent after treatment. Important contributing factors secreted by activated T cells mediate increased macrophage production of NO, leading to the killing or control of Leishmania comorbidity L. pintus SAG and IFN-γ with successful treatment of CL. MCP-1 induces L. major cell death by aggregate cells, and synergistically counteracts IFN-γ and inhibits IL-4 and IL-10, which are not produced by iNOS regulation. This was observed in a mouse model, suggesting that MCP-1 and NO are important components in the CL analysis in humans for *Leishmania tropica* (Santos, 2020). The study by Boussoffara *et al*., (2019) also failed to detect a significant increase in interleukin-8 levels and indicated that IL-8 mRNA was present at higher levels within ZCL lesions compared to SCL lesions. IL-8 is one of the oldest and most abundant chemicals produced during beneficial immune responses. It is primarily responsible for the apparent neutrophil recruitment, which could explain the large number of nuclei within ZCL lesions compared to SCL lesions. This inflammation led to early polarization of inflammatory monocytes and Leishmania cells in mice, and significant differences in the expression of interferon-gamma (IFN-γ) and interleukin-8 (IL-8) were observed. Age-related cutaneous leishmaniasis (ZCL) lesions are characterized by elevated IL-8 levels and a large number of multinucleated cells in the skin infiltrates. This study is consistent with the findings of Katara *et al*., (2013), who observed high levels of interferon-alpha (IFN-α), tumor necrosisfactor-alpha (TNF-α), monocyte attractant protein (MCP-1), and interleukins (IL-10, IL-1β, IL-8, and IL-4) in the local lesion tissue of patients with cutaneous leishmaniasis. This is consistent with histochemical reports showing that the lesion tissue in these patients was predominantly infiltrated by neutrophils and exhibited strong IL-8 expression. (Maspi *et al*., 2016). IL-8 is a cytokine that plays a key role in neutrophil recruitment and activation during its efforts, and in selecting the monthly or tissue damage. Scientists cannot find isolated IL-8 from individuals with high levels of IL-8 expression. IL-8 (a chemotactic neutrophil attractant) is involved in early defense against the Lysmania parasite in situ. In February, neutrophils play a different role in skin immune-stimulating discrimination, and represent Of the chemicals CXC, the second protein, (the macrophage inflammatory protein, and KC2 mouse homologs of IL-8) are rapidly produced by distinct cell types, which then act as chemotactic attractants for neutrophils and induce early neutrophilization, and neutrophil sorting with *L. major* Key high levels of IL-8 will increase neutrophil activity in order to increase parasite affinity, and by focusing on the programmed cells on their surface which they eliminate, reducing DC pressure leads to the suppression of Th1 cell and CD8+ T cell function and the role of death or survival of Leishmania parasites through neutrophil production.

2- Interleukin-6 (IL-6)

The current results for interleukin-6 (IL-6) showed an increase in IL-6 concentration in patients with cutaneous leishmaniasis before treatment, reaching 0.24 ± 0.73 pg/ml. This concentration decreased to 0.04 ± 0.25 pg/ml in the control group, compared to the control group. The results of this study are consistent with those of Raziuddin *et al*., (1994) studied two groups of leishmaniasis patients (males and females) aged 5–10 years and confirmed that serum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) in infected individuals did not show significant changes. This was attributed to a possible deficiency of IL-6 and IL-8 resulting from an imbalance in the production of granulocyte- and phagocytic-stimulated colony-forming factor (SCF) in cerebrospinal fluid (CSF-GM). Interleukin-6 and tumor necrosis factor-alpha (TNF-α) are considered key mediators of the pathophysiological changes associated with multisystem dysfunction syndrome and acute-phase protein responses in humans (Din *et al*., 2002). This includes the synthesis of interleukin-6 by multiple cell types, including macrophages, monocytes, B cells, and T cells, which regulate a pivotal aspect of the host's immune defense response (Lozer *et al*., 1998). It stimulates the liver to produce specific proteins, thereby promoting the proliferation of hematopoietic progenitor cells (Schmidt-Rosr-Arras and John, 2016). The beneficiaries of IL-6 include hematopoiesis, coagulation, and antibody production. Consequently, it may be associated with IL-6 and TNF-α production in distinct cutaneous leishmaniasis. Beneficial proteins, hypergammaglobulinemia, and fever induction have been reported (Narazaki and Kishimoto, 2017) in individuals with advanced leishmaniasis compared to control groups, and the immune system plays a role. Sufficient elements are naturally supplied for the parasite or bacterial product as a response, and these elements are differentiated into specific functional options by activation of M1 macrophages or M2 phagocytic cells. When M1 or type 1 macrophages encounter pathogens, they are capable of producing numerous phagocytic cytokines, as well as ROS and RNAs, which exhibit bactericidal activity against the organism. M2 macrophages, on the other hand, bind to incomplete cytokines and chemokines. Many M1 and M2 macrophages produce phagocytic cytokines such as IL-1,6-IL- and TNF-α, and secreted chemokines such as α-CCL3/MIP, CCL4/1, and MIP-β. Cytokines secreted by M1 macrophages attract

various immune cells, such as leukocytes and T cells, to the site of infection to eliminate the pathogen. Additionally, M1 macrophages initiate the differentiation of Th1 T cells. A study by Parslow *et al*., (2001) also revealed a significant increase in the number of people infected with cutaneous Leishmania parasites in Brazil, indicating that IL-6 is involved in coordinating the levels of this element in both cutaneous and mucosal lesions. It was also found that IL-6 independently influences the expression of matrix metalloprotein 1 (MMP1), a copper-containing element in macrophages. This mechanism is activated in ulcerated and damaged skin tissue, stimulating the production of MMPI enzymes, which are highly responsive to the breakdown of type I collagen. Subsequently, a proliferative remodeling occurs as keratinocytes migrate and lose their function in relation to the arrangement of type I collagen. This happens only in the presence of MMPI enzymes, except in skin folds, because these enzymes are capable of breaking down type I collagen and causing keratinocyte shedding. Furthermore, a study by Rodriguse *et al*., (2019) yielded similar results, finding elevated levels of interleukin-6 (IL-6) in mice infected with Leishmania. This suggests that immune cells recognize non-invasive forms of the parasite by detecting innate immune receptors (IL-6, IL-10, and TNF-α). Within hours of the parasite coming into contact with cancer cells, levels of cytokine 6 and interleukin-6 in blood cells increased significantly, along with the expression of tumor necrosis factor-alpha (TNF-α), which is produced by the parasite. This confirms that the presence of the parasite in liver cells strongly stimulates cytokine production.

3- Interferon Gamma (IFN-γ)

The study results regarding the immune variable interferon gamma showed no changes in interferon levels in patients before treatment compared to the control group. These parameters then returned to normal levels, registering 5.19 ± 0.34 pg/ml in patients before treatment compared to 4.29 ± 0.29 pg/ml in the control group. These parameters then returned to normal levels of 4.88 ± 0.21 pg/ml. Caldas *et al*., (2005) demonstrated that cutaneous leishmaniasis leads to abundant production of several cytokines, including IFN-β. IFN-β plays a largely accepted role in stimulating the activity of Leishmania macrophages. Its high serum levels during active CL are inconsistent with the large parasite burdens observed in this disease. IFN-β in the sera of CL patients did not exhibit the antiparasitic activity seen in the sera of tegumentary leishmaniasis. The reduced β-IFN activity may be related to the concurrent presence of elevated IL-10 levels, as IL-10 appears to be the primary cytokine inactivator in human leishmaniasis. The role of other cytokines capable of counteracting IFN-β activities, such as TGF-β, cannot be ruled out. The marked decrease in IL-10 levels after treatment coincides with the control of parasite growth, supporting an important role for this cytokine. In human CL. The study agreed with (Kima & Soong, 2013) that no significant differences were found for interferon gamma. Interferon gamma is not secreted in a vacuum. IL-10 and other Th2 cytokines exert critical effects on IFN-ϒ at the induction and response levels, which then determine the course of infection. Little is known about how IFN-ϒ and other pro-inflammatory chemokines are regulated in parasitic diseases. Cells infected with either flagellated or non-flagellated forms of the parasite are less responsive to stimuli such as LPS, which elicit the production of IFN-ϒ that activates the antiparasitic effector response of macrophages. Studies in mice with selective impairment of ϒ-IFN signaling in macrophage strain cells have clearly demonstrated the crucial role of IFN-ϒ activated macrophages in controlling primary parasitic infection in vivo. Studies evaluating these responses have used different readouts to assess cell activation. These readouts have included evidence of predicted gene transcription. The production of iNOS, evidence of intermediate signal translocation to the nucleus, and phosphorylation of signaling media in the cytosol all confirmed that Leishmania infection has a suppressive effect on the IFN-ϒ response. However, it remains unclear how and whether this reduced response in infected cells affects the infection pathway. Carneiro *et al*., (2016) did not report a significant difference, suggesting that IFN-ϒ promotes the production and activation of CXCL10 natural killer cells, leading to increased IFN-ϒ secretion. Natural killer cells may be the source of IFN-ϒ in the Leishmania response. Since we also detected IFNG mRNA and IFN-ϒ 72 hours after Leishmania stimulation, we propose that natural killer cells may play a role in the significant IFI27 activation by type I IFN, and that IFN-α stimulates IL-12 secretion, further enhancing IFN-γ production.

4- Tumor Necrosis Factor-α (TNF-α)

Our study recorded an increase in the value of this factor that affects the Leishmania cutaneous parasite. The study agreed with Singh *et al*., (2016) that there are elevated levels of broad necrosis factor in Leishmania cutaneous, as TNF-α is an important component in the control of intracellular pathogens, especially those infecting macrophages. It also suggested that TNF-α is essential for Leishmania scavenging but that alternative pathways exist. Furthermore, it proposed that IL-10 neutralization increases TNF-α production by SA cells, and that increased TNF-α could contribute to parasite killing. However, it remains to be seen whether TNF-α plays a direct role in controlling Leishmania parasite growth. Both IFN-γ and IL-10 are involved in regulating parasite proliferation in the splenic tissues of Leishmania patients. Therefore, since IFN-γ levels initially increased, subsequently joining TNF-α in promoting parasite growth, one possible explanation is that other cytokines may compensate for the reduced IFN-γ levels in this system, as both IL-17 and IL-10 are involved. IL-22 plays a role in better controlling Leishmania growth, as mice can eliminate parasites without signaling via TNF receptors. This has been found to be partly related to macrophage activation and NO production, suggesting that TNF-α is not strictly required for parasite containment. By activating TNF-α, T cells can suppress macrophages to prevent translocation and thus eliminate the Leishmania parasite, which may explain the sequence. Factors affecting new cell development show no effect on mutational growth in culture growth. Cunningham *et al*. (2014) attributed the differences

in TNF-α concentration to B1 cells, which contain B cells involved in mixed immune diversity and are not part of the adaptive immune system, as they lack memory. B1 cells are characterized by many roles other B cells play, including the production of antigen-resistant compounds. B1 cells are also effective at inducing TNF production through interaction with flagellar frontalis (Geraldo *et al*., 2016). Recent studies have shown that the protozoan stage of Leishmania parasites is capable of releasing extracellular vesicles, which stimulate bone marrow-derived phagocytic cells to increase the expression of interleukin-10 and interleukin-6. However, these vesicles exhibit a different effect on B-1 cells, increasing the expression of interleukin-6 and tumor necrosis factor-alpha (TNF-α) without affecting interleukin-10 (Barbosa *et al*., 2018). This study is consistent with the findings of Bosch-Nicolaou *et al*., (2019), both of whom found significantly elevated TNF-α levels, suggesting that an innate TNF-α-dependent mechanism stimulates cellular immunity by activating helper T cells (CD4+) and cytotoxic T cells (CD8+). TNF-α is known to play a crucial role in the primary control of infection, along with other cytokines such as interleukin-12 and interferon-gamma. These cytokines stimulate an effective response from type 1 helper T cells (Th1), thereby controlling the infection. Additionally, TNF-α and IFN-γ are responsible for activating the anti-leishmanial activity of phagocytic cells, characterized by increased production of free oxygen radicals and nitric oxide (NO), as well as inducing apoptosis in infected cells. According to the choice of expectations for healthy individuals and for leishmaniasis after recovery.

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