Detection of *Leishmania tropica* from patients in Diyala province/ Iraq

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Abstract

Background: Leishmaniasis is the second-leading cause of mortality among parasitic infections after malaria. The World Health Organization (WHO) classifies human leishmaniasis as a neglected tropical disease (NTD). It is common, appearing on every continent save Antarctica and Australia, but it mostly affects underdeveloped countries in the tropics. But due to war-related human movement and the increase of sand fly habitats brought on by changes in environmental conditions that are frequently linked to climate change, its endemicity in affluent countries is changing.

Aim of study: To determine the distribution of *Leishmania tropica* according to sex, age, occupations, residency and contact with animals in Diyala province in Iraq.

Patients and methods: This cross-sectional study included analysis of the reported cases of Cutaneous Leishmaniasis. Data of current study were collected for the period from 10th of October 2023 to 15th of February 2024. We collected 100 patients with skin manifestations who attended the dermatology clinic in Baquba Teaching Hospital. The diagnosis of cases achieved by physicians' works in these health associations and by blood tests. The privacy of the patients was preserved by coding their data into number to prevent bias. We collected a sample of 5 ml of blood in a gel tube to confirm the results in addition to information about age, gender, residency, occupation and marital status using prepared written questionnaire.

The data analysis was done by Statistical Package for Social Sciences (SPSS) version 26.

Results: 100 people were enrolled in this study. 75 patients were infected in this study. 54.7 % of them were males and 45.3% were females. The majority of the patients were under 16 years old. The majority lives in Baquba (62.7%), Khalis (26.7%), Muqdadiya (6.7%) and Baladruz (4%). 21.3% of them were married and 78.7% were not married, 14.7% of them breeding animals and 85.3% of them not breeding.

Conclusion: Cutaneous leishmaniasis is considered as major health problem in Iraq especially in Diyala governorate. The most common type is *Leishmania tropica* which has major impact on the health authorities. We recommend

performing educational campaigns about the transmission, symptoms, complications and prevention of this infection.

Introduction

Leishmaniasis is the second-leading cause of mortality among parasitic infections after malaria. The World Health Organization (WHO) classifies human leishmaniasis as a neglected tropical disease. It is common, appearing on every continent save Antarctica and Australia, but it mostly affects underdeveloped countries in the tropics. But due to war-related human movement and the increase of sand fly habitats brought on by changes in environmental conditions that are frequently linked to climate change, its endemicity in affluent countries is changing (1).

At least 20 species of the protozoan genus Leishmania (Kinetoplastida: Trypanosomatidae) are responsible for human leishmaniasis. Because of where they are found geographically—in the Western or Eastern Hemispheres, respectively—these are frequently referred to as New World or Old World species. *Leishmania infantum, Leishmania braziliensis*, and *Leishmania amazonensis* (syn. *Leishmania garnhami*) are among the species found in the New World. *Leishmania donovani* (syn. *Leishmania archibaldi), Leishmania infantum, Leishmania tropica* (syn. *Leishmania killicki*), *Leishmania major, and Leishmania aethiopica* are among the species found in the Old World (2,3).

Roughly 90% of the cases of cutaneous leishmaniasis were reported from Saudi Arabia, Iran, Afghanistan, Pakistan, Brazil, Peru, and Syria. In many parts of Pakistan, cutaneous leishmaniasis is an endemic infection. Though its disfiguring effects might lead to morbidity and social isolation, it is not a cause of death. The majority of the lesions occur in the skin's exposed regions. The affected area bears

a scar from the lesion or ulcer. Skin disfigurement and greater tissue destruction result from a secondary bacterial or fungal infection of the sores (4).

When an infected female sand fly bites a human, they get infected. A type of protozoa known as promastigotes is injected by sand flies and has the potential to spread illness. Macrophages are a type of immune cell that consumes promastigotes. (Cells that ingest are known as phagocytes; the process of a cell consuming a microbe, another cell, or cell fragments is known as phagocytosis.) Promastigotes undergo transformation into amastigotes in these cells. Amastigotes proliferate within macrophages found in several tissues. A sand fly contracts the infection by consuming blood that contains macrophages with amastigotes within it after biting an infected human or animal. Amastigotes mature into promastigotes in the midgut, or center, of the fly's digestive system. Promastigotes grow, proliferate, and move to the fly's mouth parts from the midgut. They are injected when the fly bites another person, completing the cycle (5).

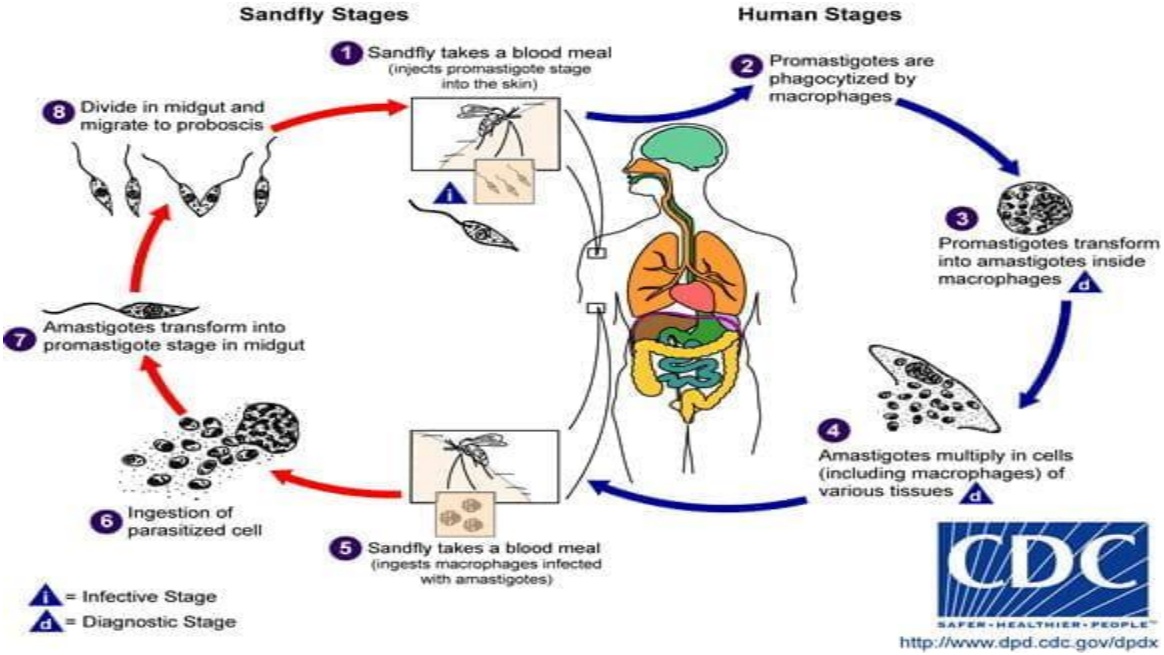

Figure 1. life cycle of *leishmania spp.*

Numerous methods have been documented for the molecular identification of *Leishmania*. These methods include random amplified polymorphic DNA, DNA fingerprinting, polymerase chain reaction (PCR), restriction fragment length polymorphism, and sequence analysis of multicopy genes (6).

Clinical case studies as well as laboratory exams such as parasitological, serological, and molecular testing are necessary for the diagnosis of CL. But conventional diagnostic techniques like smear preparation and culture medium fail to distinguish between the disease's cause species. In epidemiological and diagnostic contexts, molecular analyses are commonly employed to identify the parasite genotype. There are molecular techniques used in determination of *Leishmania* genus, species or intraspecies that depend on different target genes such as miniexon, internal transcribed spacer of ribosomal DNA (ITS-rDNA), small subunit ribosomal RNA region (SSU-rRNA), HSP-70, CPb, triose-phosphate isomerase (tim), 18S-ribosomal RNA (rRNA) , Gp63, kinetoplast DNA (kDNA) and cytochrome b (*Cyt b*) (7).

Even though leishmaniasis research and therapeutic treatments have increased, much of the focus has been on VL, with cutaneous illness receiving less attention due to its lower morbidity. Because of this, a large portion of the indexed data on the transmission of L. tropica comes from research conducted in the late 1980s and early 1990s. L. tropica is still endemic in Iraq, according to sporadic reports of outbreaks, despite the absence of rigorous follow-up and surveillance (8).

Even though CL is less severe than VL, ulcerations and subsequent infections could make the lesions worse in untreated people. Disfiguring scars and psychosocial misery brought on by the disease's social isolation and stigma constitute another complication. The majority of CL lesions heal on their own,

however depending on the type of parasite, the patient's immune system, and their genetic makeup, they may cause diffuse forms or mucosal involvement (9).

The first line of treatment for leishmaniasis is pentavalent antimonial (Sbv) drugs, which have been around for approximately 70 years. Meglumine antimoniate (MA) and sodium stibogluconate (SSG) are the two primary formulations. In an organism, Sbv functions as a prodrug and is converted to active SbIII form, a borderline metal ion that has a strong affinity for ligands including nitrogen and sulfhydryl. SbIII's antileishmanial activities most likely stem from its interactions with sulfhydryl-containing macromolecules, such as proteins, enzymes, peptides, and thiols (10).

Broad-spectrum antifungal amphotericin B deoxycholate (AmB-D) is effective against protozoan Leishmania species. When antimonial medications became less effective in India, AmB-D was chosen as the preferred first-line medication for VL. With a special affinity for ergosterol, this medication binds to membrane sterols to create complexes that organize into ion channels and improve membrane permeability (11).

It has also been demonstrated that pomomycin, an aminoglycoside antibiotic that disrupts protein synthesis and alters the fluidity of the mitochondrial membrane to prevent respiration, is a potent antileishmanial medication. In certain parts of the Indian continent, this medication is utilized as a systemic treatment for VL and as a topical treatment for CL (12).

Although there are currently no vaccinations approved for use in the general public, research and development are continually being done. There have been several attempts to date to create a leishmaniasis vaccine that has proven effective, and there are a variety of vaccine candidates, most of which are preventive.

Generally speaking, the three types of leishmaniasis preventive vaccines that are now under development are as follows: (i) live attenuated Leishmania vaccines, including new genetically modified strains; (ii) killed parasite vaccines consisting of whole killed Leishmania or fractions of the parasite; and (iii) defined vaccines, i.e. recombinant proteins, DNA vaccines and their combinations (12).

According to Avar et al. (13), in Iraq. With 1,800 VL cases recorded annually and an estimated 4,000–5,000 cases, Iraq will now be on the list of nations where 90% of VL and CL are caused by *L. major and L. tropica*. According to earlier studies, CL was mostly endemic in areas of the nation like Diyala, Kirkuk, Salah-Eldin, Wasit, and Missan in the north. Additionally, thousands of CL cases from US forces in Afghanistan and Iraq were documented. Over the past ten years, there has been an increase in leishmaniasis infections due to a variety of factors, including conflict, terrorist attacks, etc.

In the year 2001, According to the World Health Organization, the governorates of Baghdad, Dhiqar, Muthanna, Maysan, and Basra had 10.9 cases of visceral leishmaniasis for every 100,000 inhabitants. According to a study (14) the central health laboratories in Baghdad used the indirect fluorescent antibody test (IFAT) to identify 14502 cases of Kala-azar infection from sixteen provinces in Iraq in 2002. The province with the largest percentage of infections appears to have been Wasit, followed by Baghdad, Babylon, and Diyala. revealed that 310 cases of visceral and cutaneous leishmaniasis were reported among US servicemen returning from Iraq during the conflict. As to the statistics provided by the Basra governorate's health department, Qurna has 608 cases of leishmaniasis out of which 210 are in Qurna (15).

The most study (16) found that the provinces of Salah-Edin (10.3), Baghdad (11.2%), AL-Diwaniyah (15.1%), Wasit (14.5%), Najaf (13.6%), Thi-Qar (13.1%),

Basrah (11.5%), Baghdad (11.2%), and Diyala (10.8%) had reduced leishmaniasis prevalence. According to the same study, *L. major* (60%) caused the most infections, compared to *L. tropica* (40%).

Leishmaniasis requires a set of intervention strategies to prevent infection transmission because it occurs in a complex biological system including the human or animal host as well the parasite and the vector, as follows (15):

1. Effectively reducing the incidence of the infection and preventing disability or death may be possible with early diagnosis and immediate treatment. Though they are challenging to use, there are currently safe and effective medications that can be used as anti-leishmanials, particularly for visceral leishmaniasis.

2. By lowering the population of sand flies, vector management aids in the reduction or cessation of disease transmission. Insecticide spraying (home or space), the use of bed nets sprayed with fine-weave insecticides, environmental management, and personal protection are some of the control techniques.

3. Effective and timely disease monitoring can contribute to appropriate behavior and management during epidemics and reduce high treatment-related mortality rates.

4. Controlling reservoir hosts can be very effective method mainly in situation of zoonotic transmission.

Aim of study

To determine the distribution of *Leishmania tropica* according to sex, age, occupations, residency and contact with animals in Diyala province in Iraq.

Patients and methods

This cross-sectional study included analysis of the reported cases of Cutaneous Leishmaniasis. Data of current study were collected for the period from 10th of October 2023 to 15th of February 2024. In this study collected 100 patients with skin manifestations who attended the dermatology clinic in Baquba Teaching Hospital. The diagnosis of cases achieved by physicians' works in these health associations and by blood tests. The privacy of the patients was preserved by coding their data into number to prevent bias. laboratory diagnosis through Giemsa stain preparation of suspected ulcers was done, amastigotes were screened under light microscope 100x oil immersion Blood samples were centrifuged and the serum was divided into an Eppendorf tube, each containing at least 500 µl of pure serum and were then stored at -20°C for later investigation with immunofluorescent antibody test (IFAT) in addition to information about age,

gender, residency, occupation and marital status using prepared written questionnaire (16).

The data analysis was done by Statistical Package for Social Sciences (SPSS) version 26. (17)

Results:

100 patients were enrolled in this study. 75 of them were infected with leishmaniasis. Their gender is demonstrated in table 1.

Table 1. Association of *Leishmania tropica* infection with gender in present study

Gender	Frequency	Percent
Male	41	54.7
Female	34	45.3
Total	75	100.0

As shown in table 1, The males were 54.7% of the sample and the females were 45.3% of the sample.

Table 2. Association between *Leishmania tropica* and age groups in patients

Age	Frequency	Percent
1-5 years	16	21.3
6-15 years	35	46.7
16-30 years	10	13.3
31-60 years	12	16.0
More than 60 years	2	2.7
Total	75	100.0

As shown in table 2, the majority of the sample were in school age (6-15 years) as they were 46.7% of the sample, and then the under 5 years age group with percentage of 21.3%. young age group (16-30 years) was 13.3% , middle age group (30-60 years) was 16% and finally the old age group with 2.7%.

Table 3. Association between *Leishmania tropica* infection and occupation of the patients

Occupation	Frequency	Percent
Housewife	8	10.7
Free worker	9	12.0
Student	22	29.3
None	33	44.0
Employee	3	4.0
Total	75	100.0

As demonstrated in table 3, the majority of the sample were unemployed (either under 5 years or above 60 years) with percentage of 44%, and then the students with 29.3%, free workers with 12%, housewives with 10.7% and employees were only 4%.

Table 4. Association between *Leishmania tropica* infection and residency of patients

Province	Frequency	Percent
Baquba	47	62.7
Khalis	20	26.7
Muqdadiya	5	6.7
Baladruz	3	4.0
Total	75	100.0

In table 4The majority of patients lives in Baquba (62.7%), Khalis (26.7%), Muqdadiya (6.7%) and Baladruz (4%).

21.3% of them were married and 78.7% were not married as shown in figure 2.

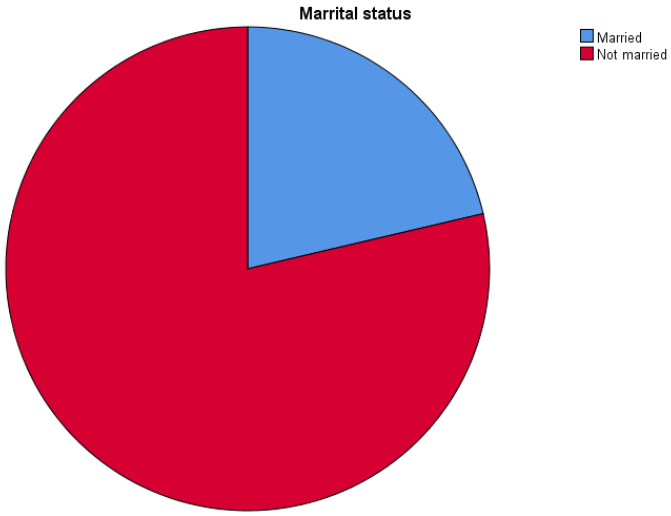

Figure 2. Association of *Leishmania tropica* with marital status of patients in present study

14.7% of them breeding animals and 85.3% of them not breeding. As demonstrated in the figure 3.

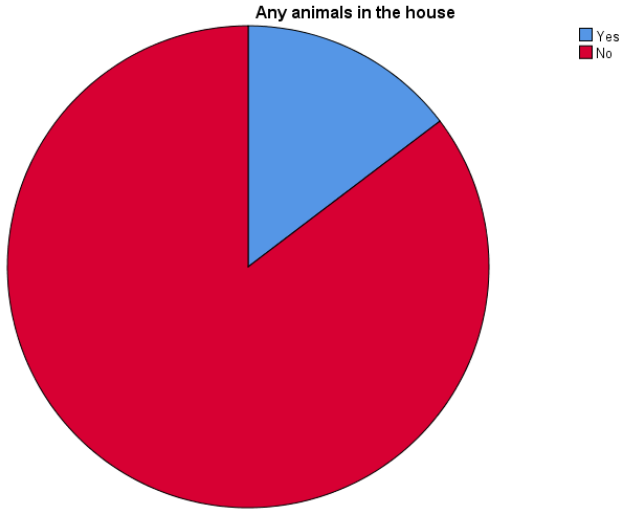

Figure 3. Association of *Leishmania tropica* infection with animal contact in present study

Discussion

Cutaneous leishmaniasis is a significant parasitic disease with importance in public health, particularly in regions where it is endemic. The disease can cause disfiguring skin lesions, leading to social stigma and psychological distress. The disease manifests in various clinical forms, ranging from self-healing skin ulcers to more severe and persistent lesions. Diagnosis can be challenging, and access to effective treatments may be limited in certain regions (18).

Cutaneous leishmaniasis is an endemic disease in several countries. Foci of L. major in Egypt, Jordan, Libya, Sudan and Tunisia, while Afghanistan, Pakistan, Iran, Saudi Arabia, Syria, Iraq, Morocco, and Yemen are endemic for both L. tropica and L. major (19).

The results of current study found no significant differences between infection of the males and the females, this may be due to the fact that Leishmania parasites infect both sexes at the same extent. Furthermore, there is no clear explanation for infection differences according to the gender. The present study

revealed that males were more susceptible leishmanasis than females, It might be individual and behavioral factors that play a role as mentioned in previous studies (20), and this agreed with Abdulwahab (21).

The highest rate of infection by *L. tropica* was among age group of 6-15 years old (46.3%), then followed by age group 1-5 years old. This can be explained as a result to previous exposure of the infection which give permanent immune for individuals. This is in consistent with the findings of Al-Difaie (20), El Hamouchi et al.(22).

The disease incidence in Iraq increases in September and October and reaches in maximum in January and February months, while leishmaniasis incidence diseases march and reach the lowest rate in July and August months (23).

The traditional treatment may not be useful in fighting this pathogen and modified amino acid should be manufactured and developed as the treatment in combination with DNA sequence in order to alter the protein synthesis pathway and thereby inhibit parasite growth and death (24).

CL is considered as an occupational infection. Soldiers, miners, policemen, farm, and forest workers are at high risk for CL infection. Our results showed that occupational exposure is an important determinant of infection with CL, where students (29.3%) were the most affected occupational category of the affected patients. A Free workers and housewives, comprising 12% and 10.7%, respectively, were the most common occupation affected and this is consistent with finding of Abdalatif (25), as they more exposed to the vector.

Throughout the present study, the occupation was determined to be another significant risk factor. Students were shown to be particularly vulnerable to insect

bites, which greatly increased their risk of contracting CL (26) In this sense, Alsamarai and Alobaidi published similar results in Iraq (27).

The presence of the disease in two married individuals is unusual as the disease often presents sporadically and in individuals. While there have been previous reports of clusters of individual infections occurring in rural areas of Iraq, these patients are unique because we are not aware of any previous cases where the infected individuals were living in the same home. Interestingly, nearly all cases in individuals who live in rural environments near livestock and farm animals, as seen in these patients. This finding raises concern for the further progression of the disease in rural communities and the need for increased awareness in these areas (28).

Animals get infected from the bite of a sandfly (vector) that has previously fed on blood from another infected animal. Dogs and cats develop the skin form of the disease and may have non-itchy, dry, flaky, hairless areas on the muzzle, around the eyes, ears or on the feet. Lesions may start on the footpads and then spread to the rest of the body. Leishmaniasis can be transmitted from dogs to people. Humans most frequently catch this disease when they are bitten by a sand fly or other insect that has previously bitten an infected animal or human (29). The present study revealed that only 14.7% of patients had contact with animals especially dogs which also could be potential risk factor for the infection.

Conclusion

Cutaneous leishmaniasis is considered as major health problem in Iraq especially in Diyala governorate. The most common type is *Leishmania tropica* which has major impact on the health authorities. In present study recommend performing

educational campaigns about the transmission, symptoms, complications and prevention of this infection.