**A THESIS SUBMITTED TO**
**THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**
**OF ÇANKIRI KARATEKIN UNIVERSITY**

# 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

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS**
**FOR**
**THE DEGREE OF MASTER OF SCIENCE**
**IN**
**BIOLOGY**

**BY**

**BAN JASIM SAADOON SAADOON**

**ÇANKIRI**

**2023**

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

By Ban Jasim Saadoon SAADOON

June 2023

We certify that we have read this thesis and that in our opinion, it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

**Advisor** **:** Asst. Prof. Dr. Banuçiçek YÜCESAN
**Co-Advisor** **:** Asst. Prof. Dr. AYSIR S. M. AL-SAMARRAI

**Examining Committee Members:**

**Chairman** **:** Asst. Prof. Dr. Banuçiçek YÜCESAN
Biology
Çankırı Karatekin University

**Member** **:** Asst. Prof. Dr. Serdal TARHANE
Biology
Çankırı Karatekin University

**Member** **:** Prof. Dr. Ayşe Serpil NALBANTOĞLU
Parazitoloji
Ankara University

**Approved for the Graduate School of Natural and Applied Sciences**

**Prof. Dr. Hamit ALYAR**
**Director of Graduate School**

**I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.**

**Ban Jasim Saadoon SAADOON**

## ABSTRACT

# 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

Ban Jasim Saadoon SAADOON

Master of Science in Biology

Advisor: Asst. Prof. Dr. Banuçiçek YÜCESAN

Co-Advisor: Asst. Prof. Dr. AYSIR S. M. AL-SAMARRAI

June 2023

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). 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.

**2023, 72 pages**

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

# ÖZET

## KORUMA VE PATOGENEZDE KUTANÖZ LEİSHMANİASİS İLE TNF-α, IL-6, IL-10, IL-13, IL-17 VE IFN-γ DÜZEYLERİNİN VE İMMÜN YANITLARININ DEĞERLENDİRİLMESİ

Ban Jasim Saadoon SAADOON

Biyoloji, Yüksek Lisans

Tez Danışmanı: Dr. Öğr. Üyesi Banuçiçek YÜCESAN

Eş Danışman: Dr. Öğr. Üyesi AYSIR S. M. AL-SAMARRAI

Haziran 2023

Kutanöz Leishmaniasis (CL), özellikle tropikal bölgelerde dünyanın en büyük ve en hızlı büyüyen sorunlarından biridir. Dünya Sağlık Örgütü (WHO), yayılma yeteneğinin hastalık dağılımını büyük ölçüde etkilediğini bulmuştur. Leishmaniasis, Leishmania spp.'nin neden olduğu bir hastalıktır. ve bir kişiden diğerine bir vektör olan tatarcık tarafından yayılır. Çalışma, Tümör Nekroz Faktörü-alfa (TNF-α), interlökinler (IL-6, IL-10) ölçülerek Leishmania parazitinin konak hücresini nasıl etkilediğini belirlemeyi amaçladı. , IL-13, IL-17) ve İnterferon-Gama (IFN- y). Ekim 2021'den Mart 2022'ye kadar 225 pozitif Kutanöz Leishmaniasis (CL) vakası ve 100 kontrol grubu örneği toplandı. Irak'ın Babil Valiliği Hastanesinde teşhis edildi. Leishmania parazitinin enfektif evresini teşhis etmek için Giemsa boyası kullanıldı. ELISA, sitokinlerin seviyesini ölçmek için kullanıldı. Sonuçlar, kentte yaşayan hastaların 86'sında (%38,22) ve kırsal kesimde yaşayan hastaların 139'unda (%61,77) hastalık bulunduğunu gösterdi; bu, iki yer arasında anlamlı bir farktır ($P < 0,05$). ikametgah Erkeklerde enfeksiyon yüzdesi (%58,22) kadınlardan (%41,77) daha fazlaydı ve erkekler ve kadınlar arasında ve çoğu yaş grubunda $p<0,05$ düzeyinde anlamlı fark vardı. Ancak KL ile hastanelere başvuranların çoğu 6-15 yaş arası erkeklerdi ve en azı 50 yaş altıydı. Ayrıca sonuçlar kadın ve erkeklerin cilt ülseri sayılarının farklı olduğunu gösterdi ($p<0.01$). Enfekte 83'ten fazla (%58.04) erkekte ülser vardı ve 41'inde (%62.1) iki ülser vardı. Üç veya dört ülser olma şansı hem erkekler hem de kadınlar için aynıydı. Serum düzeylerinin sonuçları TNF-α (46,53 pg/ml), IL-6 (9,94 pg/ml), IL-10 (115 pg/ml), IL-13 ( 58,08 pg/ml), IL- için yükselmiştir. Kontrol ile karşılaştırıldığında hastalarda 17 (58,14 pg/ml)

ve IFN-γ (38,23 pg/ml). Sonuç olarak, kırsal alanlarda Kutanöz Leishmaniasis enfeksiyonu prevalansı kentsel alanlara göre daha fazladır. Bu interlökinler, TNF-α, IFN-γ'nın artan seviyeleri, CL enfeksiyonları sırasında bağışıklık sisteminin bozulduğunu göstermektedir.

**2023, 72 Sayfa**

**Anahtar Kelimeler:** Leishmaniasis, Cutaneous Leishmaniasis, İmmünolojik parametreler, Tanı, Parazit

## PREFACE AND ACKNOWLEDGEMENTS

I want to thank my thesis advisor, Asst. Prof. Dr. Banuçiçek YÜCESAN, for her patience, guidance, and understanding.

**Ban Jasim Saadoon SAADOON**

**Çankırı-2023**

**CONTENTS**

## LIST OF SYMBOLS

| | |
|---|---|
| g | Gram |
| µL | Microliter |
| mg | Milligram |
| mL | Milliliter |
| nm | Nanometer |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACL | Anthroponotic cutaneous *Leishmania*sis |
| CL | Cutaneous *Leishmania*sis |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| HRP | Horseradish peroxidase-conjugated |
| IFN-γ | Interferon-gamma |
| IL-10 | Interleukin-10 |
| IL-13 | Interleukin-13 |
| IL-17 | Interleukin-17 |
| IL-6 | Interleukin-6 |
| NNN | Nicolle- Novy MacNeal medium |
| OD | Optical density |
| PCR | Polymerase chain reaction |
| RPMI | Roswell Park Memorial Institute |
| TNF-α | Tumour necrosis factor-alpha |
| WHO | World health organization |
| ZCL | Zoonotic cutaneous *Leishmania*sis |

**LIST OF FIGURES**

**LIST OF TABLES**

## 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).

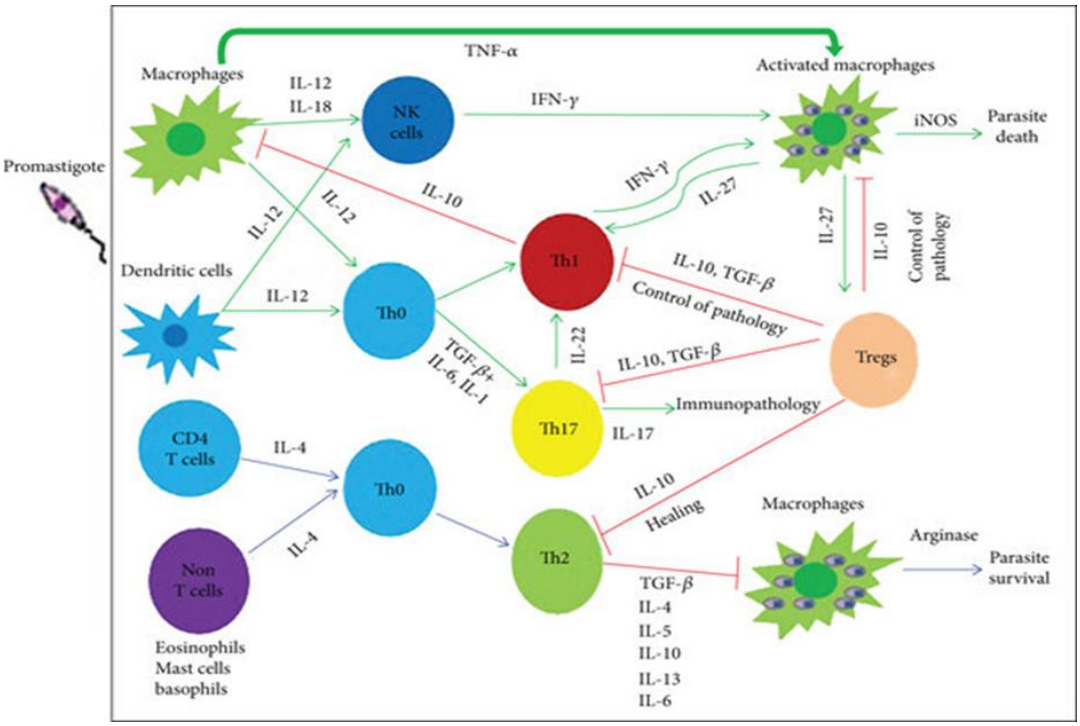


**Figure 1.1** Profile cytokines in cutaneous leishmaniasis (Dubie and Mohammed 2020)

## 1.1 Aim of the Study

1-Determination of Cutaneous Leishmaniasis (CL) infection prevalence in rural and urban areas. Also, investigation of the prevalence of the infection according to age and gender.

2-Determination of the levels of TNF-α, IL-6, IL-10, IL-13, IL-17, and IFN-γ in CL cases.

3-Detection of the relationship between infection with CL and host cell with immunological parameters.

# 2. LITERATURE REVIEW

## 2.1 *Leishmania* parasite

There are four main types of leishmaniasis: visceral, cutaneous, post-kala-azar dermal, and mucocutaneous. All of these types can be found in different parts of the World, and a female sandfly bite contributes to the transmission of the parasite from one host to another (Merkel and Voges 2006, Maxfield and Crane 2022, Gaber *et al.*2022). There are 30 types of *Leishmania* which can infect mammals, and 21, which can infect people (Knight *et al.* 2023).

The ancient World involves *Leishmania tropica*, *Leishmania major*, *Leishmania aethiopica*, *Leishmania infantum,* and *Leishmania chagasi* in the Caspian Sea and Mediterranean regions. *Leishmania amazonensis, Leishmania mexicana, Leishmania braziliensis, Leishmania peruviana, Leishmania panamensis,* and *Leishmania guyanensis* are widespread in central and South America also North America area (Prieto-Granada *et al.*2010). *Leishmania tropica* is a unicellular eukaryotic flagellate that lives as a parasite inside macrophage cells in the reticuloendothelial system of a vertebrate host. (Markell and Voges 2006, Goodman 2020).

Leishmaniasis is a disease caused by *Leishmania* spp. and is spread from one person to another by a vector, the sandfly. It is a significant cause of death worldwide, especially in poor countries. *Leishmania* is a protozoan forced to live inside the cells of its hosts. It causes disease that are called *Leishmania* diseases (Hazra *et al.* 2002, de Vries and Schallig 2022).

## 2.2 History of *Leishmania*

People in ancient Iraq discovered Leishmaniasis and wrote about the disease in the second millennium Before the Christian Era (BCE) (Bray and Chang 1985). Thousands of years ago, Leishmaniasis was found in the Americas or the New World. This was

shown by pottery and skull designs from the time of the Colombians and Peruvians, which showed that the parasite had been there since then (Vidyashankar 2002). In the 1600s, Bueno was the first to say that sandflies spread this disease. He mentioned what Peruvians call "Uta," which means sandfly (Herrer and Christensen 1975). Later, people tried to find the disease-causing organism. Cunningham thought 1885 that the pathogen was made up of nuclear bodies, but Borovsky found in 1898 that it was made up of tiny particles inside larger cellular structures (Hoare 1938).

In the Indian city of Dum Dum in 1903, a scientist named Leishman looked at a spleen biopsy of a person who had died of Dum Dum fever. He saw many things with rod-shaped chromatin that was 2–3 micrometers long. In the same year, Donovan looked at a child's spleen from Madras and saw bodies similar to those Leishman had described (Markel and Makhol 2004). In 1904, it was found that leishmaniasis was mainly caused by the Donovan bodies (Hoare 1938). Baghdad and Mosul were the first places in Iraq where leishmaniasis was found. Six children between the ages of 2 and 6 were found to have the disease in Mosul (Bashir 1954), and 4 children between 18 months and 4 years old were found to have the disease in Baghdad (Taj-Eldin and Alousi 1954).

### 2.3 Classification of *Leishmania*

The genus *Leishmania* is a hemoflagellate, a group of flagellates living in the blood and tissues of humans and animals. According to the classification within the Kinetoplastida parasites (Paniker and Ghosh 2017), hemoflagellates have a basal body, a single flagellum, and other organelles and cytoplasmic organelles (Knight *et al.* 2023).

Kingdom: Protista
Sub-kingdom: Protozoa
Phylum: Sacromastigophora
Sub phylum: Mastigophora
Class: Zoomastigophora
Order : Kinetoplastida

Suborder: Trypanosomatina.

Family: Trypanosomatidae

Genus: *Leishmania*

Species*: L. tropica*,and *L.major* etc.

## 2.4 Morphology of *Leishmania*

Leishmaniasis has two forms during its life cycle.

### 2.4.1 Amastigote phase

*Leishmania* is between 3 and 5 micrometers long at this stage and is round or ovoid to pear shape. It has one nucleus near the middle and an oval-to-rod-shaped kinetochore. The axial filament Axoneme grows from the kinetochore (Torres-Guerrero *et al.* 2017) Figure 2.2.

### 2.4.2 Promastigote phase

At this stage, the *Leishmania* parasite looks like a long spindle between 14 and 20 micrometers long in the middle of the nucleus. At the same time, the part that moves is about 2 micrometers from the front, and it is connected to a loose flagellum that can be up to 22 micrometers long (Al-Saad and Kawan 2021, Torres-Guerrero *et al.*2017). Figure 2.1. The structure of *Leishmania* is also seen.

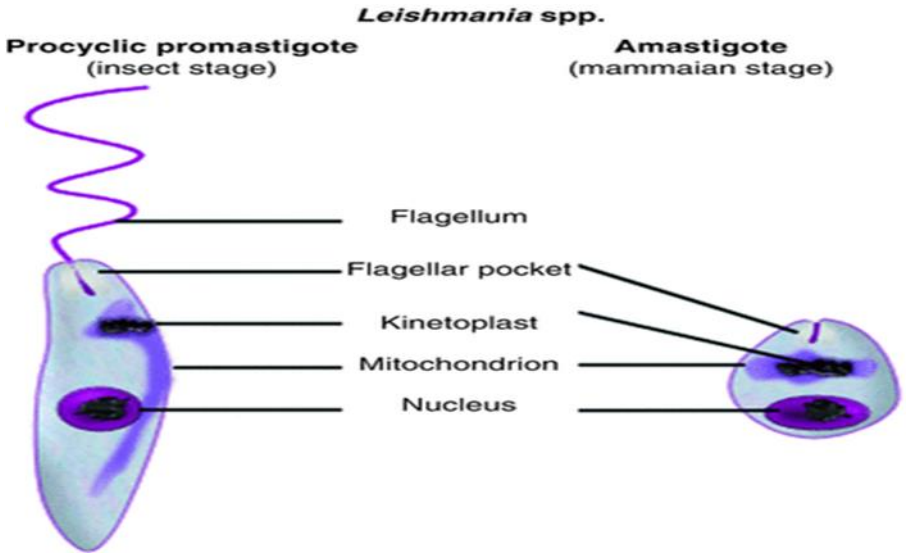


**Figure 2.1** *Leishmania* stages (promastigote and amastigote) (Florin-Christensen and Schnittger 2018)

## 2.5 Life Cycle of *Leishmania*

The life cycle of *Leishmania* occurs in two hosts: an invertebrate host and a vertebrate host. This life cycle include: (1)The promastigote phase of the parasite enters the vector when the female sandfly feeds on the blood of the infected vertebrate host. (2) Then the first step begins, Promastigotes phagocytized via macrophages. (3) Promastigote transfom to amastigote. (4) Amastigote multiply to infect phagocytes. (5 and 6) The factors such as parasite and host) influence if the infection is symptomatic and whether due to cutaneous /or visceral leishmaniasis. Where sand flie infected through ingestion of infected cell through blood meal. (7) Amastigote changes to promastigote, and (8)-development in gut, then emigrate to the proboscis (CDC 2020) (Figure 2.2).

Within 72 hours of feeding, the promastigote flagellum splits into two parts, forming the procyclic stage. The promastigote moves to the front of the middle gut of the insect, where it quickly divides into many parasites. These parasites move toward the head of the fly and settle in the pharynx. It then moves to parts of the mouth to get ready to be injected into the blood of a vertebrate host with saliva when the fly feeds again. When the fly bites a healthy person, macrophages in the skin eat the promastigote, which

quickly changes into the amastigote stage. The amastigote stage then copies itself through binary fission until the cell explodes and spreads the virus to other cells. So the process, called "incubation," continues (Loassini *et al.* 1999, Ponte-Sucre 2003).

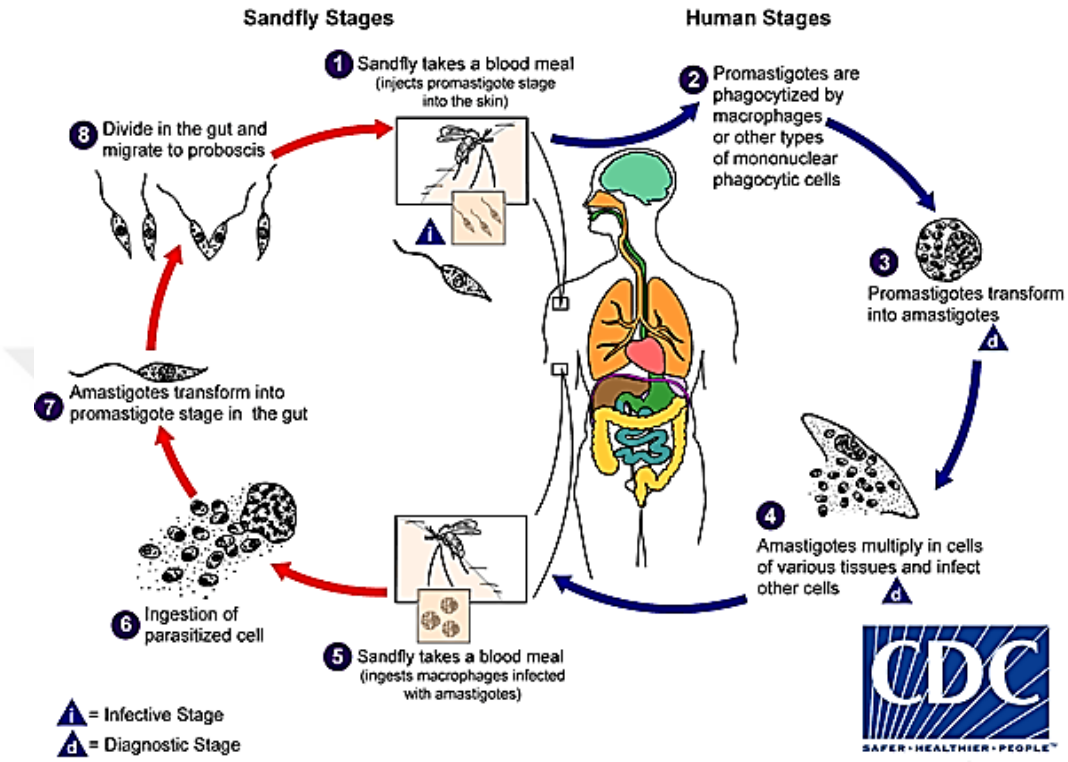


**Figure 2.2** Life Cycle of Leishmaniasis (https://www.cdc.gov/ parasites/ leishmaniasis)

## 2.6 Pathogenesis of Leishmaniasis

This disease is caused by a parasite called *Leishmania*. Female Phlebotomus sandflies pass this parasite on to other people. People can get more than 20 kinds of Leishmaniasis., Sandfly can spread more than 30 different kinds of *Leishmania* spp. It is a disease that is common in more than 88 countries. The WHO has stated that 12 million people have Leishmaniasis and 350 million are at risk of getting it (WHO 2020, Al-Saad and Kawan 2021, Bonfim *et al.*2022). The female sandfly bites and injects the *Leishmania* promastigotes into the vertebrate host. Then, these promastigotes enter the macrophages that are all over the skin. The parasite's surface has lipophosphoglycan,

essential for binding with the macrophages. The outcome of a parasite infection seems to depend on the severity of the infection and how the host's immune system reacts. Depending on the type of *Leishmania* species and the disease it causes, it can spread to all body parts through infected macrophages. When these infected cells burst, they release parasites that can infect the skin and cause one of the known types of CL, or they can infect the mucous membranes of the mouth and nose and cause mucocutaneous leishmaniasis. The parasite has flagella, and hyperplasia also happens when the bone marrow is infected. The hematopoietic tissue cells are replaced by large macrophages infected with the parasite (Rodrguez *et al.* 2006, Singh and Sivakumar 2003, Alraey 2022).

## 2.7 Cutaneous Leishmaniasis (CL)

This is called "American Leishmaniasis" (WHO 2000) and is caused in South and Central America by *L. braziliensis* and *L. mexicana*. *L. amazonensis* is another species in Mexico and South America that can cause infection. When this parasite gets into the skin and mucous membranes, it completely deforms the face by removing the membrane separating the two nostrils. It was also known as a "camel nose" (Despommier and Karapelou 2012).

The infection starts with a hard-edged ulcer that takes between 6 and 18 months to heal. The infection can affects the area around the septum of the nose, the pharynx, and even the larynx (Singh and Sivakumar 2003, Al-Daari *et al.,* 2022).

This type is the most common infection. It occurs in hot and warm places, in tropical and desert lands. Because this disease can be found in many places, it has been given many names, such as the Baghdad boil and the Aleppo boil. Several species of *Leishmania*, such as *L. tropica*, can cause this disease.

Blum *et al.* (2004) found that *L. aethiopica* is another species that makes people sick in Kenya, southern Yemen, and Ethiopia. Also, between 2003 and 2004, *L. major* and 18 cases of CL were found in the US Army in Iraq (WHO 2005).

The first sign of the disease is a small, red bump about 0.5 cm in diameter at the spot where the insect has bitten the skin. This bump is called a papule, which slowly gets bigger until it becomes a palpable nodule (Bizri *et al.*2021). The ulcer then gets bigger ntil it is a few centimeters across and usually round with ulcerated edges. The infection goes away on its own after two to six months or even up to a year. Gorbach *et al.* (1998) found that it leaves a light scar and makes the person immune to future infections (Robert and Janovy 1996). Recent studies show that places where the Human Immunodeficiency Virus (HIV) is found, have more parasite infections (Mcgwire and Satoskar 2014).

Dry type:

This is called the urban type. These anthroponotic infections can be caused by *L. tropica*. The disease takes a long time, between 2 and 8 months, to develop. Then, a single ulcer grows slowly (Lachman *et al.* 1993). It is common in southwestern Iran, western India, North Africa, the Middle East, and the countries around the Mediterranean Sea. (Andrade *et al.* 2001, Vidyashankar *et al.* 2002).

Wet type:

The *L.major* type has given birth to the rural type. Burns *et al.* (2004) found that ulcers heal in a short time. In the worst cases, it can cause wet double ulcers, and ulcers that leak into the subcutaneous tissue can cause lymph node inflammation, a common disease in Central Asian countries (Walton *et al.*1977).

Another study found that the disease is common in the northwest and center of Iran. According to the same study, both promastigotes live in the sandflies' small intestines.

At the same time, flagellated parasites were found in human skin macrophages (Al-Bajalan 2021).

## 2.8 Transmission

The disease is transmitted by one of the following methods:

1- By the bite of an infected sandfly insect.

2- Transition from reservoir animals to humans, as in biting by dogs and wild rodents.

3- Transmission by blood transfusion.

4- Transplantings of affected organs, such as the liver and spleen.

5- Mechanical transmission, especially in outbreak areas (WHO 2000).

## 2.9 The Vector Host

The sandfly mainly spreads leishmaniasis. It belongs to the family Diptera and feeds on blood (Zuckerman and Lainson 1977). There are more than 600 species in five different genera: *Phlebotomus* in the Old World and *Sergentomyia*, *Lutzomyia*, *Brumptomyia*, and *Warileya* in the New World (Tripathi and Nailwal 2021). Schmidt *et al.* (2005) find another study 80% of them were *Phlebotomus sergeant*, and 20% were *P. papatsi*.

Torres-Guerrero et al. (2017) showed that *P. sergeant* is the type of insect in Iraq that spreads the disease. Recent research confirms that *P. papatasi* is a carrier of *Leishmania* parasites in the central parts of Iraq (Al-Harazi *et al*.2021).

## 2.10 The Reservoir

Rodents and dogs are the main reservoirs of CL infection (Al-Hayali and Al-Kattan 2021). Many conditions of cutaneous infection for *L. major* infected dogs that were recorded in Iraq, Saudi Arabia, and Egypt. Dogs act as a primary reservoir (Alsaad and Kawan 2021).

## 2.11 Epidemiology of Cutaneous Leishmaniasis (CL)

Leishmaniasis is prevalent in 98 countries worldwide, with 12 million *Leishmania* cases and 350 million cases exposed to infection. Further, the yearly occurrence is 2 to 2.5 million (Syed *et al*.2006).

Many studies have been performed to detect the widespread cutaneous leishmaniasis in Iraq. Al-Samarai and Al-Obaidi (2009) recorded 571 cases of CL infection . In addition another study in Iraq recorded 57% CL infection (Daham and Al-Husseini 2011). Also, another study recorded *Leishmania* infections in Kirkuk, Iraq (Ismail and As'ad 2015). Shahatha and Salih (2018) reported 1936 *L. tropica* infections in health centers and hospitals in Anbar City, Iraq, in 2017.

This parasite spreads through the subcutaneous tissues, beginning with a single lesion and slowly spreading to the ear, face, and, worst cases, the buttocks, infecting every part of the body. This infection causes sores that destroy tissue, leading to total disfigurement that is hard to treat and could be mistaken for leprosy (Vidyashankar 2002).

## 2.12 Diagnosis

Early diagnosis of CL is essential for controlling the disease and getting treatment faster without harming the tissues where the parasite lives (WHO 2000). The clinical findings are essential to determining if someone has CL, especially the mark on their skin. A lab test is needed to confirm the parasite's presence, one of the most critical steps of the diagnosis process (Singh and Sivakumar 2003, Burza *et al.*, 2022).

### 2.12.1 Direct microscopy

This method takes a scrap from the edge of the ulcer; it is used to make smears and then stained with Leishman or Giemsa dyes. The slides are scanned for the amastigote phase

of the parasite in the monocytes, but it cannot be seen in scraps taken from the middle of the ulcer because it is an ulcerated area with bacteria (Rahi *et al.* 2013).

### 2.12.2 *Leishmania* culture

Leishmaniasis is diagnosed depending on microscopic examination using Giemsa stain and is cultivated in monophasic and biphasic cultures such as RPMI 1640 ) Roswell Park Memorial Institute) and NNN (Novy-MacNeal-Nicolle) media (Mirzaie *et al.*2013). After injecting the ulcer with Lugol solution, fluid from the ulcer's edge is taken and grown in a suitable culture medium, like a gelatinous medium or biphasic medium (Tobies medium and NNN ) (Paniker and Ghosh 2017).

### 2.12.3 Indirect immunofluorescent antibody test

This is one of the most widely used serological tests for detecting *Leishmania* parasite antibodies. It depends on the antibodies present (Singh and Sivakumar 2005).

### 2.12.4 Molecular diagnosis

Molecular detection of Leishmaniasis is based on Polymerase Chain Reaction (PCR) using primers targeting many multicopy genes such as rRNA and DNA kinetoplast (Flaih *et al*.2021).

## 2.13 Treatment of Leishmaniasis

Meglumine antimoniate and sodium stibogluconate are two antimonials drugs used in the treatment of cutaneous leishmaniasis. In endemic regions, intra-lesion meglumine antimoniate is more efficient than sodium stibogluconate in treating CL caused by *L. tropica* (Yesilova *et al.* 2016). Moreover, several treatments are used, such as allopurinol, amphotericin B, liposomal, miltefosine, and paromomycin (Sivayogana *et al.* 2022).

# 3. MATERIALS AND METHODS

## 3.1 Apparatus and Instruments

The instrumentation utilized in this study is listed in Table 3.1.

**Table 3.1** Equipment and apparatus used in the study

| No. | Apparatus | Company and Origin |
|---|---|---|
| **1** | ELISA | Rayato/ Germany |
| **2** | Microscope | Olympus, Japan |
| **3** | Water bath | Kottermann, Germany |
| **4** | Incubator | Memmert, Germany |
| **5** | Centrifuge | Hettich, Germany |
| **6** | Electric Balance | Sartorius, Germany |
| **7** | Distillator | GFL, Germany |
| **8** | Refrigerator | Concord/China |

## 3.2 Biological and Chemical Materials

The kits used in this study are listed in Table 3.2.

**Table 3.2** The kits used in the present study

| No. | Material Names | Company and Origin |
|---|---|---|
| **1** | TNF-α | Sun long Biotech/China |
| **2** | IL-6 | Sun long Biotech/China |
| **3** | IL10 | Sun long Biotech/China |
| **4** | IL13 | Sun long Biotech/China |
| **5** | IL-17 | Sun long Biotech/China |
| **6** | IFN- γ | Sun long Biotech/China |

## 3.3 Epidemiological Study

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 in Appendix 5.

### 3.4 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.

### 3.5 Giemsa stain

The method of Rotimi *et al.* (2000) was used to 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.

### 3.6 Immunological Tests

#### 3.6.1 TNF-α level

Principle test: Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the level of cytokinesTNF-α in human serum, which has the specific antibodies needed to quantitatively measure the level of cytokinetic human TNF-α 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.

##### 3.6.1.1 Components of the kit:

1- The micro-calibration plate has 96 TNF- α antibody-coated holes.

2- 360 pg/ml of the standard solution.

3- Conjucated solution.

4- Standard diluent solution.

5- Sample diluent.

6- Washing solution.

7- Chromrgenic reagent solution A.

8- Chromrgenic Reagent Solution B.

9- Stop solution

10- Plate covers.

**3.6.1.2 Standard concentrations preparation :**

1- It was prepared by taking five tubes labeled 1-5 and then 150 µl from the standard diluent was added to each tube.
2- The first standard concentration (240pg/ml) was prepared by taking 300 µl from the original standard (360pg/ml) and was added to the first tube.
3- The second concentration (160pg/ml ) was prepared by taking from the first concentration 300 µl and adding it to the second tube
4- The third concentration (80pg/ml ) was prepared by taking from the second concentration 150 µl and adding it to the third tube.
5- The fourth concentration (40pg/ml ) was prepared by taking from the third concentration 150 µl and adding it to the fourth tube.
6- The fifth concentration (20pg/ml ) was prepared by taking from the fourth concentration 150 µl and adding it to the fifth tube as showen Figure3.1.

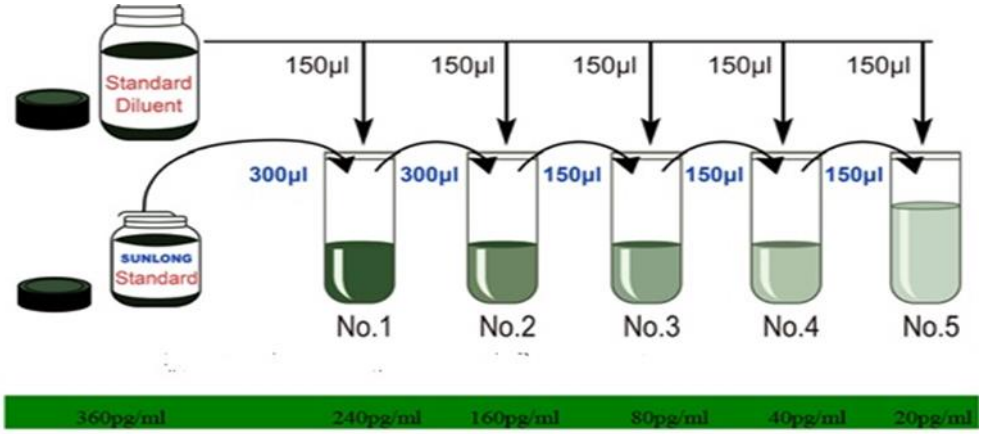


**Figure 3.1** Standards concentrations of TNF-α

### 3.6.1.3 Procedure

1- The kit components and serum samples were placed at room temperature for 15 minutes before use.

2- 40 µl of sample diluent was added to each well of plate, and then 10 µl of each sample of patients' serum was added to the wells, covered with plate paper, and incubated at 37 °C for 30min.

3- The paper plate was removed, then its contents were poured into the sink. The plate was washed by adding 300 µl of the washing solution. The washing process was repeated four times; then, the plate was struck several times to remove the water and dry the plate.

4- 50 µl of TNF-α conjugation solution was added to each field, which was covered and incubated at room temperature for 30min.

5- As in step 3, the washing and drying process was repeated four times.

6- 50 µl of chromogenic solution A and B 50 µl was added to each well and incubated in the dark for 15 minutes.

7- 50 µl of stop solution was added for terminating reaction .

8- Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

9- As shown in Figure 3.2, A standard curve generated through plotting of the absorbance value for standards values on y-axis and standards concentrations on x-axis by using Microsoft word excel drawing the standard curve to obtain equation linear to calculate the concentration TNF-α for each sample.

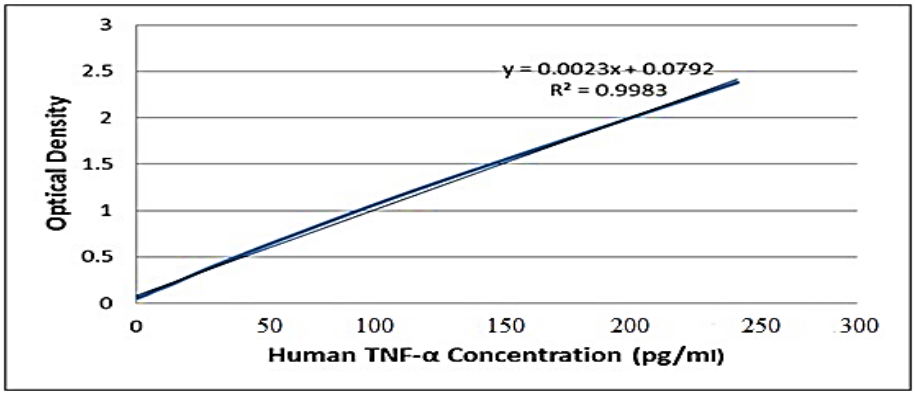


**Figure 3.2** Tumor Necrosis Factor (TNF-α) Standard curve

### 3.6.2 IL-6 level

Principle test: Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the level of cytokinetic IL-6 in human serum, which has the specific antibodies needed to quantitatively measure the level of cytokinetic human IL-6 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 to add 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.

**3.6.2.1 Components of the kit:**

1- Micro-calibration plate containing 96 wells coated with the IL-6 antibody.

2- 90 ng/ml of the standard solution.

3- Conjucated solution.

4- Standard diluent solution.

5- Sample diluent.

6- Washing solution.

7- Chromrgenic reagent solution A.

8- Chromrgenic Reagent Solution B.

9- Stop solution

10- Plate covers.

### 3.6.2.2 Standard concentrations preparation :

1- It was prepared by taking five tubes labeled 1-5 and then 150 µl from the standard diluent was added to each tube.
2- The first standard concentration (60ng/ml) was prepared by taking 300 µl from the original standard (90ng/ml) and was added to the first tube
3- The second concentration (40ng/ml ) was prepared by taking from the first concentration 300 µl and adding it to the second tube
4- The third concentration (20ng/ml ) was prepared by taking from the second concentration 150 µl and adding it to the third tube.
5- The fourth concentration (10ng/ml ) was prepared by taking from the third concentration 150 µl and adding it to the fourth tube.
6- The fifth concentration (5ng/ml ) was prepared by taking from the fourth concentration 150 µl and adding it to the fifth as showen Figure 3.3 .

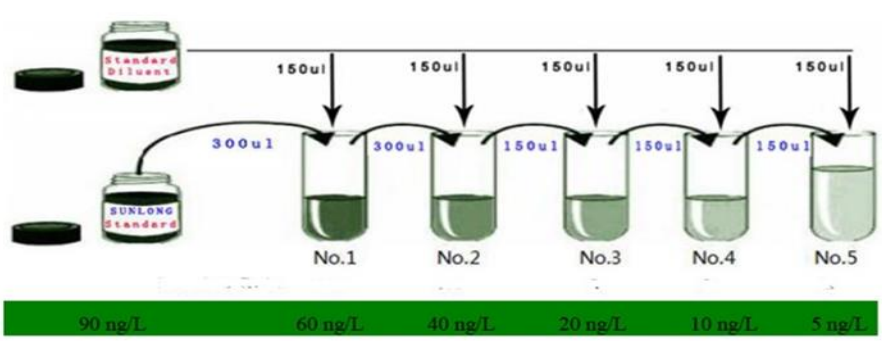


**Figure 3.3** Standards concentrations of IL-6

### 3.6.2.3 Procedure

1- The kit components and serum samples were placed at room temperature for 15 minutes before use.

2- 40 µl of sample diluent was added to each well of plate, and then 10 µl of each sample of patients' serum was added to the wells, covered with plate paper, and incubated at 37 °C for 30 min.

3- The paper plate was removed, then its contents were poured into the sink. The plate was washed by adding 300 µl of the washing solution. The washing process was repeated four times; then, the plate was struck several times to remove the water and dry the plate.

4- 50 µl of IL-6 conjugation solution was added to each field, which was covered and incubated at room temperature for 30min.

5- As in step 3, the washing and drying process was repeated four times.

6- 50 µl of chromogenic solution A and50 B µl was added to each well and incubated in the dark for 15 minutes.

7- 50 µl of stop solution was added for terminating reaction .

8- Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

9- As shown in Figure 3.4, a standard curve generated through plotting of the absorbance value for standards values on y-axis and standards concentrations on x-axis by using Microsoft word excel drawing the standard curve to obtain equation linear to calculate the concentration IL-6 for each sample

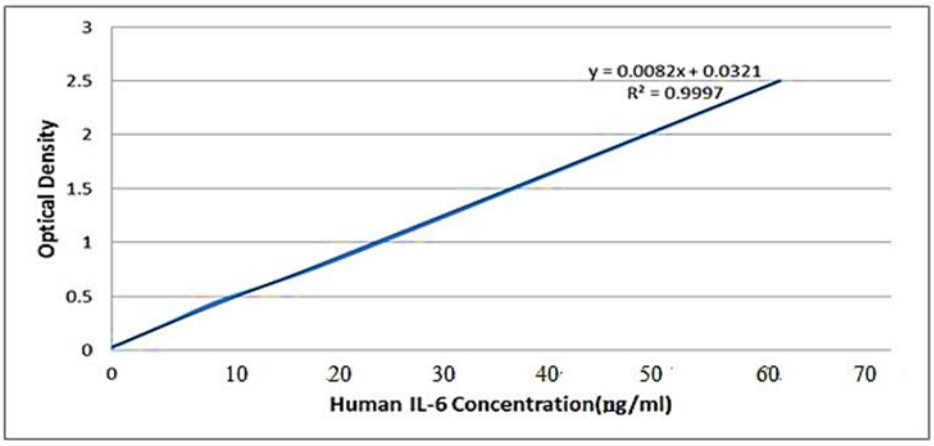


**Figure 3.4** IL-6 cytokine standard curve

### 3.6.3 IL-10 level

Principle test: Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the level of cytokinetic IL-10 in human serum, which has the specific antibodies needed to quantitatively measure the level of cytokinetic human IL-10 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 next step was to add 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.

#### 3.6.3.1 Components of the kit:

1- Micro-calibration plate containing 96 holes coated with the IL-10 antibody.

2- 540 pg/ml of the standard solution.

3- Conjucated solution.

4- Standard diluent solution.

5- Sample diluent.

6- Washing solution.

7- Chromrgenic reagent solution A.

8- Chromrgenic Reagent Solution B.

9- Stop solution

10- Plate covers.

**3.6.3.2 Standard concentrations preparation :**

1- It was prepared by taking five tubes labeled 1-5 and then 150 µl from the standard diluent was added to each tube.
2- The first standard concentration (360pg/ml) was prepared by taking 300 µl from the original standard ( 540 pg/ml) and was added to the first tube.
3- The second concentration (240pg/ml ) was prepared by taking from the first concentration 150 µl and adding it to the second tube
4- The third concentration (120 pg/ml ) was prepared by taking from the second concentration 150 µl and adding it to the third tube
5- The fourth concentration (60 pg/ml ) was prepared by taking from the third concentration 150 µl and adding it to the fourth tube

6- The fifth concentration (30 pg/ml ) was prepared by taking from the fourth concentration 150 µl and adding it to the fifth tube as shown in Figure 3.5.

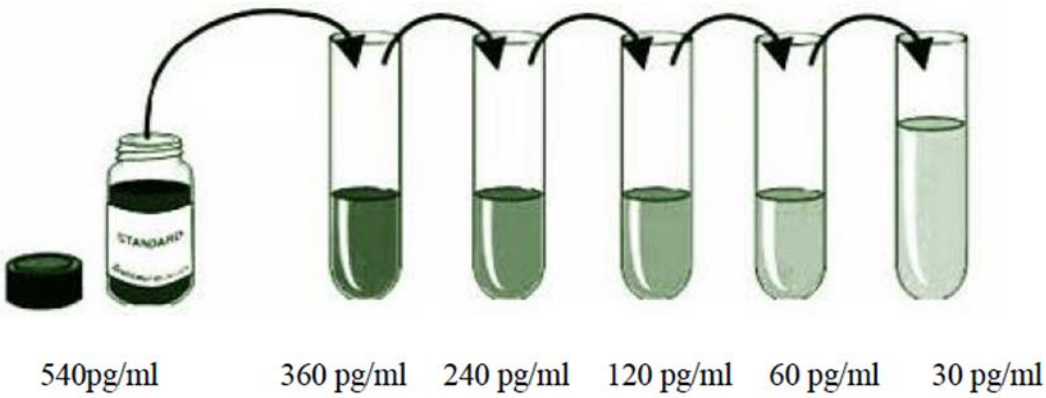


**Figure 3.5** Standards concentrations of IL-10

**3.6.3.3 Procedure**

1- The kit components and serum samples were placed at room temperature for 15 minutes before use.

2- 40 µl of sample diluent was added to each well of plate, and then 10 µl of each sample of patients' serum was added to the wells, covered with plate paper, and incubated at 37 °C for 30min.

3- The paper plate was removed, then its contents were poured into the sink. The plate was washed by adding 300 µl of the washing solution. The washing process was repeated four times; then, the plate was struck several times to remove the water and dry the plate.

4- 50 µl of IL-10 conjugation solution was added to each field, which was covered and incubated at room temperature for 30min.

5- As in step 3, the washing and drying process was repeated four times.

6- 50 µl of chromogenic solution A and B 50 µl was added to each well and incubated in the dark for 15 minutes.

7- 50 µl of stop solution was added for terminating reaction .

8- Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

9- As shown in Figure 3.6, a standard curve generated through plotting of the absorbance value for standards values on y-axis and standards concentrations on x-axis by using Microsoft word excel drawing the standard curve to obtain equation linear to calculate the concentration IL-10 for each sample

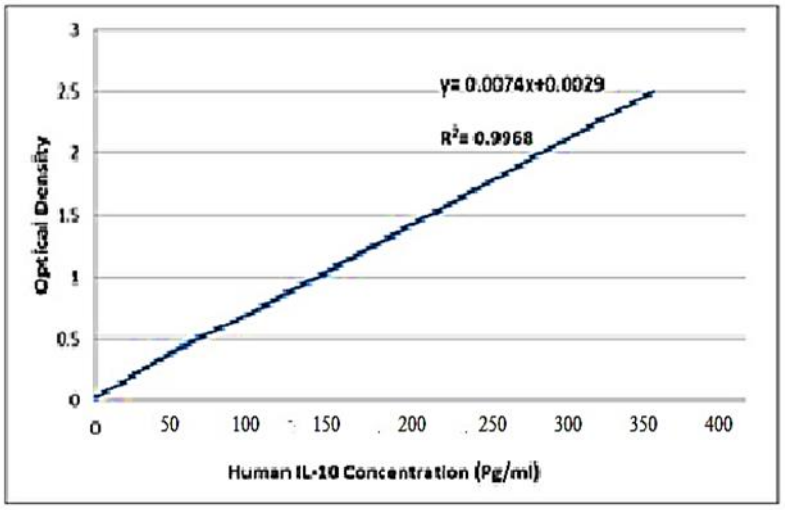


**Figure 3.6** IL-10 cytokine standard curve

### 3.6.4 IL-13 level

Principle test: Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the level of cytokinetic IL-13 in human serum, which has the specific antibodies needed to quantitatively measure the level of cytokinetic human IL-13 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 next step was to add 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.

**3.6.4.1 Components of the kit:**

1- Micro-calibration plate containing 96 holes coated with the IL-13 antibody.

2- 270 pg/mL of the standard solution.

3- Conjucated solution.

4- Standard diluent solution.

5- Sample diluent.

6- Washing solution.

7- Chromrgenic reagent solution A.

8- Chromrgenic Reagent Solution B.

9- Stop solution

10- Plate covers.

### 3.6.4.2 Standard concentrations preparation :

1- It was prepared by taking five tubes labeled 1-5 and then 150 µl from the standard diluent was added to each tube.
2- The first standard concentration (180 pg/ml) was prepared by taking 300µl from the original standard (270 pg/ml) and was added to the first
3- The second concentration (120 pg/ml ) was prepared by taking from the first concentration 300 µl and adding it to the second tube
4- The third concentration (60 pg/ml ) was prepared by taking from the second concentration 150 µl and adding it to the third tube.
5- The fourth concentration (30 pg/ml ) was prepared by taking from the third concentration 150 µl and adding it to the fourth tube
6- The fifth concentration (15 pg/ml ) was prepared by taking from the fourth concentration 150 µl and adding it to the fifth tube as shown in Figure 3.7 .

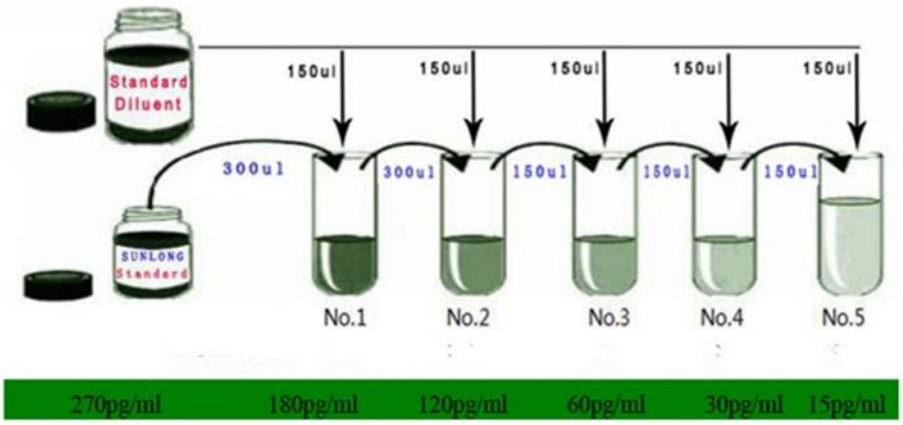


**Figure 3.7** Sandards concentrations of IL-13

### 3.6.4.3 Procedure

1- The kit components and serum samples were placed at room temperature for 15 minutes before use.

2- 40 µl of sample diluent was added to each well of plate, and then 10 µl of each sample of patients' serum was added to the wells, covered with plate paper, and incubated at 37 °C for 30min.

3- The paper plate was removed, then its contents were poured into the sink. The plate was washed by adding 300 µl of the washing solution. The washing process was repeated four times; then, the plate was struck several times to remove the water and dry the plate.

4- 50 µl of IL-13 conjugation solution was added to each field, which was covered and incubated at room temperature for 30min.

5- As in step 3, the washing and drying process was repeated four times.

6- 50 µl of chromogenic solution A and B 50 µl was added to each well and incubated in the dark for 15 minutes.

7- 50 µl of stop solution was added for terminating reaction .

8- Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

9- As shown in Figure 3.8, A standard curve generated through plotting of the absorbance value for standards values on y-axis and standards concentrations on x-axis by using Microsoft word excel drawing the standard curve to obtain equation linear to calculate the concentration IL-13 for each sample.

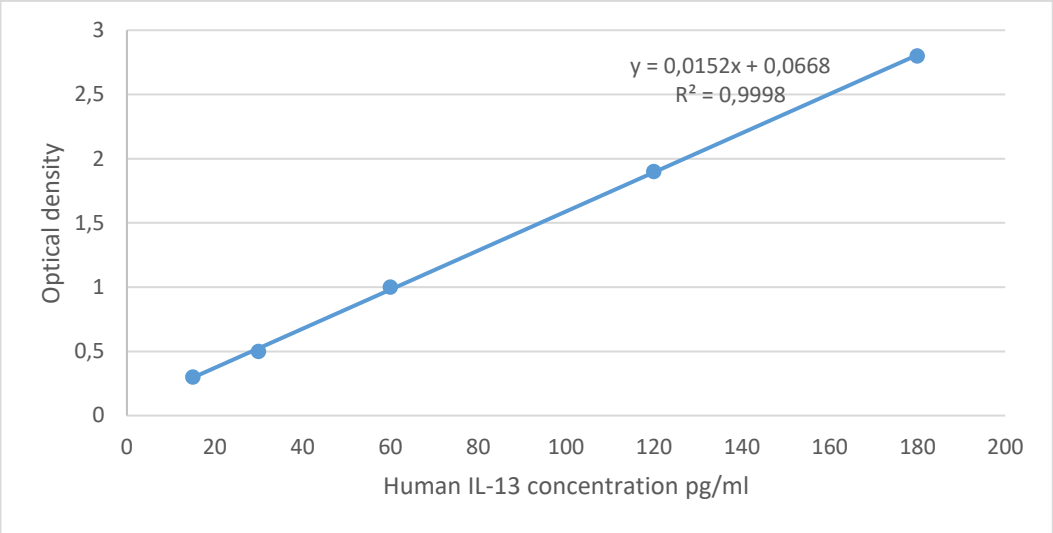


**Figure 3.8** IL-13 cytokine standard curve

### 3.6.5 IL-17 level

Principle test: An enzyme-linked immunosorbent assay (ELISA) was used to measure the cytokinetic IL-17 in human serum, which contains the specific antibodies needed to quantitatively measure the level of cytokine human IL-17 on the surface of the wells in the micro-calibration plate. When these bodies were mixed with the sample and standard solutions, antibodies only linked to each other and not to anything else. During the second incubation, secondary antibodies got rid of any antibodies that were not linked. Then, the matched enzyme was added to link the antibodies.

At the end of the process, the wells were washed to eliminate any antibodies that were not attached to anything. The substrate was then added. A blue color is produced depending on how much and how strongly the antibodies react with the antibody. Using an ELISA device and a wavelength of 450 nm, how well it lets light through.

#### 3.6.5.1 Components of the kit:

1- Micro-calibration plate containing 96 wells coated with the IL-17 antibody.

2- 270 pg/mL of the standard solution.

3- Conjucated solution.

4- Standard diluent solution.

5- Sample diluent.

6- Washing solution.

7- Chromrgenic reagent solution A.

8- Chromrgenic Reagent Solution B.

9- Stop solution

10- Plate covers.

**3.6.5.2 Standard concentrations preparation**

1- It was prepared by taking five tubes labeled 1-5 and then 150 µl from the standard diluent was added to each tube.
2- The first standard concentration 180pg/ml) was prepared by taking 300µl from the original standard ( 270pg/ml) and was added to the first .
3- The second concentration (120 pg/ml) was prepared by taking from the first concentration 300 µl and adding it to the second tube .
4- The third concentration (60 pg/ml) was prepared by taking from the second concentration 150 µl and adding it to the third tube .
5- The fourth concentration (30 pg/ml) was prepared by taking from the third concentration 150 µl and adding it to the fourth tube.

6- The fifth concentration (15pg/ml) was prepared by taking from the fourth concentration 150 µl and adding it to the fifth tube as shown Figure 3.9.

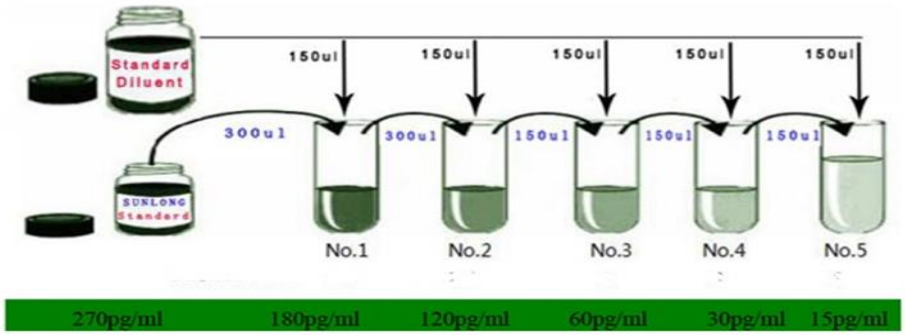


**Figure 3.9** Standards concentrations of IL-17

### 3.6.5.3 Procedure

1- The kit components and serum samples were placed at room temperature for 15 minutes before use.

2- 40 µl of sample diluent was added to each well of plate, and then 10 µl of each sample of patients' serum was added to the wells, covered with plate paper, and incubated at 37 °C for 30.

3- The paper plate was removed, then its contents were poured into the sink. The plate was washed by adding 300 µl of the washing solution. The washing process was repeated four times; then, the plate was struck several times to remove the water and dry the plate.

4- 50 µl of IL-17 conjugation solution was added to each field, which was covered and incubated at room temperature for 30min.

5- As in step 3, the washing and drying process was repeated four times.

6- 50 µl of chromogenic solution A and B 50 µl was added to each well and incubated in the dark for 15 minutes.

7- 50 µl of stop solution was added for terminating reaction .

8- Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

9- As shown in Figure 3.10, A standard curve generated through plotting of the absorbance value for standards values on y-axis and standards concentrations on x-axis by using Microsoft word excel drawing the standard curve to obtain equation linear to calculate the concentration IL-17 for each sample.

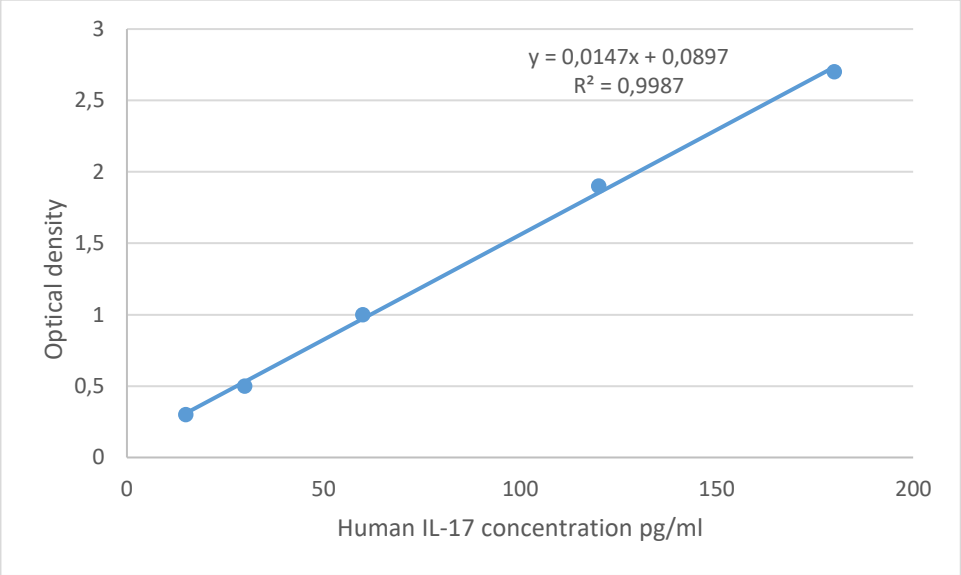


**Figure 3.10** IL-17 cytokine standard curve

### 3.6.6 IFNγ levels

Principle test: Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the level of cytokinetic IFN-γ in human serum, which has the specific antibodies needed to quantitatively measure the level of cytokinetic human IFN-γ on the surface of the wells in the micro-calibration plate. When the sample and control solutions are put on the antibodies, they stick to each other but not to other 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 next step was to add 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.

**3.6.6.1 Components of the kit:**

1- The micro-calibration plate with 96 holes coated with the IFNγ antibody.

2- 108 pg/mL of the standard solution.

3- Conjucated solution.

4- Standard diluent solution.

5- Sample diluent.

6- Washing solution.

7- Chromrgenic reagent solution A.

8- Chromrgenic Reagent Solution B.

9- Stop solution

10- Plate covers.

**3.6.6.2 Standard concentrations preparation :**

1- It was prepared by taking five tubes labeled 1-5 and then 150 µl from the standard diluent was added to each tube.

2-The first standard concentration (72pg/ml) was prepared by taking 300µl from the original standard (108pg/ml) and was added to the first tube.

3-The second concentration (48pg/ml) was prepared by taking from the first concentration 300 µl and adding it to the second tube.

4-The third concentration (24pg/ml) was prepared by taking from the second concentration 150 µl and adding it to the third tube .

5- The fourth concentration (12pg/ml ) was prepared by taking from the third concentration 150 µl and adding it to the fourth tube

6-The fifth concentration (6pg/ml ) was prepared by taking from the fourth concentration 150 µl and adding it to the fifth tube as shown in Figure 3.11.

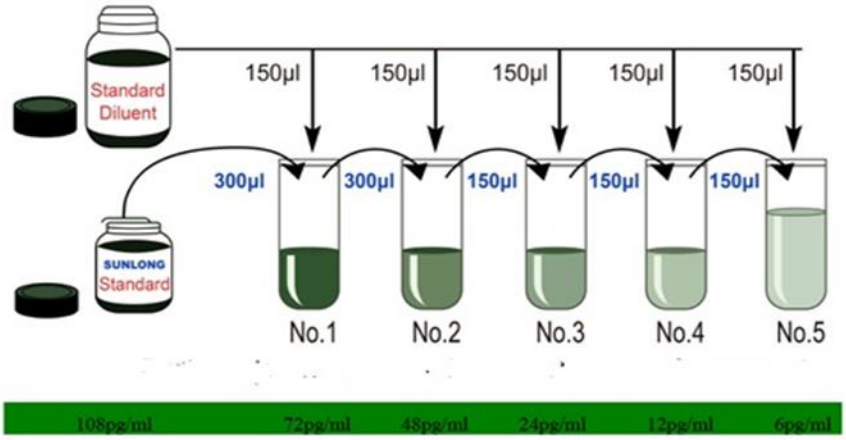


**Figure 3.11** Standards concentrations of IFNγ

### 3.6.6.3 Procedure

1- The kit components and serum samples were placed at room temperature for 15 minutes before use.

2- 40 µl of sample diluent was added to each well of plate, and then 10 µl of each sample of patients' serum was added to the wells, covered with plate paper, and incubated at 37 °C for 30 min.

3- The paper plate was removed, then its contents were poured into the sink. The plate was washed by adding 300 µl of the washing solution. The washing process was repeated four times; then, the plate was struck several times to remove the water and dry the plate.

4- 50 µl of IFNγ conjugation solution was added to each field, which was covered and incubated at room temperature for 30min.

5- As in step 3, the washing and drying process was repeated four times.

6- 50 µl of chromogenic solution A and B 50 µl was added to each well and incubated in the dark for 15 minutes.

7- 50 µl of stop solution was added for terminating reaction .

8- Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

9- As shown in Figure 3.12 a standard curve generated through plotting of the absorbance value for standards values on y-axis and standards concentrations on x-axis by using Microsoft word excel drawing the standard curve to obtain equation linear to calculate the concentration IFNγ for each sample.

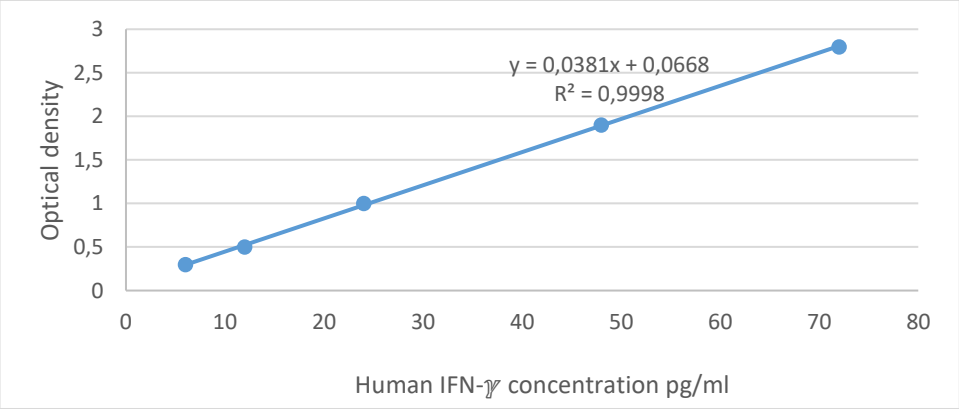


**Figure 3.12** IFNγ cytokine standard curve

### 3.7 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

## 4.1 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).

The identification of the different species of Leishmaniasis is based on clinical and laboratory diagnosis. The diagnosis and epidemiologist of Leishmania depend on several factors, such as the geographical distribution of isolates, the distribution of reservoir animals and humans, the habitat of the vector, and socio-environmental factors (Awab 2023).

## 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 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<br>NS:Non significant | | | | |

The current study showed adults between the ages of (6 -10 and (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 4.1 shows amastigote phase of CL and Figure 4.2 show the ulcer in patient Figure 4.3 and Figure 4.4.

**Figure 4.1** Microscopic examination of cutaneuos leishmaniasis

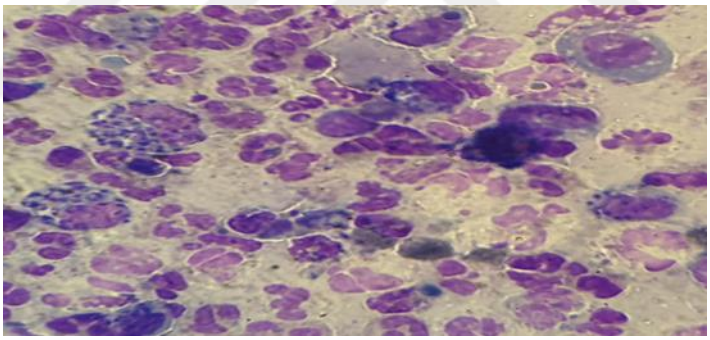

**Figure 4.2** Amastigote phase of cutaneuos leishmaniasis

**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 infection.

## REFERENCES


Al Daari, M., Al Haidari, S. and Al Amad, M., 2022. Epidemiology of Cutaneous Leishmaniasis in Yemen, 2019-2020. International Journal of Epidemiology and Public Health Research, 2(1): 1-4.

Al-Aubaidi, I.K. 2011. Serum Cytokine Production in Patients with Cutaneous Leishmaniasis Before and After Treatment. Iraqi Journal of medical sciences, 9(1): 55-60.

Al-Bajalan, M.M., Niranji, S.S., Al-Jaf, S.M. and Kato, H. 2021. First molecular identification of Leishmania major in Phlebotomus papatasi in an outbreak cutaneous leishmaniasis area in Iraq. Acta Tropica, 215: 105807.

Alexander, J. and Brombacher, F. 2012. T helper1/t helper2 cells and resistance/susceptibility to Leishmania infection: is this paradigm still relevant. Frontiers in Immunology, 3(80): 1-13

Alharazi, T.H., Haouas, N. and Al-Mekhlafi, H.M. 2021. Knowledge and attitude towards cutaneous leishmaniasis among rural endemic communities in Shara'b district, Taiz, southwestern Yemen. BMC infectious diseases, 21: 1-12.

Al-Hayali, H.L.and Al-Kattan,M.M 2021. Overview on epidemiology of leishmaniasis in Iraq. Rafidain Journal of Science, 30(1):.28-37.

Ali, M.A., Khamesipour, A., Rahi, A.A., Mohebali, M., Akhavan, A., Firooz, A. and Keshavarz, H.V. 2018. Epidemiological study of cutaneous leishmaniasis in some Iraqi provinces. Journal of Men's Health.14(4): 18–24

Alnaimy, A. and Al-Waaly, A.B. 2021. Investigation of Leishmania cutanenous Antigens in blood Specimens. Al-Qadisiyah Journal of Pure Science, 26(2):1-8.

Al-Obaidi, H.S.A. and Kamal, A. 2007. Measurement of Cytokines Concentration (IFN-γ and IL-10) in the Serum of Cutaneous leishmaniasis patients. Tikrit Journal of Pure Science, 12(1): 55-61.

Al-Obaidi, M. J., Abd Al-Hussein, M. Y. and Al-Saqur, I. M. 2016. Survey study on the prevalence of CL in Iraq. Iraqi Journal of Science, 5(23): 2181–2187.

Alraey, Y. 2022. Distribution and epidemiological features of cutaneous leishmaniasis in Asir Province, Saudi Arabia, from 2011 to 2020. Journal of Infection and Public Health, 15(7) :757-765.

Al-Rashed, A.S., Al Jindan, R., Al Jaroodi, S., Al Mohanna, A., Abdelhady, A. and El-Badry, A.A. 2022. Genotypic and phylogenic analyses of cutaneous leishmaniasis in Al Ahsa, Eastern Saudi Arabia during the coronavirus disease 2019 pandemic: First cases of Leishmania tropica with the predominance of Leishmania major. Scientific Reports, 12(1): 10753.

Alsaad, R. K. A. and Kawan, M. H. 2021. An epidemiological study of Cutaneous leishmaniasis in human and dogs. Annals of Parasitology, 67(3): 417–433.

AlSamarai, A.M. and AlObaidi, H.S. 2009. Cutaneous leishmaniasis in Iraq. The Journal of Infection in Developing Countries, 3(2): 123-129.

Al-Warid, H. S., Al-Saqur, I. M., Al-Tuwaijari, S. B. and Zadawi, K. A. M. A. L. 2017. The distribution of Cutaneous leishmaniasis in Iraq: demographic and climate aspects. Asian Biomedicine, 11(3): 255–260.

Anderson, C. F., Stumhofer, J. S., Hunter, C. A. and Sacks, D. 2009. IL-27 regulates IL-10 and IL-17 from CD4+ cells in nonhealing Leishmania major infection. The Journal of Immunology, 183(7): 4619–4627.

Andrade-Narváez, F. J., Vargas-González, A., Canto-Lara, S. B. and Damián-Centeno, A. G. 2001. Clinical picture of cutaneous Leishmaniases due to Leishmania (Leishmania) mexicana in the Yucatan peninsula, Mexico. Memorias do Instituto Oswaldo Cruz, 96: 163-167.

Awab, G.R. 2023. Leishmaniasis Epidemiology and Psychosocial Aspect. IntechOpen. doi: 10.5772/intechopen, 11: 568

Bacellar, O., Faria, D., Nascimento, M., Cardoso, T. M., Gollob, K. J., Dutra, W. O., Scott, P. and Carvalho, E. M. 2009. Interleukin 17 production among patients with American Cutaneous leishmaniasis.. The Journal of Infectious Diseases, 2(1): 75–78.

Banerjee, A., Bhattacharya, P., Joshi, A. B., Ismail, N., Dey, R., and Nakhasi, H. L. 2016. Role of pro-inflammatory cytokine IL-17 in Leishmania pathogenesis and in protective Immunity by Leishmania vaccines. Cellular Immunology, 309: 37–41.

Bari, A.U., and Simeen, B. R. 2008. CL an overview of parasitology and host-parasite-vector inter relationship. Journal of Pakistan Association of Dermatologists, 18: 42-48

Barral, A., Guerreiro, J., Bomfim, G., Correia, D., Barral-Netto, M. and Carvalho, E. M., 1995. Lymphadenopathy as the first sign of human cutaneous infection by Leishmania braziliensis. The American journal of tropical medicine and hygiene, 53(3): 256-259.

Bashir, Y. 1954. A Preliminary Report on the Occurrence of Infantile Kala-Azar in Northern Iraq. Bulletin of Endemic Diseases, 1(1): 77-80.

Bizri, N. A., Alam, W., Khoury, M., Musharrafieh, U., Ghosn, N., Berri, A. and Bizri, A.R. 2021. The association between the syrian crisis and cutaneous leishmaniasis in Lebanon. Acta Parasitologica .66: 1240–1245.

Blum, J., Desjeux, P., Schwartz, E., Beck, B. and Hatz, C. 2004. Treatment of cutaneous Leishmaniasis among travellers. Journal of Antimicrobial Chemotherapy, 53(2): 158-166.

Bonfim, N. E. S. M. T., Scott, A. L. B. and de Azevedo Calderon, L. 2022. Leishmaniasis: Molecular Aspects of Parasite Dimorphic Forms Life Cycle. Leishmaniasis: General Aspects of a Stigmatized Disease, 1: 3.

Bray, R. S., and Chang, K. P. 1985. Human parasitic disease.Elsevier, 7: 393–413.

Bryson, K. J., Millington, O. R., Mokgethi, T., McGachy, H. A., Brombacher, F., and Alexander, J. 2011. BALB/c mice deficient in CD4 T cell IL-4Rα expression control Leishmania mexicana Load although female but not male mice develop a healer phenotype. PLoS neglected tropical diseases, 5(1): e930.

Burns, T., Breathnach, S., Cox, N. and Griffiths, C. 2004. Rooks textbook of dermatology: volume (2) 7th ed . Blackwell Science Ltd .USA, 4: 76.

Burza, S., Mahajan, R., Kazmi, S., Alexander, N., Kumar, D., Kumar, V., Lasry, E., Harshana, A., de Lima Pereira, A., Das, P. and Verma, N., 2022. AmBisome Monotherapy and Combination AmBisome–Miltefosine Therapy for the Treatment of Visceral Leishmaniasis in Patients Coinfected With Human Immunodeficiency Virus in India: A Randomized Open-Label, Parallel-Arm, Phase 3 Trial. Clinical Infectious Diseases, 75(8) :1423-1432.

CDC. 2020. Leishmaniasis. https://www.cdc.gov/parasites/leishmaniasis Date of access: 12.05.2023

CDC. 2012. Web site. https://www.cdc.gov/parasites/leishmaniasis/diagnosis.html Date of access: 03.11.2022.

Chan-Bacab, M. J., and Peña-Rodríguez, L. M. 2001. Plant natural products with leishmanicidal activity. Natural Product Reports, 18(6): 674-688.

da Silva, R. R., Vasconcelos, F. D. S. F., Tavares, D. D. S., and Dos Santos, P. L., 2022. Association between interleukin 10 (IL-10) polymorphisms and leishmaniasis progression: a systematic review and meta-analysis. Scientific Reports, 12(1): 11136.

Daham, A. K. and Al-Husseini, T. I. 2011. Epidemiological survey of leishmaniasis in Salah al-Din Governorate. Diyala Journal of Agricultural Sciences, 3(2): 111-113.

de Vries, H. J. and Schallig, H. D. 2022. Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. American Journal of Clinical Dermatology, 23(6): 823-840.

Despommier, D. D. and Karapelou, J. W. 2012. Parasite life cycles. Springer Science & Business Media, 2: 14-17

Dubie, T., and Mohammed, Y. 2020. Review on the role of host immune response in protection and immunopathogenesis during cutaneous leishmaniasis infection. Journal of immunology research, 3:1-12

Ferreira, D. T., Andrei, C. C., Saridakis, H. O., Faria, T. D. J., Vinhato, E., Carvalho, K. E., and Braz-Filho, R. 2004. Antimicrobial activity and chemical investigation of Brazilian Drosera. Memórias do Instituto Oswaldo Cruz, 99: 753-755.

Flaih, M. H., 2022. Geographical Distribution of Cutaneous Leishmaniasis and Pathogenesis. Leishmaniasis: General Aspects of a Stigmatized Disease, 1: 14.

Flaih, M. H., Al-Abady, F. A. and Hussein, K. R. 2021. Phylogenetic analysis of kinetoplast DNA: kDNA of Leishmania tropica in Thi-Qar province, Iraq. Comparative Immunology, Microbiology and Infectious Diseases, 78: 101696.

Gaber, Y.A., Al-Sanabani, R., Annuzaili, D. A., Al-Danakh, A. and Ling, L. C. 2022. Research progress of health care in Yemeni children during the war. Primary Health Care Research & Development, 23: e55.

Ghatee, M. A., Taylor, W. R. and Karamian, M. 2020. The geographical distribution of cutaneous leishmaniasis causative agents in Iran and its neighboring countries, a review. Frontiers in public health, 8 :11.

Gonzalez-Lombana, C., Gimblet, C., Bacellar, O., Oliveira, W. W., Passos, S., Carvalho, L. P., Goldschmidt, M., Carvalho, E., M. and Scott, P. 2013. IL-17

mediates immunopathology in the absence of IL-10 following Leishmania major infection. PLoS Pathogens, 9(3): e1003243.

Goodman, S. R. 2020. Goodman's Medical Cell Biology, Academic Press, 3(9): 337-360

Gorbach, S. L., Bratlett, J. G., and Blacklow, N. R. 1998. Infection disease. 2nd (edn).W.B. Saunders.co, USA, 7: 123-125.

Gupta, G., Oghumu, S., and Satoskar, A.R. 2013. Mechanisms of immune evasion in leishmaniasis. In Advances in applied microbiology Vol. 82 .Academic Press, 8: 155-184.

Güran, M. 2018. An overview of leishmaniasis: historic to future perspectives. Vectors and Vector-Borne Zoonotic Diseases, 5: 76.

Hakkour, M., Hmamouch, A., Mahmoud El Alem, M., Bouyahya, A., Balahbib, A., El Khazraji, A., Fellah, H., Sadak, A. and Sebti, F. 2020. Risk factors associated with leishmaniasis in the most affected provinces by leishmania infantum in Morocco. Interdisciplinary Perspectives on Infectious Diseases, 2: 1-9.

Hazra, B., Golenser, J., Nechemiya, O., Bhattacharyya, S., Azzam, T., Domb, A., and Frankenburg, S. 2002. Inhibitory activity of diospyrin derivatives against Leishmania major parasites in vitro. Indian Journal of Pharmacology, 34(6): 422-427.

Herrer, A., and Christensen, H. A. 1975. Implication of Phlebotomus sandflies as vectors of bartonellosis and Leishmaniasis as early as 1764. Science, 190: 154–155.

Hijawi, K. J. F., Hijjawi, N. S., and Ibbini, J. H. 2019. Detection, genotyping, and phylogenetic analysis of Leishmania isolates collected from infected Jordanian residents and Syrian refugees who suffered from Cutanous Leishmaniasis. Parasitology Research, 118(3): 793–805.

Hoare, C.A., 1938. Early discoveries regarding the parasite of oriental sore. Transactions of the Royal Society of Tropical Medicine and Hygiene, 32(1): 66-92.

Holowka, T., and Bucala, R., 2020. Role of host and parasite MIF cytokines during Leishmania infection. Tropical Medicine and Infectious Disease, 5(1): 1-12.

Hölscher, C., Arendse, B., Schwegmann, A., Myburgh, E., and Brombacher, F. 2006. Impairment of alternative macrophage activation delays cutaneous Leishmaniasis in nonhealing BALB/c mice. The Journal of Immunology, 176(2): 1115–1121.

Hölscher, C., Mohrs, M., Dai, W. J., Köhler, G., Ryffel, B., Schaub, G. A., Mossmann, H., and Brombacher, F. 2000. Tumor necrosis factor alpha-mediated toxic shock in Trypanosoma cruzi-infected interleukin 10-deficient mice. Infection and Immunity, 68(7): 4075–4083.

Husain F. Hassan, Sahla Kh. Abbas and Dunya S. Shakoor. 2016. Evaluation of Interleukin IL-17A, IL-33, and Serum Levels of Zinc, Iron and Adenosine Deaminase in Cutaneous Leishmaniasis in Kirkuk, Iraq. International Journal of Current Research and Academic Review, 4(5): 179-185

Hussein, A. M., and Ali, H. Z. 2022. Detection of TNF Alpha Level as Biomarker in Different Stages of Cutaneous Leishmaniasis Infection. Iraqi Journal of Science, 63(8): 3313–3321.

Hussein, A. A., Al-Marsome, H. T., and Al-Musawi, H. N. 2015. Role of IL-17 and IL-27 in Cutaneous Leishmaniasis. International Journal, 3(6): 267-270.

Iniesta, L., Gállego, M., and Portús, M. 2005. Immunoglobulin G and E responses in various stages of canine Leishmaniasis. Veterinary Immunology and Immunopathology, 103(1–2): 77–81.

Iniesta, V., Carcelén, J., Molano, I., Peixoto, P. M., Redondo, E., Parra, P., and Corraliza, I. 2005. Arginase I induction during Leishmania major infection mediates the development of disease. Infection and Immunity, 73(9): 6085-6090.

Ismail, R. M., and As' ad, E. A. 2015. Study of local and bloody variability in children with leishmaniasis parasites in the city of Kirkuk. Journal of the University of Babylon for Pure and Applied Sciences, 23(1): 308-320.

Jameel, R. A., and Al-Qadhi, B. N. 2020. Monitoring of the Immune Response Activity in Iraqi Patients Infected with Cutaneous Leishmaniasis by IFN-Î³ and MIG Evaluation During Different Stages of Infection. Iraqi Journal of Science 61(5): 970-977

Kareem, M. A., Al-Obaidi, M. J., Al-Obaidi, M. J. ., Al-Hussein , M. Y. A., Al-Hussein, M. Y. A., and Al-Saqur, I. M. 2022. Survey Study on the Prevalence of Cutaneous Leishmaniasis in Iraq . Iraqi Journal of Science, 57(3C): 2181–2187.

Kaye, P. M., Cruz, I., Picado, A., van Bocxlaer, K., and Croft, S. L. 2020. Leishmaniasis immunopathology—impact on design and use of vaccines, diagnostics and drugs. Seminars in Immunopathology, 42(3): 247–264.

Kato, H., Gomez, E. A., Martini-Robles, L., Muzzio, J., Velez, L., Calvopina, M., Romero-Alvarez, D., Mimori, T., Uezato, H., and Hashiguchi, Y. 2016. Geographic distribution of Leishmania species in Ecuador based on the cytochrome b gene sequence analysis. PLoS Neglected Tropical Diseases, 10(7): e0004844.

Khamesipour, A., Molaei, S., Babaei-Pouya, N., and Moradi-Asl, E., 2020. Cutaneous Leishmaniasis Situation and Predicting the Distribution of Phlebotomus papatasi and P. sergenti as Vectors of Leishmaniasis in Ardabil Province, Iran. The Korean Journal of Parasitology, 58(3): 229-236.

Khan, M. Z. I., Tucker, I. G., and Opdebeeck, J. P. 2020. Antigen Delivery Systems Used to Induce Immunomodulation. In Immune Modulating Agents, CRC Press, 3(11): 385–419,

Khan, N. H., Bari, A. U., Hashim, R., Khan, I., Muneer, A., Shah, A., Wahid, S., Yardley, V., O'Neil, B., and Sutherland, C. J. 2016. Cutaneous Leishmaniasis in Khyber Pakhtunkhwa Province of Pakistan:Clinical Diversity and Species-Level Diagnosis. The American journal of tropical medicine and hygiene, 95(5): 1106–1114.

Klein, S. L., and Flanagan, K. L. 2016. Sex differences in immune responses. Nature Reviews Immunology, 16(10): 626-638.

Knight, C. A., Harris, D. R., Alshammari, S. O., Gugssa, A., Young, T., and Lee, C.M. 2023. Leishmaniasis: Recent epidemiological studies in the Middle East. Frontiers in Microbiology, 13: 1-13.

Kobets, T., Grekov, I., and Lipoldova, M. 2012. Leishmaniasis: prevention, parasite detection and treatment. Current Medicinal Chemistry, 19(10): 1443–1474.

Kumar, R., Bumb, R. A., Ansari, N. A., Mehta, R. D., and Salotra, P. 2007. CL caused by L. tropica in Bikaner, India: parasite identification and characterization using molecular and immunologic tools. The American Journal of Tropical Medicine and Hygiene, 76(5): 896–901.

Kumar, V., Das, S., Kumar, A., Tiwari, N., Kumar, A., Abhishek, K., Mandal, A., Kumar, M., Shafi, T., Bamra, T., and Singh, R.K., 2020. Leishmania donovani infection induce differential miRNA expression in CD4+T cells. Scientific Reports, 10(1): 3523.

Lachman, P. J., Peters, D. K., Rosen, F. S., and Walport, M. J. 1993. Clinical aspects of immunology Vol.3. Maple-Vial, Book, Hong Kong.

Lockard, R. D., Wilson, M. E., and Rodríguez, N. E. 2019. Sex-Related Differences in Immune Response and Symptomatic Manifestations to Infection with Leishmania Species. Journal of immunology research, Volume 20(9): 4103819

Liew, F. Y., and O'donnell, C. A. 1993. Immunology of Leishmaniasis. Advances in Parasitology, 32: 161–259.

Liu, D., and Uzonna, J. E. 2012. The early interaction of Leishmania with macrophages and dendritic cells and its influence on the host immune response. Frontiers in cellular and infection microbiology, 2: 1-8.

Lopez Kostka, S., Dinges, S., Griewank, K., Iwakura, Y., Udey, M. C., and von Stebut, E. 2009. IL-17 promotes progression of cutaneous Leishmaniasis in susceptible mice. The Journal of Immunology, 182(5): 3039–3046.

Louassini, M., Foulqie, M., Benitez, R., and Adroher ,J. 1999. Citric acid cycle Key enzyme activites during In vitro growth and metacycologensis of Leishmania infantum promastigotes. The Journal of parasitology, 85(4): 595-602.

Mahmoodi, M., Rajabalian, S., Fekri, A., and Esfandiarpour, I. 2005. Evaluation of In Vitro Production of IFN-gamma, IL-10, IL-12 and IL-13 by Blood Cells in Patients with Cutaneous Leishmaniasis Lesions. Iranian journal of allergy, asthma, and immunology, 4(1): 15-21.

Mandell, G. L., Bennett, J. E., Dolin, R., and Schwartz, D. A. 1990. Principles and practice of infectious disease. Archives of Pathology and Laboratory Medicine, 121(8): 908.

Markel, W. H., and Voge, S. 2006. Medical parasitology Elsevier, Inc, Ninth Edition.U.S.

Markle, W. H. and Makhoul, K. 2004. Cutaneous Leishmaniasis recognition and treatment. American Family Physician, 69(6): 1455-1460.

Maspi, N., Abdoli, A., and Ghaffarifar, F. 2016. Pro-and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathogens and global health, 110(6): 247-260.

Maxfield, L. and Crane, J. S. 2022. Web site. https://www. ncbi. nlm. nih. gov/books /NBK531456. Date of access: 01.11.2022.

Mcgwire, B. S., and Satoskar, A. R. 2014. Leishmaniasis: clinical syndromes and treatment. An International Journal of Medicine, 107(1): 7–14.

Mendonça, L. S. O., Santos, J. M., Kaneto, C. M., de Carvalho, L. D., Lima-Santos, J., Augusto, D. G., Carvalho, S. M. S., Soares-Martins, J. A. P., and Silva-Jardim, I. 2020. Characterization of serum cytokines and circulating microRNAs that are predicted to regulate inflammasome genes in cutaneous leishmaniasis patients. Experimental parasitology, 210: 107846.

Mirzaie, F., Eslami, G., Yosefi, M. H., and Pestehchian, N. 2013. Molecular identification of Leishmania isolates obtained from patients suspected as having cutaneous leishmaniasis referred to reference laboratories from Yazd province in central Iran. Advanced biomedical research, 2(4):1-5

Monzote, L. 2009. Current treatment of Leishmaniasis: a review. The Open Antimicrobial Agents Journal, 1(1): 9-19.

Najim, R. A., Sharquie, K. E., and Farjou, I. B. 1998. Zinc sulphate in the treatment of cutaneous leishmaniasis: an in vitro and animal study. Memórias do Instituto Oswaldo Cruz, 93: 831-837.

Novoa, R., Bacellar, O., Nascimento, M., Cardoso, T. M., Ramasawmy, R., Oliveira, W. N., Schriefer, A., and Carvalho, E. M. 2011. IL-17 and Regulatory Cytokines (IL-10 and IL-27) in L. braziliensis Infection. Parasite Immunology, 33(2): 132–136.

O'Neil, C. E., Labrada, M., and Saravia, N. G. 1993. Leishmania (Viannia) panamensis-specific IgE and IgA antibodies in relation to expression of human tegumentary Leishmaniasis. The American Journal of Tropical Medicine and Hygiene, 49(2): 181–188.

Paniker, C. J., and Ghosh, S. 2017. Paniker's textbook of medical parasitology. JP Medical Ltd, 7: 143.

Pearson, R. D., Wheeler, D. A., Harrison, L. H., and Kay, H. D. 1983. The immunobiology of Leishmaniasis. Reviews of Infectious Diseases, 5(5): 907–927.

Pissinate, J. F., Gomes, I. T., Peruhype-Magalhães, V., Dietze, R., Martins-Filho, O. A., and Lemos, E. M. 2008. Upgrading the flow-cytometric analysis of anti-Leishmania immunoglobulins for the diagnosis of American tegumentary Leishmaniasis. Journal of Immunological Methods, 336(2): 193–202.

Ponte-Sucre, A. 2003. Physiological consequences of drug resistance in Leishmania and their relevance for chemotherapy. Kinetoplastid Biology and Disease, 2: 1-10.

Prieto-Granada, C. N., Lobo, A. Z., and Mihm Jr, M. C. 2018. Skin infections. In Diagnostic pathology of infectious disease, pp. 542-647, Elsevier.

Qurtas D. S. 2018. Cutaneous Leishmaniasis in Erbil Governorate: Clinical manifestations and disease course. Medical journal of the Islamic Republic of Iran, 32: e71.

Rahi, A. A., Nsaif, S., Hassoni, J. J., Ali, M. A., and Hamza, H. A. 2013. Comparison of diagnostic methods in Cutaneous Leishmaniasis in Iraq. American Journal Bio Sciences, 1(1): 1-5.

Rassi, Y., and Hanafi-Bojd, A.A. 2006. Phlebotomine sandflies, vectors of Leishmaniases: Morphology, biology, ecology, and field and laboratory methods with pictorial key of Iranian sandflies. Tehran, Iran: Noavaran-Elm Publication, 1(2): 51.

Roberts, L.. Schmidt, G.D.and Janovy, J. 2009. Foundations of parasitology. 8th ed., McGraw-Hill Company Inc. New York, NY. Pages 701

Rodríguez, A., Solano-Gallego, L., Ojeda, A., Quintana, J., Riera, C., Gállego, M., Portús, M., and Alberola, J. 2006. Dynamics of Leishmania-specific immunoglobulin isotypes in dogs with clinical Leishmaniasis before and after treatment. Journal of Veterinary Internal Medicine, 20(3): 495–498.

Rotimi, O., Cairns, A., Gray, S., Moayyedi, P., and Dixon, M. F. 2000. Histological identification of Helicobacter pylori: comparison of staining methods. Journal Clinical Pathology, 53(10): 756-759.

Saha, A., Roy, S., and Ukil, A., 2022. Cytokines and Signaling Networks Regulating Disease Outcomes in Leishmaniasis. Infection and Immunity, 90(8):e0024822.

Schmidt, G. D., and Roberts, L. S. 2005. Foundations of parasitology 7th ed.MC. Graw Hill , companies, America, 2: 16.

Shahatha, S. S. and Saleh, T. A. 2018. An epidemiological, diagnostic, and therapeutic study of the Leishmania tropica parasite in Iraq's Anbar Province. Baghdad Science Journal, 15(4): 392-400.

Sharma, U., and Singh, S. 2009. Immunobiology of leishmaniasis. Indian journal of experimental biology, 47(6): 412–423.

Singh, S., and Sivakumar, R. 2003. Recent advances in the diagnosis of Leishmaniasis. Journal of Postgraduate Medicine, 49(1): 55-60.

Singh, S., Dey, A., and Sivakumar, R. 2005. Applications of molecular methods for Leishmania control. Expert Review of Molecular Diagnostics, 5(2): 251-265.

Sivayogana, R., Krishnakumar, A., Kumaravel, S., Rajesh, R., and Ravikanth, P. 2022. Treatment of Leishmaniasis. Leishmaniasis: General Aspects of a Stigmatized Disease, 5: 76.

Souza, M. A., Castro, M., Oliveira, A. P., Almeida, A. F., Reis, L. C., Silva, C. J., Brito, M. E. F., and Pereira, V. R. A. 2012. American tegumentary Leishmaniasis: cytokines and nitric oxide in active disease and after clinical cure, with or without chemotherapy. Scandinavian Journal of Immunology, 76(2): 175–180.

Syed, A. R., Aman, S., Hussain, I., and Kazmi, S. A. H. 2006. Kashmor: focus of cutaneous leishmaniasis. Journal of Pakistan Association of Dermatologists, 16(3): 147-150.

Taher, H. M., Mohammed, S. A., and Mahmood, A. R. 2020. Determination of IFN-γ and TNF-α levels in serum of patients with Cutaneous leishmaniasis. Tikrit Journal of Pure Science, 25(6): 50-53.

Taj-el Deen, S. D., and Al Alousi, K. 1954. Kala-Azar in Iraq: Report of Four Cases. Journal of the Faculty of Medicine, Baghdad, 18(1-2): 15-19.

Tanaka, T., Narazaki, M., and Kishimoto, T. 2014. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor perspectives in biology, 6(10): e016295.

Torres-Guerrero, E., Quintanilla-Cedillo, M. R., Ruiz-Esmenjaud, J., and Arenas, R. 2017. Leishmaniasis: A review. F1000Research, 6: e750

Tripathi, L. K., and Nailwal, T. K., 2021. Leishmaniasis: an overview of evolution, classification, distribution, and historical aspects of parasite and its vector. Pathogenesis, Treatment and Prevention of Leishmaniasis, Volume 2: 1-25.

Turner, M. D., Nedjai, B., Hurst, T., and Pennington, D. J. 2014. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1843(11): 2563-2582.

Ullah, W., Yen, T. Y., Niaz, S., Nasreen, N., Tsai, Y. F., Rodriguez-Vivas, R. I., Khan, A., and Tsai, K. H. 2023. Distribution and Risk of Cutaneous Leishmaniasis in Khyber Pakhtunkhwa, Pakistan. Tropical Medicine and Infectious Disease, 8(2): 1-14.

Urban, J. F., Finkelman, F. D., Shea-Donohue, T., and Gause, W. C. 2020. Cytokine immunomodulation of infectious diseases. In Immune Modulating Agents:169-186, CRC Press.

Vidyashankar, C. 2002. Leishmaniasis. Journal of the Indian Medical Association, 100(1): 24-26.

Walton, B. C., Intermill, R. W., and Hajduk, M. E. 1977. Differences in biological characteristics of three Leishmania isolates from patients with espundia. The American Journal of Tropical Medicine and Hygiene, 26(5): 850-855.

Weina, P. J., Neafie, R. C., Wortmann, G., Polhemus, M., Aronson, N. E., and Strausbaugh, L. J. 2004. Old world Leishmaniasis: an emerging infection among deployed US military and civilian workers. Clinical Infectious Diseases, 39(11): 1674-1680.

WHO(World Health Organization). 2020. Health sector bulletin. Health sector Syria. Reporting period: 01-01-2020 to 31-01-2020. Available online at: https://healthcluster.who.int/ docs/ librariesprovider16/meeting-reports/syria-health-sector-bulletin-jan (accessed January 9, 2023).

WHO. 2005. WHO in Iraq: weekly bulletin 6-12 Jun. 2005. https://reliefweb.int/report/iraq/who-iraq-weekly-bulletin-6-12-jun-2005 (accessed June 27, 2023).

WHO.2000. Leishmania/HIV co-infection in south-western Europe 1990-1998: retrospective analysis of 965 cases (No. WHO/LEISH/2000.42). World Health Organization, 1: 11.

Yasmin, H., Adhikary, A., Al-Ahdal, M. N., Roy, S., and Kishore, U. 2022. Host–pathogen interaction in leishmaniasis: immune response and vaccination strategies. Immuno, 2(1): 218-254.

Yesilova, Y., Surucu, H. A., Ardic, N., Aksoy, M., Yesilova, A., Oghumu, S., and Satoskar, A. R. 2016. Meglumine antimoniate is more effective than sodium stibogluconate in the treatment of cutaneous leishmaniasis. The Journal of dermatological treatment, 27(1): 83–87.

Zahirnia, A. H., Bordbar, A., Ebrahimi, S., Spotin, A., Mohammadi, S., Ghafari, S. M., Ahmadvand, S., Jabbari, N., Esmaeili Rastaghi, A. R., and Parvizi, P. 2018. Predominance of Leishmania major and rare occurrence of Leishmania tropica with haplotype variability at the center of Iran. Brazilian Journal of Infectious Diseases, 22: 278-287.

Zuckerman, A. and Lainson, R. 1977. Leishmania In :parasitic protozoa Vol.1,kreier, ed (ed). Parasitic Protozoa. Academic press, 2: 7.

## APPENDICES

**APPENDIX 1. Patient approval form**

**APPENDIX 2. Questionnaire form**

**APPENDIX 3. Facilitating the task (In Turkish)**

**APPENDIX 4. Facilitating the task (In Arabic)**

**APPENDIX 5. Leishmania lesions**

**APPENDIX 1. Patient approval form**

We invite you to the research title "Evaluation of TNF-α, IL6, IL10, IL-13, IL-17, AND IFN- γ Levels and Immune Responses with Cutaneous Leishmaniasis in Protection and Pathogenesis" conducted by Ban Jasim Saadoon . Before deciding whether or not to participate in this research, you need to know why and how the research will be conducted. If there is something that you do not understand and is not clear to you, or if you want more information, ask us. Participation in this study is completely voluntary. You have the right to withdraw from the study at any time. Your response to the study will be interpreted as your consent to participate in the study. Do not be under pressure from anyone when answering the questions on the forms given to you. The personal information obtained from these forms will be kept completely confidential and will be used for research purposes only.

**Researcher's Name**

**Phone:**

**E-mail:**

**Interview date:**

**Participant Name:**

**Adress:**

**Signature:**

**APPENDIX 2. Questionnaire form**

1. Gender: Male
   Female

2. Occupation :

3. Age:

| | |
|---|---|
| **≥1** | |
| **1-5** | |
| **6-10** | |
| **11-15** | |
| **16-20** | |
| **21-25** | |
| **26-30** | |
| **31-35** | |
| **36-40** | |
| **41-45** | |
| **46-50** | |
| **≥ 51** | |

4. Patient Governorate:

| | |
|---|---|
| Urban | |
| Rural | |

5. Diagnosis and clinical signs:
6. Number of ulcers:
7. Location of ulcers in the body:

**APPENDIX 3. Facilitating the task (In Turkish)**

**APPENDIX 4. Facilitating the task (In Arabic)**

**APPENDIX 5. Cutaneous leishmaniasis lesions in face and legs**

## CURRICULUM VITAE

### Personal Information

Name and Surname : Ban Jasim Saadoon SAADOON

### Education

| | | |
|---|---|---|
| MSc | Çankırı Karatekin University<br>Graduate School of Natural and Applied Sciences<br>Department of Biology | 2021-2023 |
| Undergraduate | Samarra University<br>Faculty of Sciences<br>Department of Biology | 2016-2020 |