International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 2s, 2025
https://www.theaspd.com/ijes.php

Evaluation of TNF-α, IL-6, IL-10, IL-13, IL-17, aND IFN- γ Levels and Immune Responses with Cutaneous Leishmaniasis in Protection and Pathogenesis

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Background: *Cutaneous Leishmaniasis (CL) is one of the World's biggest and fastest-growing problems, especially in tropical areas. The World Health Organization (WHO) found that its ability to spread greatly affects disease distribution. Leishmaniasis is a disease caused by Leishmania spp. and is spread from one person to another by a vector, the sandfly. The study aimed to determine how the Leishmania parasite affects its host cell by measuring Tumor Necrosis Factor-alpha (TNF-α), interleukins (IL-6, IL-10, IL-13, IL-17), and Interferon-Gamma (IFN- γ).*

Materials and Methods: *Two hundred and twenty-five positive cases of Cutaneous Leishmaniasis (CL) and 100 samples of control group were collected from October 2021 to March 2022. They were diagnosed in the Hospital of the Babylon Governorate, Iraq. Giemsa stain was used to diagnose infective stage of Leishmania parasite. ELISA was used to measure the level of cytokines.*

The results: *showed that disease was present in 86 (38.22%) of the patients who lived in urban and in 139 (61.77%) of the patients who lived in rural areas, which is a significant difference ($P < 0.05$) between the two places of residence. The percentage of infection in males (58.22%) was more than in females (41.77%), and there were significant differences at the $p<0.05$ between males and females and in most age groups. However, most people admitted to hospitals with CL were males aged between 6-15 years, and the fewest were people aged under 50. Also,the results showed that men and women had different numbers of skin ulcers ($p<0.01$). More than 83 (58.04%) infected males had ulcers, and 41 (62.1%) had two ulcers. The chance of getting three or four ulcers was the same for both men and women. The results of serum levels were increased for TNF-α (46.53 pg/ml), IL-6 (9.94 pg/ml), IL-10 (115 pg/ml), IL-13 (58.08 pg/ml), IL-17 (58.14 pg/ml), and IFN-γ (38.23 pg/ml) in patients compared with the control.*

In conclusion: *Cutaneous Leishmaniasis infection prevalence in rural areas was more than in urban areas .The increasing levels of these interleukins, TNF-α, IFN-γ indicated a disturbance of the immune system during CL infections.*

Keywords: *Leishmaniasis, Cutaneous Leishmaniasis, Immunological parameters, Diagnosis, Parasite*

1. INTRODUCTION

Cutaneous Leishmaniasis (CL) is one of the World's biggest and fastest-growing problems, especially in tropical areas. The World Health Organization (WHO) found that its ability to spread greatly affects disease distribution (Hijawi et al. 2019, O'Neil et al. 1993). It is also one of the most dangerous and common diseases. This disease has many names, such as Baghdad boil, oriental sore, and Aleppo boils (Mandell et al. 1990). The names were derived according to the places where the disease was found.

Leishmania, a unicellular obligatory intracellular parasite, causes this tropical disease (Schmidt and Roberts 2005). It belongs to the Trypanosomatidae family and Leishmania genus. People can be infected with different types of CL. Their symptoms depend on the pathogen's spread in the body or skin tissues (Bari and Simeen 2008).L. tropica and L. major cause the disease in Iraq (Najim 1998), and their life cycles are similar to the other species of Leishmania (Schmidt and Roberts 2005). However, the clinical signs of the disease are different, as are the host's reaction to the parasitic infection and how much it interacts with it. The female Phlebotemous spp. is the vector that spreads the disease from one host to another (Paniker and Ghosh 2017).

Chemotherapy for leishmaniasis uses highly toxic chemicals like antimonials, which are injected into the veins or muscles of the patient and cause side effects. Therefore, the person must stay in the hospital and get medical care. When someone is treated with pentamidine, it can cause low blood pressure, kidney failure, infectious diseases, and terrible hives (Chan-Bacab and Pena-Rodriguez 2001).

Helper T cells type 1 (Th1) secrete cytokines that are important in resistance infection, including interferon-γ (IFN-γ) and tumor necrosis factor (TNF-α), to form Nitric Oxide (NO), which works to kill the intracellular amastigotes. Type II helper T cells secrete a variety of cytokines, including interleukin-10 (IL-10). Proinflammatory cytokines produce to amplify the immune response against Leishmania. The main proinflammatory cytokine includes IFN-γ, TNF-α, and IL-17, while anti-inflammatory cytokine represents immunoregulatory molecules that prevent the impacts of proinflammatory cytokines for limiting the inflammation due to increasing proinflammatory cytokines production and the main antiinflammatory cytokine include IL-10, and IL-13 as shown in Figure 1.1. (Dubie and Mohammed 2020). However, Th2 cells secrete stimulating B lymphocytes to produce immunoglobulins. Thus, these cells are more effective in humoral Immunity (Schmidt and Roberts 2005).

3. Materials and Methods

Between October 2021 and March 2022, 325 samples were collected (225 positive cases with cutaneous Leishmaniasis and 100 control groups without CL disease) who went to a government hospital in the Governorate of Babylon, Iraq, and its districts were included in this study. It was diagnosed Leishmania depending on the dermatologist and the type of lesion, whether it is dry or wet. Amastigote form was seen microscopically in the samples isolated from CL patients and isolated (Zahirnia et al.2018). A questionnaire form was used to collect information about each patient with CL, such as their gender, age (1-70 years), time of onset, place of residence, number of ulcers, and the location of the ulcers on the hand, face, and legs.

3.1. Sample Collection

The area around the lesion was cleaned with 70% ethyl alcohol, and then 0.5 ml of sterile sodium chloride solution (sterile saline) was put into a syringe and injected subcutaneously at the lesion's edge. While the needle was under the skin, it was turned several times to remove small pieces of tissue from the edge of the wound (Al-Obaidi et al. 2016). A drop of blood from the infection was used to make a thin swab, left to dry at room temperature, and set with methyl alcohol for one minute. Then, giemsa dye was used. The slides were washed with plain water, dried with filter paper, and left in the air to dry completely. The slides were examined with a light microscope. The Leishmania parasite's phase without flagella was examined with a 100X immersion oil.

For the immunological tests, 5 ml of venous blood from 225 positive cases and 100 control group was taken by syringe, put into test tubes, and left to clot for 5 minutes before the serum was separated with a centrifuge at 3000 rpm for 5 minutes. The serum was then divided into three parts. Each part was put into a 0.5-ml Eppendorf tube and kept in a freezer at -20 °C until the immunological tests were performed. The level of cytokines in the patient's serum was measured and compared with the negative case. And prepare the dye solution:Stock solution .The solution was made by mixing 3.8 g of Giemsa powder with 250 ml of 70% ethanol. After heating the solution for 30 minutes at 60 °C, 250 ml of glycerin was added and filtered to remove impurities. Then, it was put in dark bottles and kept at room temperature. Working solution is 10 ml of the stock solution was mixed with 80 ml of distilled water and 10 ml of 70% methanol.

3.2. Immunological Tests

Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the level of cytokinetic IL-6 in human serum ,TNF-α level,IL-10 level,IL-13 level,IL-17 level and IFNγ levels has the specific antibodies needed to quantitatively measure the level of cytokinetic human on the surface of the wells in the micro-calibration plate. When the sample and control solutions are put on the antibodies

During the second incubation, secondary antibodies were added to get rid of any antibodies that were not linked. Then, the enzyme that matched the antibody was added to increase the bond strength between the antibodies. At the end of the process, the wells were washed to eliminate the free antibodies. The final step was added the substrate, for turn the color blue to yellow. In an ELISA device with a wavelength of 450 nm, the strength of the reaction between the antibodies can be seen by the color intensity of the reaction.

3.3 Statistical Analysis

The results were put together using the program SPSS 2021 (Version 28). The chi-square and Student t-tests were used for statistical analysis and to find significant differences when $P < 0.05$.

4.RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Isolation of Cutaneous Leishmaniasis from Ulcers in Infected Patients

In the hospitals of Babylon governorate, 225 cases of CL and 100 control groups were found between October 2021 and March 2022. The laboratory and clinic of the Babylon governorate both confirmed these cases. These CL cases were isolated from Iraq. It is essential to know what kind of parasites are causing the disease and what kind of disease it is to treat and plan a new control program. There is also a dangerous epidemic of this disease, and it is difficult to know how many people will get sick in each city and how often (Al-Warid *et al.* 2017, Ghatee *et al.*2020, Hakkour *et al.*2020).

In a study conducted in Kirkuk (Alhaweja Hospital) in Iraq, 107 KL cases were found (Alsamarai and Alobaidi 2009). In addition, Alnaimy and Al-Waaly (2021) found that the average of infections in the urban area in Diwania City/Iraq is higher than in the rural area. Al-Obaidi *et al.*(2016) showed The highest incidence of CL in Iraq from 2008 to 2015 was concentrated in the middle and western regions of the country, accounting for 53% of total cases. The southern and eastern regions had a high incidence of CL as well, representing 46% of total cases. In contrast, the northern region had the lowest incidence, with only 1% of cases. The another study further noted that the middle provinces of Iraq had a higher reported incidence of CL (33.7%) compared to the northern (0.4%) and southern regions (14.1%). Notably, the province of Salahuddin had the highest reported cases of CL in Iraq, with a mean of 288 cases over the eight years. It is worth mentioning that there were no reported cases of CL in the Duhok province in the north of Iraq. Flaih *et al.* (2021) registered 247 cases of CL in Thi-Qar. Also, Flaih (2022) appeared about 2 million leishmaniasis infections were recorded with CL. In comparison with another study such asAli *et al.*(2018) was found in their study that Diwaniyah had the highest incidence at 15.1%, while, Wasit was 14.5%, Najaf wsa 13.6%, Thi-Qar was 13.1%, Basrah was 11.5%, Baghdad was 11.2%, Diyala was 10.8%, and Salah-Edin province had the lowest incidence was 10.3% .

CL is diagnosed mainly by the appearance of lesions on the skin in places where it is expected. However, the disease can be challenging to diagnose when the symptoms are not prominent enough due to abnormal changes.This can happen if the condition is not treated correctly or another infection is contracted. This changes CL symptoms, making it harder to diagnose and take longer to treat. Even though there have been cases of CL in Iraq (Al-Hayali and Al-Kattan 2021, Kareem *et al.* 2022).

4.2 Distribution of Cutaneous Leishmaniasis by Type of Residence and gender

Table 4.1 shows that the disease was present in 86 (38.22%) of the patients who lived in urban and in 139 (61.77%) of the patients who lived in rural areas, which is a significant difference ($P < 0.05$) between the two places of residence.

Table 4.1 Distribution of cutaneous Leishmaniasis by Type of residence and gender (N=325)

Urban Regions		**Rural Regions**	
Male	Female	Male	Female
38 (44.2%)	48 (55.8%)	56 (40.3%)	83(59.7%)
(38.22%) 86		**(61.77%) 139**	
P<0.05			

In the current study, it was found that there was a significant difference between urban areas, with a rate of 38.22%, and rural areas, with a rate of 61.77%, where it was observed that residence affected the prevalence of CL. The current study compared with another studies Leishmaniasis can also spread because of how people move around in places where it is shared. During the Iran-Iraq war, for example, many soldiers stationed in places where CL was spreading became infected (Singh and Sivakumar 2003). In Iran, the highest occurrence rate of CL was 37/100000 in Bilasavar and 35/100000 in Germi (Khamesipour *et al.* 2020). Also, the prevalence of CL appeared in Ardabil city (20–30%), and 47% of cases had no travel history (Rassi and Hanafi-Bojd 2006). Similar results were obtained in this study.

4.3 Distribution of Cutaneous Leishmaniasis Among Patients According to Age and Gender

In Table 4.2., the percentage of males (58.22%) was higher than that of females (41.77%), and there were significant differences at the $p<0.05$ and $p<0.01$ levels between males and females and in most age groups. However, most people admitted to hospitals with CL were males aged between 6-15 years, and the fewest were people aged under 50.

Table 4.2 Distribution of Cutaneous Leishmaniasis among patients according to age and gender (N=325)

Age	Male	Female	Total	P value
≥1	4	3	7	P <0.05*
1-5	7	5	12	P < 0.01 **
6-10	32	16	48	P < 0.01 **
11-15	34	28	62	P <0.05*
16-20	21	8	29	P < 0.01 **
21-25	8	9	17	NS
26-30	7	5	12	P < 0.01 **
31-35	6	8	14	P <0.05*
36-40	3	3	6	NS
41-45	4	5	9	P <0.05*
46-50	3	2	5	P <0.05*
≥ 51	2	2	4	NS
Total	131(58.22%)	94(41.77%)	225 (100%)	P < 0.01 **
* P <0.05: mean significant , ** P < 0.01 : mean strong significant NS:Non significant				

The current study showed adults between the ages of (6 - 10and (11-15 years had the highest number compared with ages range(1-5 and(16-51 years. Therefore the current study compared with another Iraqi studies it was accord with Alsaad and Kawan (2021) showed that older children seemed at higher risk. Moreover, another study found that CL in Pakistani patients with age 17-31 was 89.2% (Khan *et al.*2016). In the world studies such as ,Torres-Guerrero *et al.* (2017) showed how children in Tunisia were affected by Leishmaniasis. Where People between the ages of 5 months and 15 years spread this disease like an epidemic. The age group of 5 years and under had the lowest percentage due to less exposure to the infection than other age groups. In one study, 17% of the cases were under ten years. In addition, 74.5% of cutaneous Leishmaniasis was identified in thirteen urban areas (Khamesipour *et al.,* 2020). Also, it was observed the current study was an approach to previous studies when it compared with Ullah *et al.* (2023)

showed in their study that male patients were 62.8% (2003/3188) in all ages, more than females recorded at 37.2% (1185/3188).

4.4 Distribution of Cutaneous Leishmaniasis according to the number of Ulcers

The results showed that men and women had different numbers of skin ulcers (Table 4.3, $p<0.01$). More than 83 (58.04%) infected males had ulcers, and 41 (62.1%) had two ulcers. The chance of getting three or four ulcers was the same for both men and women.

Table 4.3 Distribution of Cutaneous Leishmaniasis with the number of ulcers in males and females (N=325)

Gender	Number of Lesions				Total
	1 Lesion	2 Lesions	3 Lesions	4 Lesions	
Male	83 (58.04%)	41(62.1%)	6(46.2%)	1(33.3%)	131 (58.2%)
Female	60(41.9%)	25(37.9%)	7(53.8%)	2(66.6%)	94(41.8%)
Total	143(100%)	66(100%)	13(100%)	3(100%)	225(100%)
p<0.01					

The current study found 225 people in governorate hospitals infected with Leishmaniasis. Patients had infections all over their bodies, and men have one lesion (58.04%) more than women (41.9%) had one lesion. Most females (66.6%) with CL (four lesions) were between (6-15) years old. The results of this study agreed with one of the Iraqi studies by Qurtas (2018) showed their study CL infection in males (75%) more than in females (25%). Also, there is a significant linkage between lesion ulceration and size. The lesion number in patients was not a predictor for lesion ulceration.

Regarding the number of ulcers of people with CL had only one. These results agree with other world studies such as Barral *et al.*(1995) showed that ulcer is a sign of an infection. Also, the current study when compared with other studies such as Monzote (2009) found that most patients have two ulcers due to several bites frequency by infected insects. Multiple ulcers are common, and in some ways, they are like zoonotic cutaneous Leishmaniasis (ZCL).

The difference in infection is due to the immunological response differences between males and females, which results in differences in the natural and adaptive immune response. This difference is due to variations in hormones and genes in both gender (Klein and Flanagan 2016). Also, *leishmania* infection may be related to gender and immune response in the pathogenesis of infection (Lockard *et al.*2019). The types of leishmaniasis do not always correspond to the appearance of ulcers. Clinical symptoms might not be enough to tell if someone has a disease without a laboratory test because a new parasite could cause them. Even though the parasites are the same species, they can cause different symptoms because their genes are different. This means that molecular tests distinguish all types of *Leishmania* (Kobets *et al.* 2012, Al-Rashed *et al.*2022). Another study applied the PCR technique to diagnose the target kinetoplast of DNA which was the sensitive method to identifying (86.5%) of all positive samples that were diagnosed by other methods (Khan *et al.*2016).In one study, 65% of lesions were dry and without excretion so these signs may be associated with CL (Khamesipour *et al.* 2020).

4.5 Direct microscope examination of cutaneous leishmaniasis

It was diagnosed CL by Giemsa stain. It was examined under a microscope. Where it was obsereved the amastigote phase, this proceudre is accurate 70–80% (CDC 2012). Figure 4.2 show the ulcer in patient Figure 4.3 and Figure 4.4.

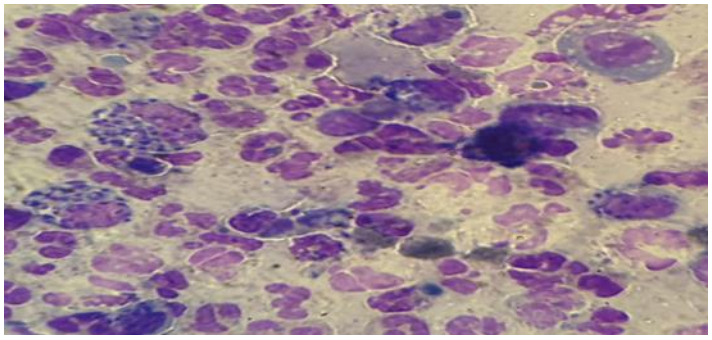

Figure 4.2 Amastigote phase of cutaneuos leishmaniasis

Figure 4.3 The ulcer in patient legs

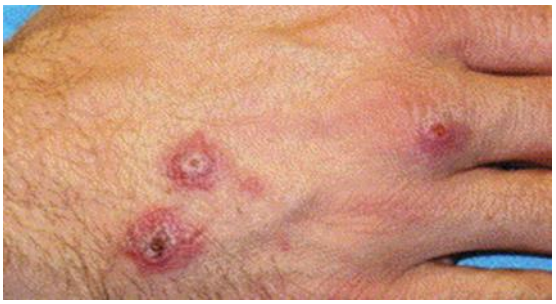

Figure 4.4 Multiple ulcer in patient

4.6 Level of TNF-α in the study cases

Comparisons of TNF-α values with control group and positive samples are given in Table 4.4 . TNF-α levels were higher in CL patients (46.53 ± 12.06 pg/ml) than the control group (23.0 ± 4.86 pg/ml).

Table 4.4 Level TNF-α in the study cases (N=325)

TNF-α	
Groups	**Mean±SE (pg/ml)**
Positive	46.53±12.06
Control	23.0±4.86
Significant difference at (P < 0.05)	

The rate of TNF-α in the CL group's serum was higher when compared to the rate of TNF-α in the control group's serum. This was a significant difference at a probability level of less than 0.05. The results of this study were similar to one of Iraqi studies by Hussein and Ali (2022), who found in a study that patients with CL had a higher level of TNF than a healthy group. The observed rise of TNF-α level can be an obvious marker of this pro-inflammatory cytokine in predicting disease development. Also, the results of this study were similar to one of the Iraqi studies by Taher *et al.* (2020) appeared their study the level of IFN-γ and TNF-α increased in CL patients

Also, the current study agreed with other world studies when compared with them such as Maspi *et al.* (2016) found increased TNF-α production in CL infection. This is due to macrophages producing more TNF-α to eliminate the parasite. Because TNF-α generates nitric oxide (NO), which aids in eliminating *Leishmania*. TNF-α also plays a role in the IFN-γ /Th1 response, especially against *L. major*. The current results agreed with another study (Iniesta *et al.* 2005) with 50 patients infected with the CL parasite. This study found that the blood serum of the infected group increased TNF-α. The infection rate in the infected group was (6.23 ± 15.77 pg/ml), while it was (4.92 ± 21.83pg/ml) in the control groups. These immune-regulating cytokines are released mainly by activated macrophages in response to different stimuli and have been linked to these changes (Pissinate *et al.* 2008). The differences in TNF-α levels were caused by B1 cells. B1 cells are a type of B-cell involved in the humoral response but are not part of the adaptive immune system because they do not remember things. By interacting with the early stages of the flagellum, the B1 cell can induce TNF-α production (Kaye *et al.* 2020, Holowka and Bucala 2020).

4.7 Level of IL-6 in the study cases

Comparisons of IL-6 values with control group and positive samples are given in Table 4.5 .The level of IL-6 in CL patients was (9.94 ±3.35 pg/ml)higher than control group (6.50 ± 2.11 pg/ml).

Table 4.5 Level of IL-6 in the study cases (N=325)

IL-6	
Groups	**Mean±SE (pg/ml)**
Positive	9.94±3.35
Control	6.50±2.11
Significant difference at (P < 0.05)	

The results of this study were similar to one of the Iraqi studies by Al-Aubaidi (2011) appeared their study the level of IL-6 increased in CL patients compared with the control group It has appeared the current study agreed with other world studies in increasing the level of IL-6 in CL patients when compared with them. Where Liew and O'Donnell (1993) observed before treatment, the level of IL-6 in people with CL was (12.85 ± 1.28 pg/ml) , while after treatment, it was (5.08 ± 0.70 pg/ml) where it was found that the interleukin-6 levels in the infected serum changed significantly. The same results were in the blood samples of 28 people with CL compared to the control group, where the infection rate was (1.61 ± 4.6pg/ml)there was significant increase in the number of people with infection.Where Cytokines are stimulated of the body's defenses (Kumar *et al.* 2007).

IL-6 is one of the most essential cellular cytokines. It is released by macrophages, dendritic cells, and T cells, and it controls the function of B lymphocytes and the acute phase response proteins (Turner *et al.* 2014, Tanaka *et al.* 2014).Moreover, in other world studies showed during *Leishmania* infection, the macrophages produce high levels of pro-inflammatory cytokines like TNF-α, IL-6, and IFN-γ (Yasmin *et al.* 2022). Kumar *et al.* (2007) found that the levels of IL-6 were slightly higher in people with CL. They also found that the innate immune system and macrophages with IL-6 cytokines play a crucial role in fighting pathogens when infection happens.

4.8 Level of IL-10 in the study cases

Comparisons of IL10 values with control group and positive samples are given in Table 4.6. The level of IL-10 (115±8.8 pg/ml) in CL patients was more than the control group (11.52±5.4 pg/ml).

Table 4.6 Level of IL-10 in the study cases (N=325)

IL-10	
Groups	**Mean±SE (pg/ml)**

Positive	115±8.8
Control	11.52±5.4
Significant difference at (P < 0.05)	

The results of this study were similar to one of the Iraqi studies by Al-Obaidi and Kamal (2007) showed their study the level of IL-10 increased in CL patients compared with the control group Also, the current study agreed with another world study where it appeared IL-10 is related to the development of leishmaniasis because of it prohibits the leishmanicidal function of macrophages and the stimulation of mediators like IFN-γ (da Silva *et al.*2022). In most parasitic diseases, the cellular (TH) or humoral (TH2) response is better at controlling pathogens. A successful immune response depends on the T helper cell response, and the TH1 cell has molecules that affect the delayed hypersensitivity and the release of interleukin. IFN-γ are essential cell-mediated immune system effectors (Khan *et al.* 2020). The Th2 cell, on the other hand, does not transduce delayed hypersensitivity. Instead, it makes IL-10, contributing to the B cell to make immunoglobulins (IgG and IgA) (Urban *et al.* 2020, Kaye *et al.* 2020). T cells have a role in immunity responses. The production of nitric oxide to remove the parasite is stimulated by Th1 cells that produce IFN-γ. Th2 has a role in susceptibility due to able the production of cytokines like IL-13. Also, T regulatory cells preserve the expression of IL-10 and the continuity of Immunity against parasites (Gupta *et al.* 2013).

4.9 Level of IL-13 in the study cases

Comparisons of IL13 values with control group and positive samples are given in Table 4.7.The level of IL-13 in CL patients was (58.08 ± 8.34 pg/ml) higher than control group (18.86 ± 7.14 pg/ml).

Table 4.7 Level of IL-13 in the study cases (N=325)

IL-13	
Groups	**Mean±SE (pg/mL)**
Positive	58.08±8.34
Control	18.86±7.14
Significant difference at (P < 0.05)	

The current study agreed with another study where it appeared IL-13 levels after induction with *Leishmania* infection were increased in patients compared to control with significant differences (Mahmoodi *et al.*2005). While there is much disagreement about IL-4/IL-13 effects during CL may be because of the different ways target cells interact with IL-13 and how important they are in a hierarchy when the illness of the host (Alexander and Brombacher 2012). Studies on mice showed that IL-4R signaling in CL positively affected cells other than CD4+ T cells. Since IL-13 does not affect murine lymphocytes, populations that are not lymphocytes could compete for this function. So, in two separate studies, researchers investigated how IL-4R signaling affected macrophages and neutrophils in LysMcre IL-4R/lox BALB/c mice infected with *L. major*. Despite the abundance of type 2 immune responses, the lack of IL-4R on macrophages and neutrophils slowed the progression of *L. major* illness (Güran 2018). Macrophages and neutrophils do not express IL-4R (Bryson *et al.* 2010, Saha *et al.* 2022).

4.10 Level of IL-17 in the study cases

Comparisons of IL17 values with control group and positive samples are given in Table 4.8.The level of IL-17 (58.14 ± 16.14 pg/ml) in CL patients was higher than the control group (16.14 ± 3.45 pg/ml).

Table 4.8 Level of IL-17 in the study cases (N=325)

IL-17	
Groups	**Mean± SE(pg/mL)**
Positive	58.14±16.14
Control	16.14±3.45
Significant difference at (P < 0.05)	

The current study was similar to one of the Iraqi studies that showed the level of IL-17 was increased in CL patients compared with the healthy group. These outcomes indicate the role of IL-17 in CL pathogenicity (Husain *et al.*2016). Also, another Iraqi study appeared an increase in the level of IL-17 in CL patients in comparison to control groups (Hussein *et al.*2015). The current study agreed with another world study where it appeared IL-17 levels increased in patients with CL compared with healthy groups (Mendonça *et al.*2020). Anderson *et al.* (2009) found CL lesions worsen when IL-17 expression is high in a mouse model. More importantly, they show how important it is for IL-27, a negative regulator of Th17 cells, to keep the balance between protective Immunity and disease by making IFNc and IL-10 from CD4 T cells and stopping the formation of pathogenic Th17 subsets. Nevertheless, no one has shown what role IL-17 plays in CL caused by *L. tropica* in humans.

When *L. tropica* is the source of CL, protective immunity is linked to developing *Leishmania*-specific CD4 and CD8 cells that make IFNc (Lopez Kostka *et al.* 2009). New studies have shown that neutrophils were also crucial for developing CL disease in another way. CD4 T cells and neutrophils from BALB/c mice vulnerable to *L. tropica* significantly make much more IL-17 than C57BL/6 mice resistant to *L. major*. It has been shown that parasite-infected Dendritic cells (DCs) release IL-23, which may help BALB/c mice make more Th17 cells compared to normal BALB/c mice, infected IL17-deficient animals had smaller lesions with fewer parasites. This study shows that *L. major*-infected DCs make IL-23, which keeps Th17 cells alive. Th17 cells affect the development of disease by controlling neutrophil recruitment (Lopez Kostka *et al.* 2009, Kumar *et al.*2020). Neutrophils make IL-17, contributing to tissue damage and helping lesions grow (Gonzalez-Lombana *et al.* 2013).

By directly linking the amount of cellular infiltration with higher levels of IL-17 production, (Bacellar *et al.* 2009) found that people with an *L. braziliensis* infection made more IL-17. That proved the role of IL-17 in the pathogenesis of an *L. braziliensis*-mediated mucocutaneous leishmaniasis (MCL). Also, patients with active *L. braziliensis* human CL infections had higher levels of IL-17 in their Peripheral blood mononuclear cell (PBMC) culture supernatants than patients who had already recovered from the infection (Souza *et al.* 2012). Other research shows that the illness is controlled when humans make IL-17 with *L. braziliensis* infections that are not yet serious. Evaluating the role of IL-17-producing cell types in this protection is essential to make treatments more effective (Banerjee *et al.* 2016, Novoa *et al.* 2011).

4.11 Level of IFN-γ in the study cases

Comparisons of IFN-γ values with control group and positive samples are given in Table4.9. The level of IFN-γ in CL patients was (38.23±13.95 pg/ml) higher than the control group (21.98±5.69 pg/ml).

Table 4.9 Level of IFNγ in the study cases (N=325)

IFN γ	
Groups	**Mean±SE (pg/ml)**
Positive	38.23±13.95
Control	21.98±5.69
Significant difference at (P < 0.05)	

In the current study, the level of IFN **γ** was higher in the serum of people with CL compared with control group. This difference was statistically significant at a probability level of less than 0.05. The current study was similar to another Iraqi study such as Jameel and Al-Qadhi (2020) who showed the incraese level of IFN γ in CL patients Moreover, this study's findings are consistent with world studies such as Sharma and Singh (2009), who found that people with CL significantly increased (212 ± 0.4102 mg/dl) in IFN- γ compared with control group ,where which is needed to keep a balance between Th2 and Th2 responses. The levels of IFNγ have a role in the regulated balance between Th1 and Th2. This rise was caused by the host's response to parasite antigens and the increase of T-helper T cells type I Th1, which leads to the production of IFNγ and its high level in the serum of patients with CL, where IFN **-γ** acts on the activation of macrophages, increasing the formation of Inducible nitric oxide synthase (iNOS2). This enzyme causes more production of NO, which kills intracellular amastigotes (Liu and Uzonna 2012).

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1- Cutaneous Leishmaniasis infection prevalence in rural areas was more than in urban areas. The infection was most common in those aged 6-15 years and more prevalent in males than females.

2- The serum levels of TNF-α, IL-6, IL-10, IL-13, IL-17, and IFN-γ were increased in Cutaneous Leishmaniasis patients compared to the control group.

3- The increasing levels of these interleukins indicated a disturbance of the immune system during CL infections.

5.2 Recommendations

1- Conduct more research on how infections affect the production of other cytokines outside the body to determine how these kinetics work and their effect on CL.

2- Study gene expression for cytokines in patients with leishmaniasis.

3- Use genetic and molecular methods to detect genes that have a role in causing infection.

4- Study gene expression for genes that have a role in causing infectio

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