Çankırı Karatekin University
Graduate School of Health Sciences

Master of Science Thesis

IMMUNOLOGICAL EVALUATION OF PENTOSTAM AND IVERMECTIN USED IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN KIRKUK. IRAQ

GOLYAR ABBAS RASOOL RASOOL

Advisor

Asst. Prof. Dr. Banuçiçek YÜCESAN

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BY

GOLYAR ABBAS RASOOL RASOOL

The Institute of Health Sciences
The Department of Veterinary Parasitology

The Degree of Master of Science

SUPERVISOR
Asst. Prof. Dr. Banuçiçek YÜCESAN

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ACCEPTANCE AND APPROVAL

Golyar Abbas Rasool RASOOL, the graduate student of The Institute of Health Sciences with the student number of 208203201, has successfully presented her thesis entitled “Immunologıcal Evaluatıon of Pentostam and Ivermectın Used in the Treatment of Cutaneous Leıshmanıasıs in Kırkuk, Iraq” before the jury whose signatures are below, after fulfilling all of the requirements determined by the relevant regulations for the degree of Master of Science:

Advisor **:** Asst. Prof. Dr. Banuçiçek YÜCESAN
Co-Advisor **:** Asst. Prof. Dr. Shahad Abbas AL-ATTAR

Examining Committee Members:

Chairman **:** Asst. Prof. Dr. Banuçiçek YÜCESAN
Sağlık Bilimleri Fakültesi (Veteriner Parazitolojisi)
Çankırı Karatekin University

Member **:** Prof. Dr. Özcan ÖZKAN
Biyoloji Bölümü (Zooloji Anabilim Dalı)
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Aycan Nuriye GAZYAĞCI
Veteriner Fakültesi (Veteriner Parazitolojisi)
Kırıkkale University

Approved for the Graduate School of Health Sciences

Doç. Dr. Nazan KAYTEZ
Director of Graduate School

ETHICS STATEMENT

The thesis entitled “Immunologıcal Evaluatıon of Pentostam and Ivermectın Used in the Treatment of Cutaneous Leıshmanıasıs in Kırkuk, Iraq” which was prepared and presented as a thesis, was written by myself and in accordance with the scientific, academic rules and ethical conduct. The idea/hypothesis of my thesis solely belongs to my supervisor and to me. The research pertaining to the thesis was conducted by myself and therefore, all of the used sentences and interpretations within the work belongs to me.

I declare the aforementioned issues to be correct.

Signature

2023

Golyar Abbas Rasool RASOOL

ABSTRACT

IMMUNOLOGICAL EVALUATION OF PENTOSTAM AND IVERMECTIN USED IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN KIRKUK, IRAQ

Golyar Abbas Rasool RASOOL

Master of Science in Veterinary Parasitology

Advisor: Asst. Prof. Dr. Banuçiçek YÜCESAN

Co-Advisor: Asst. Prof. Dr. Shahad Abbas AL-ATTAR

January 2023

The aim of this study was to obtain information about the parasitic disease Cutaneous Leishmaniasis (CL), which has started to spread recently, in Iraq. To investigate the prevalence of parasites in the hospitals in the study area and to obtain information about the detected cases and to compare the currently used drugs (Pentostam and Ivermectin) with each other and to determine which one is more effective. The current study included patients infected with CL who attended a number of government hospitals in Kirkuk governorate and its affiliated districts, which include Hawija, Daquq, Debs, Riyadh, Taza and Altun Kobri for the period between 01/03/2022 to 15/08/2022.

The current study showed that the highest rate of infection was in Hawija district.

The rate was higher in males than in females. The study also included age groups ranging from less than one year to 70 years, and the most vulnerable age group to infection was the age group (1-10) years.

There was a significant difference between the two treatments regarding the levels of immunoglobulins. The ivermectin group significantly inhanced the levels of the immune globulines (IgG, IgM) more than Pentostam but the IgE levels was higher in Pentostam group. The results suggest that treating of CL with different drugs, showed that the efficacy of ivermectin was higher than Pentostam in treatment of CL.

2023, 77 pages

Keywords: Cutaneous leishmaniasis, Parasite, Treatment, Ivermectin, Pentostam

ÖZET

IRAK, KERKÜK'TE KUTANÖZ LEİSMANİASİS TEDAVİSİNDE KULANILAN PENTOSTAM VE İVERMEKTİN'İN İMMÜNOLOJİK AÇIDAN DEĞERLENDİRİLMESİ

Golyar Abbas Rasool RASOOL

Veteriner Parazitolojisi, Yüksek Lisans

Tez Danışmanı: Dr. Öğr. Üyesi Banuçiçek YÜCESAN

Eş Danışman: Dr. Öğr. Üyesi Shahad Abbas AL-ATTAR

Ocak 2023

Bu çalışmanın amacı, Irak'ta son yıllarda yayılmaya başlayan parazite hastalıklarından biri olan Kutanöz Leishmaniasis (KL) hakkında bilgi elde etmektir. Çalışma alanındaki hastanelerde parazit prevalansını araştırmak ve tespit edilen vakalar hakkında bilgi edinmek ve halihazırda kullanılan ilaçları (Pentostam ve İvermectin) birbirleriyle karşılaştırmak ve hangisinin daha etkili olduğunu belirlemektir. Mevcut çalışma, Kerkük şehrı ve Hawija, Daqug, Debs, Riyad, Taza ve Altun Kobri gibi bağlı ilçelerdeki devlet hastanelerine başvuran KL hastalarını içermektedir. Epidemiologik olarak alınan bilgiler 01/03/2022 - 15/08/2022 tarihleri arasındadır. Mevcut çalışmada en yüksek enfeksiyon oranının Hawija bölgesinde olduğunu tespit edildi. Cinsiyet açısından karşılaştırma yaptığımızda ise CL hastalığının erkeklerde kadınlara göre daha yüksek olduğu tespit edildi. . Çalışma ayrıca bir yıldan az ile 70 yaş arasında değişen yaş gruplarını da içermekte olup enfeksiyona karşı en savunmasız yaş grubu 1-10 yaş grubudur.Yapılan Çalışmalar da İmmünoglobulin seviyeleri açısından iki ilaç arasında önemli farklılıklar vardı. İvermectin grubu, immünglobulin seviyeleri (IgG ve IgM) Pentostam'a göre daha fazla ölçüde artırdı. Ancak IgE seviyeleriPentostam grubun da daha yüksekti. Sonuçlar farklı ilaçlarla tedavi yöntemlerinde de KL tedavisinde İvermectin ilacının daha fazla etkinliğinin olduğunu düşündürmektedir.

2023, 77 sayfa

Anahtar Kelimeler: Kutanöz Leishmaniasis, Parazit, Tedavi , İvermektin, Pentostam

PREFACE AND ACKNOWLEDGEMENTS

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Golyar Abbas Rasool RASOOL

Çankırı-2023

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g	Gram
mg	Milligram
mL	Milliliter
nm	Nanometer
μL	Microliter
mm	Milimeter

LIST OF ABBREVIATIONS

ACL	Anthroponotic cutaneous Leishmaniasis
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
HRP	Horseradish peroxidase conjugated
IVM	Ivermectin
NNN	Nicolle- Novy MacNeal medium
OD	Optical denisty
PCR	Polymerase chain reaction
TMP	Trimethoprim
WHO	World health organization
ZCL	Zoonotic cutaneous Leishmaniasis
CDC	Communicable Disease Control Center

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1. INTRODUCTION

Leishmaniasis is a parasitic disease that is found in more than 92 countries, including Iraq, Iran, Brazil, Afghanistan, Syria, India, Bangladesh, and Sudan. Each year, more than a million cases of leishmaniasis are reported (Aksoy *et al*., 2017).

It is one of the nine largest parasitic diseases that infect humans and cause many health problems. Despite its seriousness and rapid spread, it is still neglected globally (Alvar *et al*., 2012). The disease is caused by a protozoan parasite that is forced to parasitize inside living cells. A common zoonotic disease that infects humans, dogs, and wild rodents and is spread from host to host by the bite of a female sand fly (*Phlebotemous* spp.) (Aksoy *et al*., 2017 ; Evans *et al*., 1989).

CL is also known as Oriental sore, Baghdad boil, and Aleppo bean. For other names, depending on the regions you get into, in Iraq. There are two types that cause CL, namely, *Leishmania tropica* and *Leishmania major*, and they are similar in the life cycle. The parasite lives in the cells of the reticuloendothelial system in the skin, especially the phagocytic cells that grow and multiply inside and cause the appearance of skin ulcers. It causes visceral (VL), cutaneous (CL), mucocutaneous (MCL), diffuse cutaneous (DCL) and post kala-azar dermal (PKDL) forms of Leishmaniasis (Clem, 2010).

Visceral Leishmaniasis (VL) has many names, such as *Leishmania donovani*, Kala azar,and black fever. An infection of this type will lead to death (Iddawela *et al*., 2018).

(VL) is a endemic parasite illness spread by the biting of sandflies. Every year, half a million individuals worldwide are afflicted with (VL), commonly known as kala-azar (Schleier et al., 2009). Leishmania spp. are parasites that cause VL, which is spread through the bite of females phlebotomine sand flies that are infected. The symptomatic disease frequently affects children who are underweight and immunocompromised, and if it is ignored, it can be fatal (Oshaghi et al., 2009).

Leishmania infantum and Leishmania donovani, both of which have also been detected in nearby Saudi Arabia, Iran, and Turkey, are the parasites responsible for VL, which is endemic in Iraq (with an estimated population of 32 million) (Oshaghi et al., 2009). The most frequent vector species, according to recent detailed entomological research of the vector on an American military post in Iraq, are Phlebotomus alexandri and, Phlebotomus papatasi (Coleman et al., 2007; Coleman et al., 2009). In Iraq and the rest of the Middle East, feral dogs and jackals serve as the principal reservoirs for Leishmania (Coleman et al., 2006). The Ministry of Health (MOH) is in charge of reporting communicable diseases and carrying out control and preventative programs. This center is called the Communicable Disease Control Center (CDC Baghdad). The national (VL) control strategy calls for weekly report via a passive population based surveillance system, entomological studies, reservoir control through the eradication of rodents and feral dogs, vector control through night fogging, as well as pesticide spraying and delivery of insecticide-treated bed nets to high-risk households, and ensuring that diagnosed cases receive appropriate care at the hospital level (Majeed et al., 2012). Healthcare professionals in Iraq are obligated to use the national surveillance system to notify the provincial Health Department (DOH) of (VL) cases. CDC Baghdad receives aggregated VL data from DOHs, which also carry out the national (VL) control strategy's operations (Jassim, 2006). A weekly report of VL to the CDC Baghdad's Surveillance Section became required in 2008. Using data from nationwide surveillance collected in Iraq between 1990 and 2009, we looked at changes in VL prevalence.

Only aggregated data was available for analysis for the years 1990 to 2006; however, data on the case's age, sex, place of residence, & date of diagnosis was available for the years 2007 to 2009. The Surveillance Section's Populatiokn statistics were utilized to estimate the prevalence of illness in children under the age of five (Majeed et al., 2012).

Therefore, the existing surveillance data show that this potentially fatal disease poses a persisting and large health burden for the community, particularly in young children, despite major interruptions in recording of cases in Iraq during the previous 10 years. Therefore, in order to more correctly track the full extent of illness and properly focus control efforts, we advise a systematic review of (VL) control programs, including all

the epidemiological and entomological vector control capabilities and surveillance systems (Majeed et al., 2012).

Mucocutaneous leishmaniasis also has many names, such as *Leishmania braziliensis* and Espundia. This paracite will lead to deformation due to erosion of the mucous membranes of the mouth and ear (Sachdeva and Sharma, 2016).

Pharmacological treatment of leishmaniasis is currently limited to a limited number of drugs. The most famous chemical drugs used are the pentavalent antimony compounds, which are among the oldest and most widely used compounds. Pentostam and glucantime have been used for more than fifty years to treat both cutaneous and visceral leishmaniasis. But treatment with them requires a long time and several doses. In addition, it is characterized by several negative effects, such as high toxicity, high cost, and loss of efficacy with increasing treatment periods (Croft *et al.,* 2005; Sundar and Chakravarty, 2015).

CL can be diagnosed in a number of ways, including blood smears from the edge of the infection, staining with Giemsa stain, direct examination with a microscope to find the flagelless phase, culture on MacNeal–Nicolle (NN) medium, histological diagnostic methods, immunological examinations, and highly sensitive and specific molecular techniques based on the discovery of the parasite's DNA in a polymerase chain reaction (Singh and Sivakumar, 2003; Sundar and Rai, 2002).

ELISA is one of the most widely used diagnostic laboratory techniques in most laboratories due to the possibility of analyzing a large number of samples in a relatively short time, its high sensitivity, and the possibility of determining the phase of the disease, whether it is chronic or acute. The formation of polyclonal antibodies (IgG and IgM) in the sera of patients with this disease is one of the hallmarks of this infection (Kalter, 1994; Pourmohammadi *et al.,* 2010; Singh and Sivakumar, 2003).

1.1 Aim of the Study

1. The aim of this study is to find out more about CL, a parasitic disease that has recently started to spread in Iraq.

2. To investigate the prevalence of parasites in the hospitals in the study area to obtain information about the detected cases and to compare the currently used drugs (Pentostam and Ivermectin) with each other and determine which one is more effective.

2. LITERATURE REVIEW

CL and VL are ancient diseases known to man for several centuries. They gave it many local names, but he was not aware of its pathogen, which caused much suffering and death (Aksoy *et al.,* 2017). On clay tablets from the time of King Ashurbanipal in the seventh century BC, as well as on pharaonic papyrus, some of them were mentioned in the Torah (2500–1500 BC) (Oumeish, 1999).

The Sheikh of Muslim Scholars, Avicenna (980–1037 AD), was the first to characterize this disease in northern Afghanistan in detail and called it the "pill of the city of Balkh," pointing out that it occurs as a result of the sting of flying insects such as small mosquitoes, as mentioned in his book The Law of Medicine, by saying: "The spleen becomes fattening and the body withers." There will be a fever and less blood flow, and its color will be yellowish and black. The physician Al-Razi (1500 AD) was the first to mention the disease in Iraq in his book "The Summary of Experience," describing it as "one of the most common diseases in Baghdad," and people call it the "Baghdad grain" (Aksoy *et al.,* 2017 ; Oumeish, 1999).

There is some evidence that proves the presence of Leishmaniasis in the ancient world, specifically in the North and South American continents thousands of years ago. As designs of pottery and skulls dating back to the Colombians and Peruvians were found that prove the presence of the parasite since that time (Al-Hussaini *et al.,* 2017; Oumeish, 1999).

The most important clinical description of CL was written by Alexander Russell in 1756 after examining a Turkish patient (Hawgood *et al.,* 2001).The researcher Cunningham was the first to refer to the *L. tropical* parasite, as he described it in 1885 in Delhi (Hart, 1986). The New World CL was described in a writing by Spanish missionaries in the sixteenth century, and it was thought that the same factor caused both the Old World and the New World leishmaniasis (Hoakk, 1938).

As for New World CL, research indicated that similar evidence for the cutaneous form of this disease was found in Ecuador. Before the Spanish colonists and the Spanish Incas, cutaneous leishmaniasis was known as Valley disease, Andean disease, or white leper disease. There are pictures of skin lesions and facial deformities on pottery from the pre-Inca civilization that dates back to the first century BC. The literature from the fifth and sixteenth centuries shows similar cases (Ngure *et al.,* 2009; Oumeish, 1999; Torpiano and Pace, 2015).

In 1900, researcher William Leishman used a blood smear from an English soldier with Dum Dum fever in India to describe the parasite in more detail. He said that the parasite is made up of many bodies that are between 2 and 3 micrometers long and have a kinetochrome (Alsaad and Kawan, 2021; Reithinger *et al.*, 2007).

While in 1903, the researcher Donovan noticed the presence of bodies similar to those described by Luchman while examining the spleen of a child from India and named them *leishmania donovani* bodies. (Dutta, 2003; Al Awadi, 2019; Alsaad and Kawan, 2021). In 1903, Wright was the first person to say that the parasite *L. tropica* caused this disease (Adler and Theodor, 1926). In 1911, Wenyon was the first person to find the parasite that causes the disease in a number of skin ulcers in Baghdad (Wenyon, 1911).

2.1 Old World Cutaneous Leishmaniasis

Leishmania tropica, L. major, L. aethiopica, and *L. infantum* cause Old World CL (Baily and Lockwood, 2007).

Because *L. tropica* infection is transmitted from person to person by *Phlebotomus sergenti* and *Phlebotomus papatasii*, which are located in cities and have no other reservoir, it is called "Anthroponotic Cutaneous Leishmaniasis" (ACL). Lesions caused by *L. tropica* begin as a small, painful red papule at the ridge. The bottom of the papule appears bloodshot. In some cases, the papule remains flat but scaly, grows slowly and widely, eventually forming a tubercle or nodule (Mehlhorn, 2016).

Some ulcers remain bare and may attach to additional infections. Lesions are usually 1 cm in diameter, but sometimes they can be larger or smaller. If infections like lymphangitis and pyodermas aren't present. The lesion is painless and doesn't swell the surrounding lymph nodes. Healing begins with the appearance of granulation tissue after a few months, which is usually 9–12. It is better to leave a trace between months (Özbel *et al*., 2007).

Leishmania major infection: It is more common in rural areas. The incubation period is less than four months, and the lesion is usually seen on the legs. *Leishmania* causes a wet, ulcer-like skin lesion at the bite site. The infection that starts with a papule turns into an inflamed ulcer with an acute course within 1-3 weeks. *L. tropica*, *Psammomys obesus* (Israel), and *Meriones crassus* (Israel). Meriones libycus (Iran), *Meriones sacramenti* (Egypt), *Rhombomys opimus* (Iran), and *Meriones sacramenti* (Mis). *Rhombomys opimus* (Iran) infects other hosts, including small rodents, dogs, and humans, via vector sandflies of the *P. papatasi* species (Klaus *et al*., 1999).

Leishmania infantum is reported as a sporadic CL agent in Old World countries. It usually forms a small, single ulcerated lesion that is 0.5–1 cm in diameter and is often located on the face (Bensaid *et al*., 2006).

Leishmania aetropica infection sometimes manifests itself in the form of skin, sometimes mundane membranes, and sometimes diffuse skin leishmanioses. Lesions are usually seen in the mouth and nose. Lesions develop very slowly, and ulceration occurs very late or not at all. Recovery may take 1–3 years or more (Özbel *et al*., 2007; Unat *et al*., 1995).

2.2 New World Cutaneous Leishmaniosis

Leishmania braziliensis, L. mexicana, L. amazoensis, L. guyanensis, L. panamensis, L. peruviana cause New World Cutaneous Leishmaniosis" (Korotoski, 1999).

The disease caused by *L. braziliensis* is called "Espundia."

A metastatic lesion involves the nasal and oral mucosa, affecting cartilage and soft tissues and causing damage and deformation. In *L. mexicana* infections, the painless lesion usually heals in a few months. In general, the lesion is single, and approximately 60% of it is located in the ear. If the vector bites on the auricle, it results in a chronic lesion called "Chiclero's Ulcer." Because the vascularization in the cartilage of the auricle is low, the immune response is weak, and the auricle is lost in 40% of the cases. (Özbel *et al*., 2007)

Leishmania guyanensis infection: whole body; open, oozing, and flattened lesions present as ulcerated plaques. This infection in Uruguay and Venezuela is called "pian bois." It is often compared to fungal infections and misdiagnosed (Gustavo *et al*., 2001)

Leishmania amazonensis causes solitary or multiple lesions that rarely heal spontaneously. Infection is common in forest rodents, and humans are less infected. (Krotoski, 1999*).*

Leishmania peruviana infection manifests itself as a single or several painless lesions. It heals spontaneously in 4-5 months, and this infection is called "uta." (Özbel *et al*., 2007)

Leishmania panamensis causes ulcerous lesions that cannot heal spontaneously. Its reservoirs are dogs and monkeys. *L. venezuelensis* usually causes single, painless nodular lesions. *L. garnhami* usually causes single or multiple lesions that heal spontaneously in six months (Valez *et al.,* 2015).

2.3 *Leishmania* spp. Parasite

The *Leishmania* parasite is a unicellular eukaryotic flagellum that lives as a parasite inside macrophage cells spread throughout the vertebrate reticuloendothelium system (Torres-Guerrero *et al*., 2017). There are two distinct forms during its life cycle: the

amastigote form in the phagocytic cells of the vertebral hosts and the promastigote form in the invertebrate hosts (sand flies) (Masmoudi *et al*., 2013).

Cunningham is credited with being the first to refer to the parasite *Leishmania tropica* as the cause of CL, which he described in a skin infection in Delhi in 1885 AD (Hart, 1986). Followed by the first description in 1903 AD by William Leishman and Donovan separately in the infection of an English soldier with a fever (Dum Dum fever) during a blood smear examination, and this parasite was named *Leishmania* in memory of William Leishman (Hart, 1986).

2.4 Classification of *Leishmania* spp.

Depending on the sequence of the nitrogenous bases of DNA and isoenzymes, *Leishmania* was classified according to (Chappuis *et al*., 2006) to:

Kingdom: Protista

Subkingdom:Protozoa

Phylum:Sarcomastigophora

Subphylum:Mastigophora

Class:Zoomastigophora

Order:Kinetoplastida

Suborder:Trypanosomatina

Family:Trypanosomatidea

Genus:*Leishmania*

Species:*L. tropica, L.major, L. donovani, L. infantum, L. aethiopica ……*

2.5 Cutaneous Leishmaniasis Distiribution

CL is one of the common and endemic skin lesions in hot and warm areas, and because of its wide geographical distribution, this disease has taken several names, including Baghdad boil (its sister), Aleppo boil, Delhi boil, and Nile seed. It is also called the Oriental pimple (Bailey and Lockwood, 2007; Reithinger *et al*, 2007). In southern Yemen and Ethiopia, the genus *Leishmania* contains a number of species, including *L. tropica* and *L. aethiopica*, which are the main causes of this disease (Zakai, 2014).

Iraq is one of the countries in which this disease is highly prevalent, as two types of CL have been diagnosed (*L. tropica*) and 600 cases of CL were diagnosed in Iraq, Kuwait, and Afghanistan within the ranks of the US military between 2003 and 2004 (Alsaad and Kawan, 2021; Alvar *et al.*, 2012).

The clinical manifestations of the disease begin with the appearance of a small, reddish swelling at the site of the insect bite called a papule, which gradually increases in size to become a prominent nodule. Then the ulcer increases in size until it reaches a few centimeters and is usually circular with ulcerated edges. The infection heals itself in two to six months and may last a year, but it leaves a light atrophic scar called a "scar" and results in permanent immunity (Özbilgin *et al*., 2019).

In some cases, there may be 200 ulcers in various parts of the body, particularly the arms, legs, and face, but cutaneous Leishmaniasis is not fatal and heals on its own, but it leaves permanent scars that cannot be removed (Mcgwire and Satoskar, 2014; Monzote, 2009). It is indicated that the success of the treatment of this disease leads to the occurrence of immunity against re-infection, and sometimes these ulcers become infected with bacteria, which increases the severity of inflammation at the site of the ulcers. Often, the infection of this type of disease appears on the skin and does not

include the internal organs of the body (Croft *et al.*, 2005; Kobets *et al.*, 2012; Monzote, 2009; Sundar and Chakravarty, 2015).

2.6 Phenotypic Form of Leishmaniasis

The *Leishmania* parasite has two phenotypic stages during its life cycle: the promastigote and the amastigote (Evans *et al*., 1989).

2.6.1 Amastigote stage

The flagellum-less phase, also known as the Leishmanial form, has a round to oval shape. It is flagellum-less, and ranges in size from 3-5 microns. This phase has a single nucleus located near the center of the parasite, as well as an oval or rod-shaped kinetoplast from which the axoneme extends. The flagellaless phase grows and reproduces compulsorily in the vertebral host within phagocytic cells (Evans *et al*., 1989).

The amastigote contains the myxomatium, which is in the form of cysts of different sizes and contains inside it a number of protease enzymes and acid phosphatase enzymes, which play an important role in the survival of the amastigote phase within the phagocytic cells of the host. It reflects the low activity of glycolysis in the amastigotes, and the mitochondria are less efficient and developed in the amastigotes. So their way of life is anaerobic (Clem , 2010; Evans *et al*., 1989).

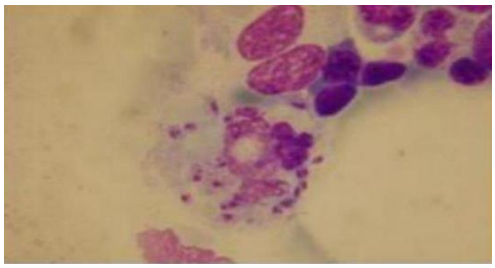

Figure 2.1 Amastigote stage of *Leishmania* that taken from skin ulcer (De vries *et al.* 2015)

2.6.2 Promastigote stage

The anterior flagellum is also called the Leptomonas form. The parasite takes the shape of an elongated spindle in this phase, and its size ranges between 14 and 20 microns. It has an anterior flagella that travels about the length of the body, and the motility generator is approximately 2 microns from the anterior end. The anterior flagella grows and reproduces outside of the sand fly's extracellular cells, as well as when cultured in culture media (Al-Hussaini *et al.*, 2017; Mcgwire and Satoskar, 2014).

The glycosomes appear as small bags containing a dense substance that appear in the form of plates and spread in huge numbers in the promastigote phase, and this reflects the high glycolysis efficiency in the promastigote compared to the promastigote. Less than 0.4% will experience a loss of motility, a decrease in protein synthesis, and a decrease in parasite size, followed by cell death (Lata *et al*, 2021; Singh and Sivakumar, 2003).

The electron microscope showed that the body of the *Leishmania* parasite in its two phases, without flagella and anterior to the flagella, is surrounded by a bilayer plasma membrane (2-4 nm) in diameter and contains a dent of the plasma membrane called a flagellar pocket, located at the anterior end of the parasite, which surrounds the base of the flagellum. Under the electron microscope, the flagella appears as a double membrane supported by a thin layer consisting of 2-9 fibers extending from the other side of the flagellar sinus, and the nucleus is surrounded by a nuclear envelope consisting of two thin layers characterized by containing minute holes (Kumar *et al*, 2007; Reithinger *et al*, 2007).

As for the endoplasmic reticulum, it is composed of a group of tubular cisternae surrounded by thin membranes connected to each other to form a network inside the cell, and it is of two types, one rough (rough) that contains ribosomes and the other smooth (smooth) that is devoid of ribosomes. A small reticulum is located near the lash depot (Reithinger et al., 2007).

The kinetoplast is a filamentous, disc-shaped body that arises from the flagellum and contains DNA in the form of a network of 25-250 large circles estimated at approximately 30 kbp and from 5000-10,000 small circles estimated at Kbp 2 that form the mitochondrial genome together. It was discovered that the kinetoplast-mitochondrion complex consists of the majority of the electron-carrying molecules in eukaryotes (Claborn, 2014; Zakai, 2014).

2.7 The Storage Host of the *Leishmania* spp.

The main source of leishmaniasis is dogs, which pose health problems and are among the most common carriers of zoonotic diseases in the world (Al Bajari, 2019). Also, there are approximately five types of leishmaniasis that have been recorded in cats all over the world, although most cases include *L. infantum* (Pennisi, 2013). Recent studies have found that there are infections of leishmaniasis in domestic cats (Vides *et al*., 2011). And it is unknown whether domestic cats acted as the main host or an occasional host (pennisi, 2015) (Maia and Campino, 2011).

Figure 2.2 Street dogs infected with CL and act as a storage hosts
(These photos were taken by us in kerkuk)

2.8 Vector Host

The sandfly is the host vector for leishmaniasis and is a small bloodsucking insect. It belongs to the order Diptera, family Psycodidae, under the family Phlebotominae. All types of sand flies that transmit leishmaniasis belong to the genus *Phlebotomus*, and this genus has more than 600 species. The family of phlebotomies includes five genera:

Phlebotomus and *Sergentomyia* (in the countries of the Old World) and *Lutzomyia*, *Brumptomyia*, and *Warilea* (in the countries of the Modern World). The genus *Phlebotomus* is the main vector of leishmaniasis in tropical and subtropical regions (the Old World). The genus *Lutzomyia*, which is spread only on the American continent, is the main vector of leishmaniasis in the countries of the modern world (Al-Awadi, 2019; Naucke *et al.*, 2008).

There are about 30 different species of the genus *Lutzomyia* and *Phlebotomus*, which differ in the basis of their transmission to different genera of the parasite *Leishmania*. It can transmit at least 20 different species of *Leishmania* parasites (Al-Awadi, 2019).

In the diagnosis of harmus in the period 1786–1925 AD, it was based on its external appearance, as it was adopted as a classification characteristic, including the number and spread of hairs on the male organs of the genitalia, the size, color, and spread of hair and scales on the body, the wing venation, the length of the palpate pieces, and the measurement of the head and abdomen in females, and after each of the cavities was described: the buccal cavily, phraynx, cibarial teeth, and spermatheca (Naucke *et al*., 2008).

The genus *Phlebotomus* is considered the most important and includes species that attack humans and mammals to feed on their blood and thus contribute to the transmission of many pathogens, including *Leishmania*. The vector insect in Iraq is represented by the type *P. sergenti*. Research confirms the dominance of *P. papatasi* in the central region of Iraq as a vector for leishmaniasis in general and CL in particular (Al-Awadi, 2019; Naucke *et al.*, 2008).

Many studies indicate that the peak of the spread of the vector occurs in two different time periods: the first period falls in May and June, and the second period falls at the end of September a nd the beginning of October. The insect begins to disappear in the winter in the central regions of Iraq, only to reappear between the months of March and November, and it is present in large numbers in the months of May, September, and October. Sukkar (1986) confirmed the spread of the sand fly in the central regions of

Iraq starting in April, with its numbers gradually increasing, reaching the peak in September, and then disappearing completely in December (Al-Awadi, 2019; Maikan *et al*, 2022).

Figure 2.3 Sandy fly (Stebut, 2015)

2.9 *Leishmania* spp. Life Cycle

The life cycle of the *Leishmania* spp. parasite is characterized by the fact that it takes place in two hosts:

•The invertebrate host is a female sandfly in which the promastigote stage lives.

•Vertebrate host, which is mammals, in which the amastigote phase lives.

The first step begins when the female sandfly takes a blood meal from the infected vertebrate host. The flagellar phase of the parasite amastigote enters the vector and transforms into the promastigote, which divides to give a procyclic stage in the posterior part of the intestine, where it multiplies and becomes elongated in shape. In the nectomonad phase, which is longitudinal and mobile, the phospholipids play an important role in the adhesion and maturation of the parasite, and within five days it reaches the anterior part of the intestine, after which the heptamonad promastigote sticks to the gate of the stomach. The movement appears in the cavity of the foregut containing the flagellum and reaches its final length within four hours, which helps the parasite to move and stick to the intestines of the fly (Murray *et al*, 2005; Steverding, 2017).

Then the parasite moves in front of the promastigote and moves towards the anterior part of the middle intestine of the insect, which in turn actively divides inside the middle intestine of the insect to produce large numbers of the parasite. The parasite then moves towards the head of the fly to settle in the pharynx, and then moves towards the mouth parts to be ready for injection into the blood of the vertebral host with saliva during a second feeding of the fly, in which it turns into a non-proliferative, metacylic promastigote.

When the fly stings, it implants its proboscis in the host's skin, where the parasite enters with saliva and attacks the phagocytic cells. As the parasite multiplies by simple binary division inside the lysosomal vacuole, two structural parasites are formed, and their numbers increase until the cell explodes, infecting other cells. The process continues during what is known as the incubation period.

It is worth noting that the sting is transferred from the 10-200 promastigote to enter the skin and reach the bloodstream and phagocytic cells, and it was found in the experiments conducted in glass that the promastigote connects to the macrophage through the end of its flagella, is devoured by the pseudofeet, is surrounded by a gap, and turns into an amastigote (Franco, 2012). The *Leishmania* parasite can live inside phagocytic cells, resist destruction by lytic enzymes, and use the cell as a food source as it settles inside a vacuole in the phagocytic cell (Bailey and Lockwood , 2007; Salam *et al*, 2014).

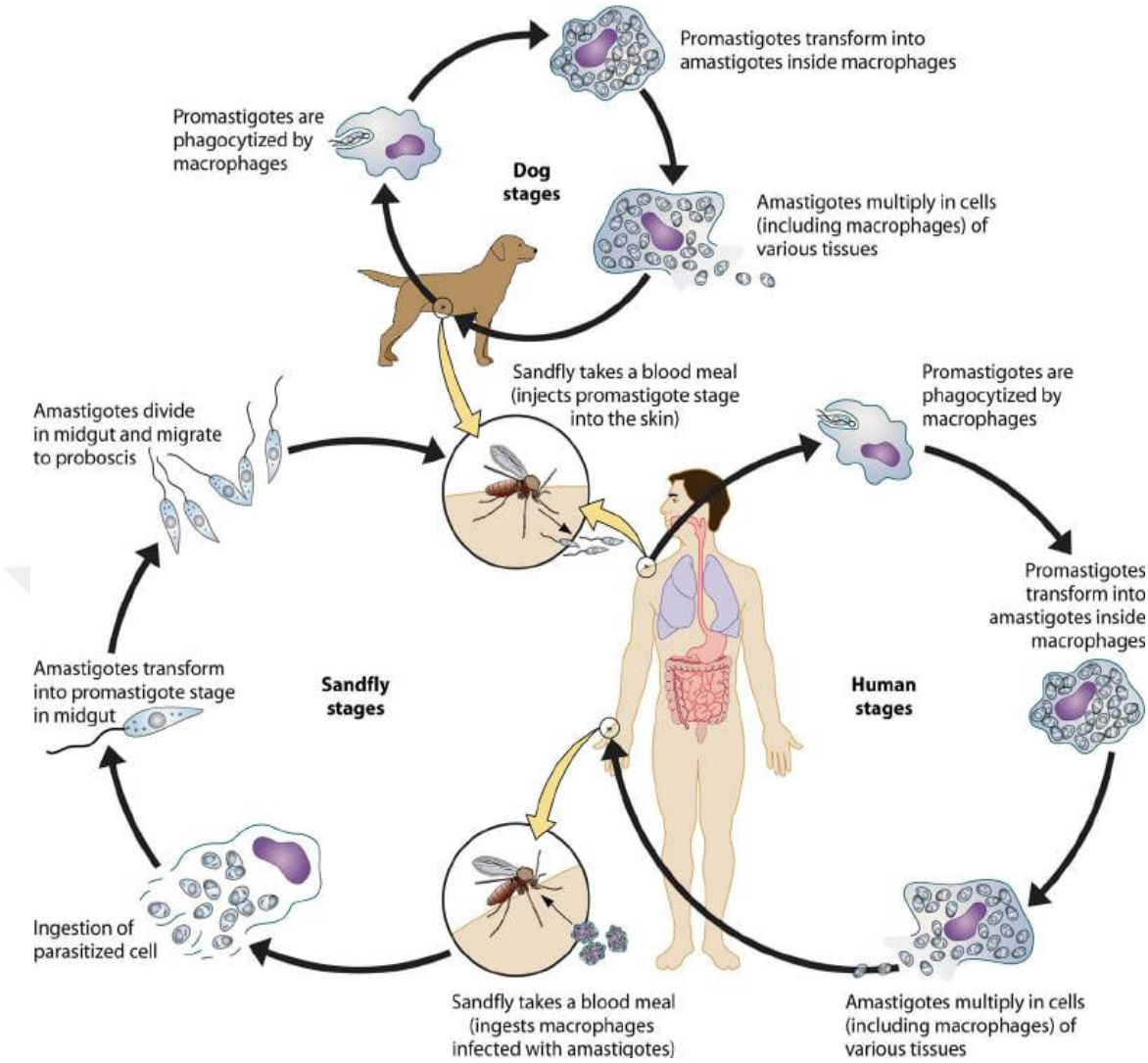

Figure 2.4 The life cycle of Leishmaniasis (Petersen, *et al*., 2022).

2.10 Diagnosis Methods

Early CL diagnosis is critical for disease control and treatment to prevent damage to the tissues in which the parasite is found (Chappuis *et al*, 2006; Noori *et al*, 2017). This is dependent on the pathological condition and clinical examination, after which the presence or absence of the parasite is confirmed or not based on Laboratory Diagnostics (Al-Hussaini *et al*. 2017).

2.10.1 Direct examination

2.10.1.1 Direct diagnosis

This method is done by taking a sample from the edge of the ulcer and making smears, including smears and staining with Giemsa stain, to reveal the flagellum-less amastigote in macrophages, which is of a circular shape, contains a nucleus near the center. It has rod-shaped motility, 70-80% sensitivity (Singh and Sivakumar, 2003; Sundar and Rai, 2002).

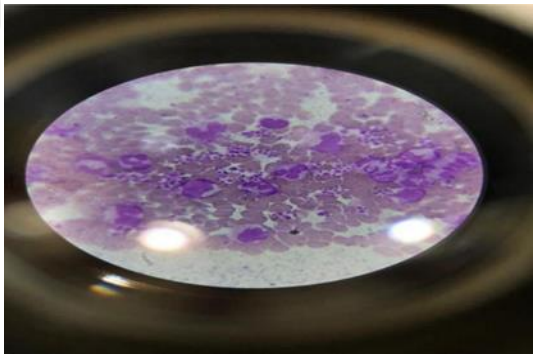

Figure 2.5 Amastigot stage of leishmaniasis under microscope (Direct examination by using Giemsa stain)

2.10.1.2 Culture

This method is carried out by taking samples of CL using the fine-needle aspiration technique (Kubba, 1987), and it is grown on special media where the promastigote phase grows. The most common medium is the biphasic medium (NNN) of Nicolle-Novy and MacNeal, after which it is incubated. At a temperature of 26 °C for a week, this method takes a long time until the results appear (Kubba *et al*, 1987). The anterior flagella stages can also be grown in the laboratory in different media, including Schneider's insect medium, and with nutrients that help the parasite grow, such as adding fetal calf serum under a temperature of 22–26 °C as growth media within the culture medium, as well as the use of other nutritional supplements that help maintain

the development of the anterior flagella in the culture medium (Kalter, 1994 ; Kobets *et al*, 2012).

The anterior flagella stages within the culture media differ in shape and size, as some of them are short and stumpy while others are thick and elongated. When necessary, the whip's frontal phases will be at the peak of its ability to cause injury (Claborn, 2014; Ejazi and Ali , 2013; Torpiano and Pace , 2015).

2.10.1.3 Skin test

Delayed hypersensitivity is an important characteristic of CL in humans and plays a key role in the healing of the injury site.

Name the Montenegro reaction. This test gives positive results 5-7 weeks after infection. The test is performed by injecting parasite antigens into the dermis of the skin (in an amount of 0.1 ml) and then measuring the sensitivity of the site and the extent of its hardening 24-48 hours after the antigen is injected. The test is considered positive whenever the hardening rate increases by more than 5 mm in the injection area (Pearson *et al.*, 1983; Sundar and Rai, 2002).

2.10.2 Serological Methods

2.10.2.1 Indirect tests

It is one of the most sensitive tests for diagnosing leishmaniasis. This test is based on the detection of antibodies that are present in the early stages of infection, as the antibodies are not detected after six to nine months or after taking the treatment dose. Low antibody levels are an excellent predictor of relapse and an increase in the severity of the infection (Kalter, 1994; Kubba *et al*., 1987).

This test has a high sensitivity of 96% and a specificity of 98%. Commercial strip test strips have been manufactured according to the basis of this test for the purpose of detecting parasite-specific antibodies (Kalter, 1994; Kubba *et al.*, 1987).

2.10.2.2 Enzyme linked immunosorbent assay (ELISA)

It is a highly valuable technique in the serodiagnosis of leishmaniasis. It is useful in both laboratory and field applications. However, the antigen used has a significant impact on the ELISA test's sensitivity and specificity. Certain antigens have been detected in the ELISA test called to detect the Gene B protein for CL type *L. major* (Liew and O'donnell, 1993; Sharma and Singh, 2009).

ELISA is one of the most common diagnostic laboratory techniques in most pathological analysis laboratories because it analyzes a large number of samples in a relatively short time and is more sensitive than other tests, as its sensitivity is 99%. Through this technique, we can also determine the stage of the disease, whether it is chronic or acute. However, the sensitivity and specificity of the test are greatly affected by the antigen used (Liew and O'donnell, 1993; Sharma and Singh, 2009).

2.11 Treatment

Before starting treatment for CL, it should be determined whether the wound is infected with secondary bacteria. If present, antibiotics and antifungal drugs should be added to the treatment in addition to antimony compounds. Intramuscular administration is preferred if the drugs used are not suitable for injection into the lesion in the upper part of the eye. (Hepburn and Omer, 1999; Herwaldt, 1999).

In the treatment of CL, one or more of systemic drug therapy, intralesional therapy, topical therapy, or physical methods should be applied depending on the type of leishmaniasis, the leishmaniasis clinic, the location, the severity, the number of lesions, and the immunological status of the person (Ramos *et al.,* 2002) Lesions of different types also differ in terms of clinical course and response to treatment. For example the CL caused by *L. tropica* and *L. major*, protective immunity develops and spontaneous

recovery can occur without treatment. Therefore, in the infections of these species, it may be sufficient to follow up without the use of drugs, especially in endemic areas. Since healing may take a year or more, it is recommended to treat the lesion in order to reduce the recovery time, especially in endemic areas. CL caused by *L. mexicana* and *L. braziliensix* is more serious and longer lasting. Since *L. braziliensis* species causes mucocytanemia, systemic treatment is given to patients in whom this species is detected (Hepburn, 2000; Hepburn, 2003; Berman, 2003).

2.11.1 Physiological treatment

It includes the Scraping the lesion with a sharp instrument, or cauterization, is a treatment method that has been used since ancient times. However, this method has a high probability of recurrence. Critotherapy, hot application, radiotherapy, laser, curettage, and surgical excision are other physical methods used in CL (Akisu and Korkmaz, 2003).

2.11.2 Chemical treatment

Leishmania treatment involves systemic or local use of pentavalent antimony and aromatic diamidines. The two most commonly used compounds are the two pentavalent antimonials, sodium stibogluconate (Pentostam) and neglumine antimonite (Glucantime). It is given daily for at least 20 days (Miranda *et al*., 2005; Arevalo *et al.*, 2007), and in the patients who did not respond, the treatment had to be repeated 4 to 5 times. For patients who did not respond to pentavalent antimony treatment, we used the second line of treatment, so pantamidin was given at a dose of 4 mg/kg of body weight three times a week for a period of 25 weeks or more, depending onIf these drugs are not effective, pentamidine and amphotericin B are used. Amphotericin B is given daily at a dose of 0.5–1 mg/kg of body weight for a period of 8 weeks (Murray *et al.*, 2012; Blum *et al.*, 2014). Stibogluconate is the drug of choice for the treatment of leishmaniasis, but it is expensive and toxic.

2.11.2.1 Ivermectin

Ivermectin (IVM) is a broad-spectrum antiparasitic medication that was developed and supported by Merck & Co. in 1974 in order to control and eradicate onchocerciasis in West Africa, which was caused by the parasitic worm *Onchocerca volvulus* and afflicted around 340,000 individuals in the 1980s (Burki, 2020; WHO, 2021). During that historical period, Africa did not possess the resources that were required to search for cures for this ailment. At the Kitasato Institute in Tokyo, Professor Satoshi Mura discovered the avermectins, of which IVM is a member (Ikeda and Ōmura, 1997). These avermectins were found to be fermentation products of the bacteria *Streptomyces avermitilis.* IVM is a member of this family. IVM is administered to patients suffering from onchocerciasis, lymphatic filariasis, strongyloidiasis, and scabies. Just lately, it has also been used to treat and prevent lice. IVM was recommended for inclusion in the twentieth list of essential medicines and the sixth list of vital medicines in children by the expert committee of the World Health Organization (WHO) in 2019 (WHO, 2021; Somerville *et al,* 2021; Agarwal *et al,* 2021; Ghazy *et al*, 2020). This was a result of the drug's low cost, high efficacy, safety, and marked tropism for helminths, as well as the fact that it has almost no impact on human biochemistry. The fact that it has a specific affinity for ion channels is what gives it its favorable safety profile (Skipper and Boulware, 2021; Ghazy *et al.,* 2020).

Therapeutic levels of avermectin formulation, despite the fact that they are extremely poisonous to insects, do not often have any effect on mammals (Clark *et al*., 1995). Rasheid and Morsy (1998) conducted research to investigate the impact that ivermectin has on the infectious potential of *L. major* promastigotes in Syrian golden hamsters. They demonstrated that the development of skin lesions at the site of infection in hamsters that had been infected with promastigotes and given a single dosage of ivermectin in culture medium at a concentration of 100 mg/ml was prevented. After being exposed to 90 mg/mL for two days, promastigotes either perished or lost their ability to spread the disease.

2.11.2.2 Pentostam (Sodium stibogluconate)

Sodium stibogluconate is a medicine used to treat leishmaniasis and is only available for administration by injection (Murray *et al*., 2005).

This includes leishmaniasis of the cutaneous, visceral, and mucosal types (Herwaldt and Berman, 1992). Some combination of miltefosine, paramycin and liposomal amphotericin B, however, may be recommended due to issues with resistance (WHO, 2010; Oray and Akbari, 2016).

Sodium stibogluconate has been studied as early as 1937 and has been in medical use since the 1940s (Sneader and Walter, 2005; Jager *et al.,* 2013). It is on the World Health Organization's List of Essential Medicines (WHO 2019). In the United States, it is available from the Centers for Disease Control (CDC 2016).

The mode of action of sodium stibogluconate is not clearly understood. In vitro exposure of amastigotes to 500 mg pentavalent antimony/ml results in a greater than 50% decrease in parasite DNA, RNA protein and purine nucleoside triphosphate levels. It has been postulated that the reduction in ATP (adenosine triphosphate) and GTP (guanosine triphosphate) synthesis contributes to decreased macromolecular synthesis. (Murray *et al.,* 2005).

Side effects are common and include loss of appetite, nausea, muscle pains, headache, and feeling tired (WHO 2010, Oray and Akbari, 2016). Serious side effect may include an irregular heartbeat or pancreatitis (Oray and Akbari, 2016). Sodium stibogluconate is less safe than some other options during pregnancy (WHO 2010). It is not believed to result in any problems if used during breastfeeding (drugs.com). Sodium stibogluconate is in the pentavalent antimonials class of medication (Oray and Akbari, 2016).

2.12 Immunity in Cutaneous Leishmaniasis

There is no inherent human resistance to cutaneous and mucocutaneous leishmaniasis. However, in areas where the disease is endemic, for example, it is found in people who

do not have CL. Age and gender have no effect. However, the infection is usually seen in children (Saygı, 1998).

After CL is healed, people develop a lifelong, permanent immunity. This immunity is species-specific, and those who are immune to one species are susceptible to the other. Therefore, *L. donovani* infection does not provide immunity against *L. tropica* infection, but there is such a relationship between *L.braziliensis* and *L.mexicana* that only *L. major* can give immunity to *L. tropica*, but *L. tropica* cannot (Soulsby, 1987).

3. MATERIAL METHODS

3.1 Apparatus of Research Insturmentation

Table 3.1 Apparatus of the study

Apparatus	**Company**	**Origin**
Sensitive balance	Sartorius	Germany
Centrifuge	Hettich	Germany
Microscope Light	Olympus	Japan
Digital Microscope	Micros	Germany
Incubator	Memmert	Germany
ELISA	Biotek	Spain
Autoclave	Vacum B	England
Vortex	VM-300	Taiwan
pH-meter	Orient Research	USA
Freezer	Beko	Turkey
Incubator	Termal	Turkey
Micropipette	Sugitoh	Japan
Pipets tips	JRL	Lebanon
Slide	Sali Brand	China
Slide Jar	Clifton	England
Disposable tube	Plastilable	Lebanon
Filter paper	Whatman	UK
Syringes	Medeco	UAE
Gloves	Bilim	Jordan
Cotton	Samara	Iraq

3.2 Chemical Materials of the Study

Table 3.2 Chemical materials of the study

Chemicals	**Company**	**Origin**
HCl	Scharlau	Spain
KH2PO4	Scharlau	Spain
Agar	Scharlau	Spain
Cacl2	Scharlau	Spain
Peptone water	Scharlau	Spain
KCL	Scharlau	Spain
Absolute ethanol	Fluka	Germany
EDTA(ethylene diamante triacetate)	scienceBio	Germany
Giemsa stain	Atom-Scientific	U.K
Nacl	Scharlau	Spain
Pentostam	Sigma Aldrich	Germany
Ivermectin	Sigma Aldrich	Germany

3.3 Epidemiological Study

The current study included patients infected with CL who attended a number of government hospitals in Kirkuk governorate and its affiliated districts, which include Hawija, Daquq, Debs, Riyadh, Taza, and Altun Kobri, for the period between March 1, 2022, and August 15, 2022. In each hospital, a dermatologist treated the disease. The ages of the patients ranged from less than one year to 70 years, and information about each patient with CL was collected, such as sex, age, time of onset, place of residence, number of ulcers, and location of ulcers in the body, according to a form.

3.4 Evaluation of the magnitude of the lesion

The size of the lesions at the base of the tail was measured for its size every other day after the inoculation using a digital caliper (Chuan Brand, Beijing, China) in two diameters (D and d) that were at right angles to each other. The size in millimeters (mm) was determined using the formula: $S = (D + d)/2$

3.5 Immunological study of infected patients

A sufficient amount of blood was withdrawn from each infected person attending the governorate hospital (Kirkuk governorate). Blood from the 75 samples that were followed up on during treatment periods was placed in anticoagulant vessels and EDTA tubes to study humeral and cell-mediated immunity. These participants were divided into three groups based on the drug used: 25 were given Pentostam at a dose of 20 mg/kg/day for 20 days, and 25 were given ivermectin (Al-Sark Co., Syria) at a dose of 200 g/Kg/day orally and as an ointment for 20 days (after 3 weeks of using a treatment). While the final 25 were people who had never used drugs and were recruited as controls, The blood was separated to obtain the serum by a centrifuge, and the separated serum was divided into parts to avoid repeated freezing and thawing and stored at -20 C until use. to determine the values of IgM, IgG, IgE, and IgA.

In the second, the blood sample was put in anticoagulant-free plastic tubes so that enough serum could be collected. The serum was then separated in a centrifuge machine at 3500 rpm for 10 minutes. The serum was then taken out and put in small special tubes.

3.5 Preparation of dyes and media

3.5.1 Giemsa stain

The dye solution was prepared according to the method of Rotimi et al. (2000).

The solution was prepared by dissolving 3.8 g of Gimesa powder in 250 ml of 70% ethanol. The solution was heated at 60 °C for 30 minutes, then 250 ml of glycerine was added to the solution, filtered to remove unwanted particles, and stored in dark bottles at room temperature.

3.6 Estimation of anti-leishmania IgG

Human Anti-*Leishmania* IgG ELISA kit (Sun Long Biotech Co., LTD, China) was employed to assess the anti-Leishmania IgG level in human serum on a qualitative basis.

3.6.1 Test principle

The qualitative enzyme immunoassay is the foundation of the ELISA technique. An antigen specific to anti-Leishmania IgG has been pre-coated on the microplate of this kit, turning it into a solid-phase antigen. In the wells of a microplate, samples are added along with the relevant antigen. Following that, antigens specific for anti-Leishmania IgG is added to each microplate well and incubated, yielding an antigen antibody enzyme labeled antigens complex. Each well underwent the TMB substrate procedure after being washed to get rid of any unbound chemicals. Only the wells containing anti-Leishmania IgG and HRP-conjugated anti-Leishmania IgG antigen will initially show

blue before becoming yellow after the stop solution is applied. Using a spectrophotometer, optical density (OD) was calculated at 450 nm. By comparing it to the cutoff value, anti-Leishmania IgG qualitative determination can be obtained..

Table 3.3 The kit (IgG) includes materials

Materials included in the kit	96 measurements	Storages
Microplate	1	2 to 8℃
Negative-control	0.5ml-×1 (bottle)	2 to 8℃
Positive-control	0.5m-l×1 (bottle)	2 to 8℃
HRP-Conjugate reagent	6ml-×1 (bottle)	2 to 8℃
Sample-diluent	6ml-×1 (bottle)	2 to 8℃
Chromogen-Solution A	6ml-×1 (bottle)	2 to 8℃
Chromogen-Solution B	6ml-×1 (bottle)	2 to 8℃
Stop-Solution	6ml-×1 (bottle)	2 to 8℃
Wash-solution	20ml -×1(bottle)	2 to 8℃

As quickly as feasible following sample collection, sample extraction with ELISA tests, were carried out. In accordance with the pertinent literature. The samples can be kept at -20 °C if ELISA is postponed. It is best to prevent frequent freeze-thaw cycles.

3.6.2 Procedure

1. Number the micropores in the sample's corresponding order on the microplate; leave two wells unfilled for blank controls; two wells for positive controls; and two wells for negative controls. (The remainder of the step procedure is the same; for the blanks control hole, do not add sample or HRP-conjugate reagent.)

2. 50µl of each of the positive, negative, and both controls are added to the corresponding wells. 10µl of sample and 40µl of sample-dilution buffer are added to each sample well. Without hitting the wall, samples-should be loaded onto the bottom. Shake gently to thoroughly combine.

3. After fastening with Closure Plate Membranes, incubate for 30 minutes at 37° °C.

4. Distilled water should be used to dilute a concentrated wash buffer (30-times of 96T)

5. Washing involves carefully peeling off the closing plate membrane, aspirating, and refilling with the wash solution. After 30 seconds of relaxing, discard the wash solution. Repeat the washing method 5 times more.

6. Except for the blank control well, add 50µl of HRP-conjugate reagents to each well.

7. Incubate according to Step 3.

8. Step 5: Wash as directed.

9. Coloring: To each well, add 50µl Chromogen Solutions A and 50 l Chromogen Solution B, gently shake, then incubate at 37 °C for 15 min. Please avoid direct sunlight while coloring.

10. To halt the reaction, fill each well with 50µl of stop solution. The well's hue should shift from blue -to- yellow.

11. Using a microtitre plates plate reader, calculate the absorption O.D. at 450 nm. The blank control well's OD is set to zero. The assay should be finished within 15 minutes of introducing the stop solution.

3.7 Estimation of Anti-*Leishmania* IgM

Human Anti-Leishmania IgG ELISA kit (Sun Long Biotech Co., LTD, China) was employed to assess the anti-Leishmania IgM level in human serum.

3.7.1 Test principles

The quantitative enzyme immunoassay approach outlined with in IgG determination principle is used in the ELISA.

Table 3.4 The kit (IgM) includes materials

Materials included in the kit	96 measurements	Storages
Microplate	1	2 to 8℃
Negative-control	0.5ml - ×1 (bottle)	2 to 8℃
Positive-control	0.5ml - ×1 (bottle)	2 to 8℃
HRP-Conjugate reagent	6ml - ×1 (bottle)	2 to 8℃
Sample-diluent	6ml - ×1 (bottle)	2 to 8℃
Chromogen-Solution A	6ml - ×1 (bottle)	2 to 8℃
Chromogen-Solution B	6ml - ×1 (bottle)	2 to 8℃
Stop-Solution	6ml - ×1 (bottle)	2 to 8℃
Wash-solution	20ml - ×1(bottle)	2 to 8℃

As quickly as feasible following sample collection, sample extraction with ELISA tests, were carried out. In accordance with the pertinent literature. The samples can be kept at -20 °C if ELISA is postponed. It is best to prevent frequent freeze-thaw cycles.

3.7.2 Procedures

1. Number the sample's matching micropores in sequence in the microplate; Leave two wells empty as negative control group, two wells filled with control sample, and one well empty as a blank control. (Do not add samples or HRP-conjugate reagent to the blank control hole; the rest of the step operation is the same.)

2. Samples addition: 50µl of each of the positive and negative controls should be added to the corresponding wells. A total of 40µl of samples dilution buffer & 10µl of sample are introduced to the sample wells. Samples must be loaded onto to the floor of the well without contacting the wall. Shake gently to combine well.

3. After fastening with Closure Plate Membranes, incubate for 30 minutes at 37° °C.

4. Distilled water should be used to dilute a concentrated wash buffer (30-times of 96T)

5. Washing involves carefully peeling off the closing plate membrane, aspirating, and refilling with the wash solution. After 30 seconds of relaxing, discard the wash solution. Repeat the washing method 5 times more.

6. Except for the blank control well, add 50 l of HRP-conjugate reagents to each well.

7. Incubate according to Step 3.

8. Step 5: Wash as directed.

9. Coloring: To each well, add 50µl Chromogen Solutions A and 50 l Chromogen Solution B, gently shake, then incubate at 37 °C for 15 min. Please avoid direct sunlight while coloring.

10. To halt the reaction, fill each well with 50 l of stop solution. The well's hue should shift from blue -to- yellow.

11. Using a microtitre plates plate reader, calculate the absorption O.D. at 450 nm. The blank control well's OD is set to zero. The assay should be finished within 15 minutes of introducing the stop solution.

3.8 Estimation of Anti-*Leishmania* IgE

The quantitative enzyme immunoassay approach outlined with in IgE determination principle is used in the ELISA.

3.8.1 Test principles

The qualitative ELISA outlined in the IgE diagnostic principle serves as the foundation for the ELISA.

Table 3.5 The kit (IgE) includes materials

Materials included in the kit	96 measurements	Storages
Microplate	1	2 to 8℃
Negative control	0.5ml ×1 (bottle)	2 to 8℃
Positive control	0.5ml ×1 (bottle)	2 to 8℃
HRP-Conjugate reagent	6ml×1 (bottle)	2 to 8℃
Sample diluent	6ml×1 (bottle)	2 to 8℃
Chromogen Solution A	6ml×1 (bottle)	2 to 8℃
Chromogen Solution B	6ml×1 (bottle)	2 to 8℃
Stop Solution	6ml×1 (bottle)	2 to 8℃
wash solution	20ml (30X) ×1(bottle)	2 to 8℃

As quickly as feasible following sample collection, sample extraction with ELISA tests, were carried out. In accordance with the pertinent literature. The samples can be kept at -20 °C if ELISA is postponed. It is best to prevent frequent freeze-thaw cycles.

3.8.2 Procedures

1. Number the sample's matching micropores in sequence in the microplate; Leave two wells empty as negative control group, two wells filled with control sample, and one well empty as a blank control. (Do not add samples or HRP-conjugate reagent to the blank control hole; the rest of the step operation is the same.)

2. Samples addition: 50µl of each of the positive and negative controls should be added to the corresponding wells. A total of 40µl of samples dilution buffer & 10µl of sample are introduced to the sample wells. Samples must be loaded onto to the floor of the well without contacting the wall. Shake gently to combine well.

3. After fastening with Closure Plate Membranes, incubate for 30 minutes at 37° °C.

4. Distilled water should be used to dilute a concentrated wash buffer (30-times of 96T)

5. Washing involves carefully peeling off the closing plate membrane, aspirating, and refilling with the wash solution. After 30 seconds of relaxing, discard the wash solution. Repeat the washing method 5 times more.

6. Except for the blank control well, add 50µl of HRP-conjugate reagents to each well.

7. Incubate according to Step 3.

8. Step 5: Wash as directed.

9. Coloring: To each well, add 50µl Chromogen Solutions A and 50 l Chromogen Solution B, gently shake, then incubate at 37 °C for 15 min. Please avoid direct sunlight while coloring.

10. To halt the reaction, fill each well with 50µl of stop solution. The well's hue should shift from blue -to- yellow.

11. Using a microtitre plates plate reader, calculate the absorption O.D. at 450 nm. The blank control well's OD is set to zero. The assay should be finished within 15 minutes of introducing the stop solution**.**

3.9 Estimation of Anti-Immunoglobulin A

The quantitative enzyme immunoassay approach outlined with in IgA determination principle is used in the ELISA.

3.9.1 Test principles

The qualitative ELISA outlined in the IgA diagnostic principle serves as the foundation for the ELISA.

Table 3.6 The kit (IgA) includes materials

Materials provided with the kit	96 determinations	Storages
Microplate	1	2 to 8℃
Negative-control	0.5ml - ×1 (bottle)	2 to 8℃
Positive-control	0.5ml - ×1 (bottle)	2 to 8℃
HRP-Conjugate reagent	6ml - ×1 (bottle)	2 to 8℃
Sample-diluent	6ml - ×1 (bottle)	2 to 8℃
Chromogen-Solution A	6ml - ×1 (bottle)	2 to 8℃
Chromogen-Solution B	6ml - ×1 (bottle)	2 to 8℃
Stop-Solution	6ml - ×1 (bottle)	2 to 8℃
Wash-solution	20ml - ×1(bottle)	2 to 8℃

As quickly as feasible following sample collection, sample extraction with ELISA tests, were carried out. In accordance with the pertinent literature. The samples can be kept at -20 °C if ELISA is postponed. It is best to prevent frequent freeze-thaw cycles.

3.9.2 Procedures

1. Standards Diluting A Micro-ELISA strip plate has ten typical wells built up for it. 100 µl of the solutions of standard and 50 µl of the standards dilution buffer were added to wells 1 and 2 and thoroughly mixed. 100 µl of the solutions to Wells 1 and 2 are added to Wells 3 and 4, respectively. After adding 50 µl of Standard Dilution buffer, thoroughly combine. 50 µl of the solution are dumped from Wells 3 and 4. 50 l of the solutions from Wells 3 and 4 are added to Wells 5 and 6, respectively. After that, 50 µl of Standards Dilution buffer were added and thoroughly mixed. 50 µl of solution to Wells 5 and 6 are added to Wells 7 and 8, respectively. Then 50 µl of Standard Dilution buffer is added and thoroughly mixed. 50µl solution from Wells 7 and 8 is added to Wells 9 and 10, respectively. Then 50 µl of Standard Dilution buffer is added and thoroughly mixed. Wells 9 and 10 had 50l of solution dumped. After dilution, the combined volume in each well is 50 l, and the corresponding concentrations are 180ng/ml, 120ng/ml, 60ng/ml, 30ng/ml, and 15ng/ml, respectively.

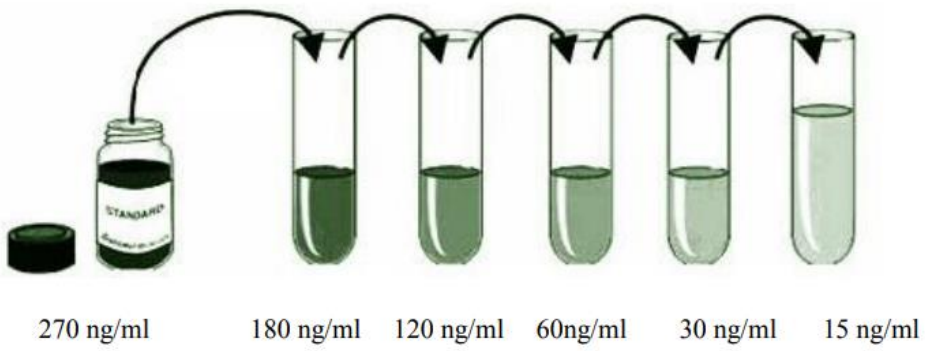

Figure 3.1 Procedure human anti-immunoglobulin A

2. Leave a well empty as a blank control in the Micro-Elisa-strip plate. 10 µl of sample and 40µl of sample dilution buffer are added to the sample wells (dilution factor was 5). Samples must be put onto the floor of the well without contacting the wall. Shake gently to combine well.

3. After fastening with Closure Plate Membranes, incubate for 30 minutes at 37° °C.

4. Distilled water should be used to dilute a concentrated wash buffer (30-times of 96T)

5. Washing involves carefully peeling off the closing plate membrane, aspirating, and refilling with the wash solution. After 30 seconds of relaxing, discard the wash solution. Repeat the washing method 5 times more.

6. Except for the blank control well, add 50µl of HRP-conjugate reagents to each well.

7. Incubate according to Step 3.

8. Step 5: Wash as directed.

9. Coloring: To each well, add 50µl Chromogen Solutions A and 50 l Chromogen Solution B, gently shake, then incubate at 37 °C for 15 min. Please avoid direct sunlight while coloring.

10. To halt the reaction, fill each well with 50µl of stop solution. The well's hue should shift from blue -to- yellow.

11. Using a microtitre plates plate reader, calculate the absorption O.D. at 450 nm. The blank control well's OD is set to zero. The assay should be finished within 15 minutes of introducing the stop solution.

3.9.3 Calculation of Results

The OD measurement and known concentrations of the Human anti-IgA-Ab Standard are shown on the log scale's x- and y-axes, respectively. Plotting the sample's O.D. on the Y-axis allows one to determine the concentration of human anti-IgA-Ab in the

sample. By multiplying the dilution factor by the original concentration, the calculation is made.

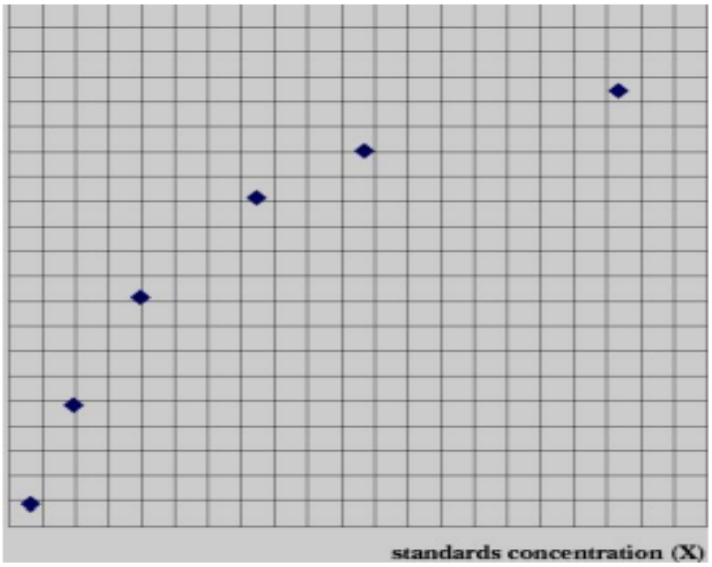

Figure 3.2 The concentration of the human anti IgA-Ab

3.10 Statistical Analysis

The results were organized using the SPSS program version 21, and statistical table analyzes were performed using different equations and the adoption of the Chi-Square Test, and the t-student test was used for the purpose of knowing the statistical differences significantly at the probability level of $P<0.05$ to analyze the levels of IgG, IgM, while The Chi-square test was used to find out the significant differences for the remaining percentages and the studied variables, and P value ≤ 0.05 was considered statistically significant.

(Apermission was taken from the Iraqi Ministry of health to conduct the practical section of the research in the file numbered (1027) in the date (2022/9/7). The file is in the Appendix (4) section.

4. RESULTS AND DISCUSSION

4.1 Epidemiological Study

728 cases of CL infection were recorded in Kirkuk governorate for the period from 1/10/2021 to 31/8/2022.

4.1.1 Distribution of Cutaneous Leishmaniasis by sex

The results of the current study showed an increase in the number of infected males with CL, which amounted to 424 compared to the number of infected females 304, and this recorded a significant difference with statistical significance at the probability level of $P < 0.05$ between males and females, and the value of ^{2}X was 39.560, Table (4.1)

Table 4.1 Distribution of CL infections by sex

Gender	No. of subjects	%
Male	424	58.2
Female	304	41.8
Total	728	100
$^{2}X = 39.560$ P-Value< 0,0.5		

The current study recorded a higher percentage of infection with CL in males than in females, as the percentage of male infection was 58.2%, while the percentage of female infection was 41.8%. It was agreeing with the results of (Kobets *et al*., 2012).

Al-Warid *et al.,* (2017) ecorded an increase in the incidence of male infection by 50.66% compared to the rate of female infection (49.34%).

Rahi *et al*., (2013) that the number of males infected was 50.90%, and the number of females was 49.09%, in relation to the total number of injuries in Al-Diwaniyah Governorate.

The results of the current study do not agree with the findings of Salam et al., (2014), as it recorded an increase in the percentage of infected females, amounting to 61.6%, while it was 38.4% in males. It was also inconsistent with what was recorded by Al-Hucheimi *et al*., (2009) that CL affects equally both sexes.

The reason for this rise may be due to the social and behavioral environment, as males are more in contact with the external environment and their time outside the home more than females, and therefore they are more susceptible to sand fly bites and infection. In addition to the role of social traditions in that most parts of the body in females are covered compared to males and thus they are less likely to be bitten by the vector insect (Majeed *et al*., 2012).

4.1.2 Distribution of Cutaneous Leishmaniasis infections by age

The results showed that the infections spread within a wide range of age groups, as the ages of the injured ranged from less than one year to 70 years, and the highest number of injuries was in the age group 0-10 years which amounted to 342. It followed 172 cases of infection by the age group 11-20. The number of infections in the age group 21–30 years reached 81 cases of infection, while the age group 31–40 years recorded 50 cases of infection, and the age group 41–50 years recorded 42 cases of infection. The number of infections among the age group 51–60 years old was 18 cases of infection, which was the lowest number of cases recorded, and finally the age group 61–70 years old recorded 23 cases of infection (Table 4.2).

Table 4.2 Distribution of CL infections by age

Ages	No. of subjects	%
0 _10	342	47
11 _20	172	23.6
21 _30	81	11.1
31 _40	50	6.9
41 _50	42	5.8
51 _60	18	2.4
61 _70	23	3.2
Total	728	100
$^{2}X = 92.638$ P-Value = 0.00003		

There was a statistically significant difference at the probability level of $P < 0.05$ between the different age groups, and the value of ^{2}X was 92.638, and the injuries spread within a wide range of age groups, as the ages of the injured ranged from less than one year to 70 years, and the results of the current study indicated that age groups less than 10 years are the most susceptible to infection, as the rate of infection reached 47%.

The current results are in agreement with the findings of Rahi (2013), who recorded the highest rate of infection (67%), and it was among children under 15 years of age, while the rates of infection decreased with age. They are also consistent with the findings of Aksoy *et al*., (2017) who recorded a higher rate of infection found in the children.

Evans *et al*., (1989) indicated that the highest percentage of patients in the age group 5–10 years was 38.73% of the total number of positive cases, while the injuries were few in the age group less than 25 years. Clem (2010) recorded the highest infection rate in the age group less than 10 years with a percentage of 47.27%. Al-Hussaini *et al*., (2017) indicated that the highest rate of infection for the age group less than 10 years was 47.83%, and the lowest was in the category 71–80 years, reaching 0.87% in Diyala Governorate.

It was reported (Al-Awadi, 2019) in the Thi-qar Governorate that the incidence rates in the three age groups (1-10 years, 11-20 years, and the age group older than 20 years) amounted to 20.7%, 48.9%, and 30.4%, respectively. For both sexes, which is in agreement with the results of the current study. As the age group decreases in incidence.

It differs from what was reached by Majeed *et al.,* (2012). The result of their study shows that the adult infection rate is the highest at 49.2%, which also does not agree with what was reached by Rahi (2013), where the highest infection rate was recorded in the age group of more than 15 years (56.13%) and less. A percentage was in the age group of 1-4 years (23.34%), where it was shown through his study that the severity of CL increases with age. As the Al-Warid *et al.*,(2017) indicated that children under 15 years of age constituted the vast majority of leishmaniasis infections. Dermatology

recorded among the local population in Pakistan and accounted for over 33% (Claborn, 2014).

Al-Obaidi *et al*., (2016) recorded 57.83% of infection rates in the age group of 16–40 years, in the age group of 6–15 years 23.37%, in children under 6 years of age 11.57%, and in patients over 40 years of age 7.23%.

It differs from what was found by Salam *et al*., (2014), where the age group older than 20 years had an infection rate of 53.2%, followed by the age group from 10 to 19 years, where the infection rat was 38.8%, and finally the age group from 1 to 10 years, where the infection rate was 8% for both sexes.

Children account for the vast majority of CL infections, which is consistent with what Al-Hucheimi *et al*., (2009) reported. It was noted that this increase in numbers may be explained by What is correct is that children are active outside the home, and thus they are vulnerable to sandfly bites (Al-Warid et al., 2017). The reason may also be due to children's inability to expel insects, especially those under a year old, and children after the age of one year increase their movement and activity, with the majority of their time spent playing. Al-Warid *et al.,* (2017), Thus, they are more in contact with the external environment, and also, children under 12 years of age have a weak immune system compared to adults, so they are more susceptible to a sand fly sting (Majeed *et al.*, 2012).

As for the age groups (51–60), the infection rate was lower compared to other age groups.the reason is that the immune system develops with age in most individuals and that it is possible that they were exposed to infection in an earlier period, which gave them a kind of immunity (Rahi, 2013).

In the other hand the fatty secretions of the face in adults may play a repellent role, which repels the vector insect when it approaches the face, unlike children who lack this condition (Oumeish, 1999).

4.1.3 Distribution of Cutaneous Leishmaniasis by districts of Kirkuk Governorate

The results of the current study showed that the number of infected people who live in Hawija district is higher than the number of infected people in other districts of the city of Kirkuk. The district of Kirkuk recorded the lowest infection cases, which amounted to 104 cases. A significant difference with statistical significance was recorded at the probability level of $P<0.05$ among the infected in the districts of Kirkuk governorate, and the value of ^{2}X was 51,795.

The results of the current study showed a noticeable increase in the rates of infection with CL, distributed geographically among the districts of Kirkuk governorate. The highest infection rate was recorded in Hawija district, which amounted to 46.2% and the lowest infection rate recorded in the center of the governorate Kirkuk 14.2%, (Table 4.3).

Table 4.3 Distribution of CL by districts of Kirkuk governorate

Area	No of subjects	%
City of Kirkuk	104	14.2
Dibs Distric	115	15.8
Daquq Distric	173	23.8
Hawija district	336	46.2
	728	100%
$^{2}X = 51.795$ P-Value = 0.0005		

The results of the current study were in agreement with the results of the study (Al-Warid *et al.*, 2017) on an epidemiological study of CL in Kirkuk governorate. they recorded the highest infection rate in Hawija district at 20.5% and the lowest infection rate in Kirkuk district at 3.5%. They were also consistent with what was found by Al-Obaidi *et al.*, (2016) and Al-Warid *et al.* (2017) for a molecular and immunological study of the CL in Karbala governorate. The highest infection rate was recorded in Ain Tamr district (40.8%) and the center (3%).

The findings contradict the findings of Rahi (2013), who found that the governorate center had higher rates of infection than the other districts in Al-Diwaniyah governorate, and that the difference in leishmaniasis infection rates between districts and places is likely due to the different environmental and social conditions of the injured.

The reason for the high rates of CL infection in Hawija district may be due to the fact that it represents the appropriate environment for the growth and reproduction of the sand fly insect in terms of the presence of water bodies, humidity, density of trees, and green spaces, all factors that help the growth and reproduction of the insect, in addition to the large spread of leishmaniasis hosts represented by dogs and rodents. While it was recorded in the Kirkuk district, which represents the governorate's center, the lowest rates of infection were due to social conditions, pest control inside homes, and a lack of animal husbandry, which represent the parasite *Leishmania'*s storage hosts (Salam *et al*, 2014; Al-Hucheimi *et al*, 2009).

4.2 Lesions of the Patients

There was a wide range of morphologies seen among the lesions 31.9 % were nodular, 63.9 % were ulcerative, 2.8 % were both nodular and ulcerative, and 1.4 % were fungating and ulcerative. Many of these lesions were found where clothing wouldn't normally be worn. Overall, 87.5% of individuals suffered some sort of injury to their upper or lower extremities.

Different patients had different numbers of lesions. Patients had an average of 5.5 lesions, for a total patients. The prevalence of multiple lesions was high. Each patient could have up to 16 lesions. One lesion was seen in the plurality (22.2%) of the 61.1% of CL cases that had between 1 and 5 lesions. Six to ten lesions were present in 25% of CL cases; eleven to fifteen lesions were present in 8.3% of CL cases; and only 5.6% of patients had sixteen or more lesions. Lesions ranged in size from millimeters to more than 3 centimeters in diameter. They lasted anywhere from a few days to almost a year, with the vast majority (91.6%) of the time falling between a few days and three months.

It is possible that the lack of discomfort associated with CL contributed to the delay in reporting symptoms (63.9% of cases were reported after ulceration). Ulceration and subsequent infection are often what prompt patients to seek treatment. Study by El Safi *et al.,* (1991) corroborated this result.

The results of the investigation showed that an infection might spread to every organ. The majority of infections (87.5%) are located in the limbs. Perhaps this is because sand flies prefer to attack exposed skin, such as the limbs, while it is dark outside. Similar findings were reported by (El Safi *et al*., 1988; Gaafar *et al*., 1995).

Lesions could last anywhere from a few days to well over a year. The median survival time was three months (91.6%). consistent with research by El Safi *et al*.,(1988). Our research found that 2.8% of instances had lesions that persisted for more than five months. This finding suggests that lesions from CL can persist for longer than a year if patients aren't given treatment. Abdalla *et al*.,(1973) report that 4.7% of cases developed lesions that lasted up to 18 months.

Lesions ranged in size from a few millimeters to more than 3 centimeters. Lesion sizes ranged from 1 cm to 6 cm, which agrees with findings from El Safi *et al*.,(1991) and Abdalla *et al*., (1973).

Individual patients' lesion counts ranged from one to sixteen, with a median of 5.5. Multiple lesions were reported in 80% of cases, according to research by (El Safi *et al*. 1991).

According to the World Health Organization (2000), this disease can generate up to 200 lesions, leading to severe impairment, persistent fear, and shame, all of which can lead to severe social prejudice (WHO, 2000).

Lesion size in this study

The results revealed a significant decrease in lesions size after three weeks of treatment, the ivermectin group showed a greater decrease than Pentostame group at 0.05 significance level. As showed at table (4.4)

Table (4.4): the evaluation of lesion size

Lesion size (mm)	**Before treatment**	**After treatment**
Ivermectin group	18	11*
Pentostame group	16	13*
Control	15	-
p-value	P < 0.05	

In this study we finding that both types of drugs have a good effect clinically by causing healing in the lesion but the ivermectin showed a better efficacy than Pentostam.

Our findings are in agreement with those of Antonelli and other researchers who discovered a favorable link between the size of CL patients' lesions and the frequency of total immunoglobulins and cytokines in their blood. (Antonelli *et al.*, 2004)

The findings of Sahar *et al.* (2020) demonstrated that there was a statistically significant reduction in the size of the lesion by day 30 in mice that had been injected with antimony medications. This was in contrast to the mice in the control group.

4.3 Diagnosis Cutaneous Leishmaniasis

4.3.1 Direct Microscopic Examination

The diagnosis of leishmaniasis types depends on the microscopic examination of smears stained with Giemsa dye, as it is considered one of the good tests to see the flagellaless

phase of leishmaniasis by taking materials from the edge of the ulcer and staining them with Giemsa dye (Atlas, 2004). When performing a direct dye smear, the sensitivity of this method reaches 70-80% (Al Hucheimi *et al*., 2009). Taken from patients' ulcers, the flagellumless phase was seen within the macrophage cells in 34 (54%) while 28 (42%) of the smears did not show anything.

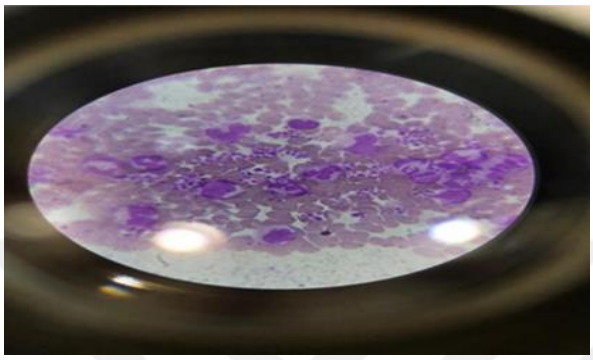

Figure 4.1 Direct examination of CL by staining with Giemsa stain

The main reason for staining cells is to see their shape, size, and components under a light microscope by Aksoy *et al.,* (2017) and Rahi (2013). The results of the current study are consistent with what was reached by Rahi (2013), where he diagnosed 60% of positive cases by direct microscopy. In another study, there were positive laboratory results using direct examination techniques (63.2%), and the percentage of negative samples was 36.8% (Al-Obaidi *et al*., 2016).

The Giemsa dye is a good and distinctive dye that is used to detect Leishmaniasis and shows details that are not clear with the other dye, especially in cells (Younis *et al.,* 2017), but there are some smears of the Giemsa dye that give negative results and the parasite cannot be seen.This is due to several reasons, including taking The patient has been treated for treatment reasons, errors in the time required for pigmentation and the thickness of the smear, or because the percentage of parasite presence in the ulcer of the skin of the infected person is very small, especially in the case of chronic infection (Al Hucheimi *et al*., 2009; Al-Warid *et al.,* 2017; Rahi, 2013). They stated that the success of microscopic diagnosis to determine the flagellum stage is different depending on the number of parasites and the experience of the person examining the slide.

A. The level of total immunoglobulin IgG:

The average level of IgG immunoglobulin in patients with CL (*L. major)* was (1811.1 + 523.1 mg / d.l), which is statistically significant compared to the average of its value in control group, which amounted to (775.0 + 162.4 mg) / d.l) where the significant difference was $P < 0.05$ (Table 4.5).

Table 4.5 The levels IgG

Immunoglobulins	**IgG mg/ dl± SE**
Ivermectin group	2141± 304.2
Pentostame group	1690± 219.7
Total Patients	1811.1± 523.1
Control	775.0 ± 162.4
p-value	P < 0.05

B. The level of IgM

It was found that the average level of IgM of the total patients in leishmaniasis patients was 170.7 mg / dl (184,9 in Ivermectin patients and 149,1 mg/ dl in the Pentostame patients' group). It increased from the control group (119.0 mg / dl) with a significant difference ($P < 0.05$) (Table 4.6).

Table 4.6 The Levels of IgM

Immunoglobulins	**IgM mg/ dl± SE**
Ivermectin group	184.9± 41.7
Pentostame group	149.1± 18.5
Total Patients	170.7± 23.6
Control	119.0± 21.5
p-value	P < 0.05

There are many ways to diagnose diseases and measure the amounts of antibodies in the blood of people who have them. Each method, starting with isolation, led to negative results. The researcher (Solano-Gallego *et al*., 2003) said that the parasite wasn't found in people who got sick right away.

Where many researchers pointed to the importance of the ELISA examination for diagnosing diseases as it determines the stage of the disease, whether it is chronic or acute, the ELISA test is suitable for diagnosing the condition of the disease by diagnosing the type of immunoglobulin. There is a sensitive test for determining IgG or IgM if used together, as it showed results consistent with the tubular agglutination test and can be adopted for the diagnosis of disease in humans and leads to the best results related to clinical signs (Mollett *et al*, 2019; Reis *et al*, 2006; Solano-Gallego *et al*, 2003).

The results indicated an increase in the levels of immunoglobulin (IgG and IgM) in the sera of patients with CL. The reason for this increase could be due to the stimulation of *Leishmania* antigens, which stimulate the proliferation and differentiation of B cells and their transformation into plasma cells that secrete antibodies, as well as cytokines secreted from cells as T-helper cells and responsible for regulating B-cell activation factors (Reis *et al*, 2006; Rodriguez *et al*, 2006; Solano-Gallego *et al*, 2003).

Th2 cell cytokines were identified as adjuvants to B lymphocytes and stimulating the production of high levels of IgE, IgM, and the unproven complement of human antibodies by Iniesta *et al*., 2005 and Pissinate *et al*., 2008.

C. The level of IgE

Serum IgE was determined in patients. IgE levels in the CL group (1294 mg/ dl in Ivermectin patients and 1348 mg/ dl in the Pentostame patients' group) were significantly higher than those for the control group (557 ± 91 mg/ dl) (Table 4.7).

Table 4.7 Levels of IgE

Immunoglobulins	**IgE mg/ dl± SE**
Ivermectin group	1294 ±192
Pentostame group	1348 ±434
Total Patients	1334 ± 418
Control	557 ± 91
p-value	P < 0.05

The clinical form of leishmaniasis, known as CL, is characterized by a pronounced Th1 immune response, which is supported by lymphocyte proliferation and IFN-y release. High parasitic-specific serum IgE levels, however, are also seen without B-cell polyclonal activation or hypergammaglobulinemia (Iniesta *et al*., 2005).. In contrast to VL, this antibody was not associated with disease activity or resistance to pentavalent antimonial treatment (Iniesta *et al*., 2005; Sousa-Atta *et al*., 2002).

IgE antibodies against *Leishmania* promastigotes were found in the serum of 48% of a group of CL patients According to (Neil *et al*., 1993). The fact that antibody activity was only detected in a small percentage of persons from the endemic area who appeared to be free of infection and in none of the individuals from a non-endemic zone suggests that this was an aspecific reaction against the protozoan. Intestinal parasitosis severity and socioeconomic status were equivalent across the three study groups (Reis *et al*., 2006; Rodriguez *et al*., 2006).

According to the immunoperoxidase test's pattern of staining, a large amount of the IgE antibody was directed towards the organisms' surface components. It was unusual to see IgE react with the intracellular amastigote form of the parasite, and this wasn't just due to technical difficulties in detecting intracellular antigens. As Anti-*Leishmania* monoclonal antibodies produced potent reactions under identical conditions (Gardinassi *et al*., 2014). It has been noted that promastigotes and amastigotes have different antigenic compositions (Iniesta *et al*, 2005; Lynch *et al*, 1982; Reis *et al*, 2006; Rodrguez *et al*, 2006; Sousa-Atta *et al*, 2002), promastigotes may also favorably promote IgE antibody responses. The discovery that some of the patients' sera contained IgE antibodies that appeared to react only with *L. mexicana* or *L. brasiliense* was intriguing. Although the specificity was not 100% with the various *Leishmania* strains used, the discovery that one of these sera could distinguish seven out of eight strains of *L. mexicana* from four out of six members of the *L. braziliense* complex stands in contrast to the general lack of specificity of the humoral and cellular immune responses against the parasite (Iniesta *et al*, 2005; Lynch *et al*, 1982; Pissinate *et al*, 2008). Additionally, the outcomes of the IgE anti-IgE immune peroxidase test supported the findings of the Anti-*Leishmania* monoclonal antibody study, where one of the two

strains of *L. guyanensis* used had peculiar staining features. It is doubtful that the test employed was simply detecting different subclasses because the monoclonal anti-IgE antibodies utilized in this investigation exhibit isotype specificity comparable to that of a polyclonal antiserum (Pissinate *et al.,* 2008) instead of the IgE antibodies' actual strain-specificity. The IgE-immunoperoxidase test's ability to distinguish between different *Leishmania* strains may, at least in part, be attributable to the activity that was found, which was primarily directed against the parasite's surface components rather than the whole parasite extracts typically used in such studies (Khan *et al*, 2020; Pellizzari and Pacheco, 2020; Pissinate *et al*, 2008; Suzuki *et al*, 2006).

It's interesting to note that the immune response can be very strain-specific while fighting *Leishmania*. According to Lynch *et al*., (1982) and Iniesta *et al*., (2005), these findings do in fact raise the question of whether IgE antibodies may play a part in the host's ability to resist infection (Al-Obaidi *et al*, 2016; Iniesta *et al*, 2005; Lynch *et al*, 1982; Rahi, 2013; Sousa-Atta *et al*, 2002). By facilitating mast cell degranulation, IgE antibodies, for instance, may promote resistance to *Leishmania* re-infection by making the environment undesirable for newly penetrating parasites. Similarly, the connection between IgE and macrophages or eosinophils (Iniesta *et al.,* 2005) might exert cytotoxic effects against the parasite (De Macedo, 2021).

It's probable that a significant portion of the IgE produced is not specifically directed against the parasite. But rather is the consequence of polyclonal activation of IgE-producing B cells by substances coming from activated T cells. However, there is growing proof that a parasite-specific element may play a role in particular IgE immunity (Iniesta *et al*, 2005; Pissinate *et al*, 2008).

D. The level of IgA

Serum IgA was determined in study subjects (Table 4.8). No significant differences between patients group (231 mg/ dl in total patients, 228 mg/dl in Ivermectin patients and 236 mg/dl in the Pentostame patients group) and control group (218±52) in IgA levels in the current study (Table 4.8).

Table 4.8 Levels of IgA

Immunoglobulins	IgA mg/ dl± SE
Ivermectin group	228± 18
Pentostame group	236± 27
Total Patients	231 ± 43
Control	218±52
p-value	P > 0.05

Positive IgA test result indicated that the parasite had invaded the mucosa; in this investigation, the rupture of the mucosa in certain cases served to highlight the infection's invasive nature even more (O'Neil *et al*, 1993; Rahi *et al*, 2013; Todol *et al*, 2009).

We were unable to detect IgA-class antibodies in mucocutaneous leishmaniasis cases that were advanced and had a history of destructive lesions. When we did notice them, they were linked to individuals who had upper respiratory tract mucous membrane irritation and a shorter illness history; tissue loss, if any, was not severe. This makes us believe that this particular antibody class may be particularly linked to an infection stage in which amastigotes are actively invading the mucous membranes. If this stage is not effectively treated, cartilaginous tissue may be mutilated and destroyed (Cantos-Barreda *et al*, 2017; Reis *et al*, 2006; Rodriguez *et al*, 2006; Shaw and Lainson, 1981).

Contrary to the findings of Pissinate *et al*., (2008), who detected this antibody in persons with acute and chronic Chagas' illness, trypanosomal IgA antibodies were only discovered in people with recent infections. Although its removal in one of the patients we analyzed could have been explained by medication, we believe that this is improbable given that we were unable to detect it in the sera of 15 patients with the chronic condition who had not received any treatment. Children treated for Chagas disease had their serum proteins examined by Pissinate *et al*., (2008) and Rodriguez *et al*., (2006). In the acute phase, they discovered that 6 out of 16 kids had IgA levels that were noticeably greater than those of healthy kids. Only two children had IgA levels that were considerably higher than those of the healthy control group after one year, and these levels had returned to normal after around two and three years. It appears

plausible that other chronic illnesses may have been the cause of these high IgA concentrations, as both of these cases involved children whose serology turned negative after therapy (De Carvalho *et al*, 2021; Hijawi *et al*, 2019; Ngouateu and Dondji , 2022; Rodríguez *et al*, 2006).

5. CONCLUSION AND RECOMMENDATİON

5.1 Conclusions

1- Leishmaniasis is one of the common and endemic transitional diseases in Kirkuk Governorate. The current study showed that the highest rate of infection was in Hawija district.

2 to Both sexes were infected with CL, and males were infected at a higher rate than females. The study also included age groups ranging from less than one year to 70 years, and the most vulnerable age group to infection was the age group (1–10) years.

3- The ELISA technique, which detects immunoglobulins IgG, IgM, IgE, and IgA, is regarded as an appropriate technique for detecting infection at an appropriate cost.

4- When compared to the controls, the treatments significantly increased the percentage of immunoglobulins (IgG, IgM, and IgE) in the treated groups.

5- There was a significant difference between the two treatments regarding the levels of immunoglobulins. The ivermectin group significantly enhanced the levels of the immune globulines (IgG and IgM,) more than Pentostam, but IgE levels was higher in Pentostam group. Conversely, there were no significant changes in IgA levels prior to treatment.

6- Subcutaneous inoculation with different drugs showed that the efficacy of ivermectin was higher than Pentostam in the treatment.

7-The ivermectin may be the best drug of choice for the treatment of leishmaniasis clinically and immunologically

5.2 Recommendations

1- Conducting field trips on an ongoing basis for the epidemiological survey and finding out the extent of CL in Kirkuk governorate, and educating and educating citizens about the disease, its seriousness, methods of transmission, and ways to prevent it. The virulence factors of the parasite need to be examined at the molecular level.

2 to Control of the vector host (sandfly) of CL in endemic areas with the use of mosquito nets, spraying them with an effective insecticide, and avoiding sleeping in the open.

3- Controlling the hosts that store the disease, represented by dogs and rodents, and controlling them, and emphasizing the breeding of pets in their breeding places and under healthy conditions.

4- More researches are recommended with a larger scale sample regarding the use of ivermectin for leishmanial treatment.

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APPENDICES

APPENDIX 1. Some Pictures of Patients Infected with Cutaneous Leishmaniasis in Kirkuk

APPENDIX 2. Informed Patient Consent Form

APPENDIX 3. Questionaire Form

APPENDIX 4. Facilitating the task (in Arabic)

APPENDIX 5. Facilitating the task (in Turkish)

APPENDIX 1. Some Pictures of Patients Infected with Cutaneous Leishmaniasis in Kirkuk (These photos were taken by us in kerkuk hospital)

APPENDIX 2. Informed Patient Consent Form

Gönüllü katılım

APPENDIX 3. Questionaıre Form

APPENDIX 4. Facilitating the task (in Arabic)

APPENDIX 5. Facilitating The Task (İn Turkish)

CURRICULUM VITAE

Personal Information

Name and Surname : Golyar Abbas Rasool RASOOL