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Research Article

Clinical picture of cutaneous leishmaniasis in Wasit, Iraq

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Abstract: **Background:** Cutaneous leishmaniosis in Iraq has 2 forms, zoonotic cutaneous leishmaniasis (ZCL), which is mainly caused by Leishmania major, and anthroponotic cutaneous leishmaniasis (ACL), which is mainly caused by Leishmania tropica . The main insect vector for transmission of L.major is the sand fly species Phlebotomus papatasi. The aims of present study to report the current status of CL in Iraq as much as is possible. **Methods:** Sixty skin samples were taken from suspected patients with CL and checked for Leishmania amastigote using Giemsa- smeared and Nested-PCR methods, during the period from October 2018 to February 2019 in Al-Karamah teaching hospital of Kut city, Iraq. **Results:** The highest infection (70 %) appeared by using Nested-PCR method while the lowest infection (51.6%) appeared by Giemsa-smeared method. The prevalence of CL were 31(51.6) in males and 29(48.4) in females and high prevalence (31.6%) in age groups (1≤ -10) and (11-20) years old. In Iraq, cutaneous leishmaniasis (Baghdad boil) caused by two species L. major zoonotic disease and L.tropica anthroponotic disease. The present study revealed that prevalence of L. major (63.3%) were higher than L.tropica (6.7 %) in the studied areas. **Conclusion:** L.major is the main species responsible of cutaneous leishmaniasis in areas of Wasit Province mid-southern of Iraq and Nested-PCR can be used for diagnosis and identification of Leishmania species**.**

Keywords: Cutaneous leishmaniasis, Nested-PCR, , Giemsa, Human.

INTRODUCTION:

Leishmaniases have been considered tropical afflictions that together constitute one of the entities on the World Health Organization/Tropical six Disease Research (WHO/TDR) lists of most important diseases (Desjeux, 2001; Herwaldt, 1999). The disease is endemic in 88 countries of 5 continents with a total of 350 -million people at risk and 12 million cases. Among the 88 endemic countries, 22 are in the New World and 66 in the Old World with an estimated incidence of 1-1.5 million cases of cutaneous leishmaniasis (CL) and 500, 000 cases of visceral leishmaniasis (VL) (Desjeux, 2001).

Cutaneous leishmaniosis in Iraq has 2 forms, zoonotic cutaneous leishmaniasis (ZCL), which is mainly caused by *Leishmania major*, and anthroponotic cutaneous leishmaniasis (ACL), which is mainly caused by *Leishmania tropica* (Mahnaz, *et al.*, 2006). The main insect vector for transmission of *L.major* is the sand fly species *Phlebotomus papatasi*.

In some urban centers of Middle East and Asia exist completely anthroponotic life cycles of the parasites, i.e. human beings are the main or only reservoir host. In such places cutaneous leishmaniasis caused by *L.tropica* can be highly endemic, but no animal reservoir is to be recognized (Postigo, 2010).

In recent decades conventional identification and taxonomic procedures for *Leishmania* microscopic examination slides, geographic distribution, clinical manifestations, pathogenic features, and culturing patterns were identified and categorized. These lack the necessary precision because there is a high similarity among species that makes the morphologic identification difficult, and also there are epidemiologic distributions for multiple *Leishmania* species coexisting in both nonendemic and endemic areas (Marfurt, *et al.*, 2003).

Polymerase chain reaction (PCR)-based methods have provided the capability of diagnosis and also identification of *Leishmania* species. Different spe-

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cies may have the criteria for the treatment phase (Blum, *et al.*, 2004). The main biological samples used for diagnosis and identification of CL species by PCR are dermal scrapings or biopsies (Chargui, *et al.*, 2005; Safaei, *et al.*, 2002). The aims of present study to report the current status of CL in Iraq as much as is possible.

MATERIALS AND METHODS:

Population study

This study was carried out during the period from October 2018 to February 2019 in Al-Karamah teaching hospital of Kut city, Iraq. A total of 60 skin samples were taken from suspected patients with CL. All patients were divided into four age groups. Samples were taken from the skin leishmanial lesion, and kept into two tubes; one stored in freeze at -20 °C for Nested- PCR and the second tube for direct smear. After the smears dried completely, they were fixed with 100% methanol, allowed to dry again, and stained with Giemsa stain for microscopic examination for presence of amastigotes (Ramírez, *et al.*, 2002).

DNA Extraction

Genomic DNA was extracted from skin lesions and aspirates by using AccuPrep®Genomic DNA extraction kit (Bioneer. Korea) and done according to company instruction. The extracted genomic DNA was checked by using Nanodrop spectrophotometer (Thermo, USA), and measured the purity of DNA through reading the absorbance at (260/280 nm) (Noyes, *et al.*, 1998).

Nested-PCR

Nested – PCR was performed as follows: in the first stage two external primers CSB1XR (CGAGTAGCAGAAACTCCCGTTCA) and CSB2XF (ATTTTTCGCGATTTTCGCAGAA CG) and in the second step, two internal specific primers 13Z (ACTGGGGGTT GGTGTAAA ATAG) and LiR (TCGCAGAACGCCCCT) was used for amplification of variable minicircles of *Leishmania* kDNA. All primers provided from (Bioneer, Korea) company. Two *Leishmania* species produced the amplified fragments of about 750 bp for *L. tropica* f and 560 bp for *L. major* (Karamian, *et al.*, 2008). Amplification reactions visualized in 1.5 % Agarose Gel Electrophoresis, using a 100 bp DNA ladder (Motazedian, *et al.*, 2006).

RESULTS:

Table.1 Nested- PCR in relation of Clinical Features

Clinical features	*L.major* %	*L.tropica* %
Hand	11(18.3)	2(3.4)
Leg	12(20)	1(1.7)
Mixed	15(25)	1(1.7)
Total	38(63.3%)	4(6.7)
Number of Lesions		
1	23(38.3)	3(5)
2	12(20)	1(1.7)
≥ 3	3(5)	0(0)
Total	38(63.3%)	4(6.7%)

Table.2 Distribution of CL cases in relation of Age, Gender

Age group (year)	Male (%)	Female (%)	Total (%)
1≤ -10	8(13.3)	11(18.3)	19(31.6)
11-20	10(16.6)	9(15)	19(31.6)
21-30	11(18.3)	4(6.7)	15(25)
> 30	2(3.4)	5(8.4)	7(11.8)
Total (%)	31(51.6)	29(48.4)	60(100)

Table.3 Comparison between Giemsa-smeared and Nested-PCR in Diagnosis of CL

Test	Positive (%)	Negative (%)	Total (%)
Giemsa-smeared	31(51.6)	29(48.4)	60(100)
Nested-PCR	42(70)	18(30)	60(100)

Table.4 Distribution *Leishmania* sp. according to the District

Leishmania sp.	No. of patients in Rural areas	No. of patients in Urban areas	Total (%)
L.major	28	10	38(63.3%)
L.tropica	3	1	4(6.7%)
Total (%)	31(51.7%)	11(18.3%)	42(70%)

DISCUSSION:

Correct diagnosis of *Leishmania* species is essential to determine the clinical prognosis and a species-specific therapeutic approach. Specification of different species of genus *Leishmania* depends on several factors such as the geographical distribution of an isolate, the clinical finding of the disease and the epidemiology of the vector and the animal reservoir (PAN, *et al.*, 1993).

The diagnosis of CL classically relies on microscopic examination and in vitro cultivation. These classical methods require the presence of a relatively high number of viable or morphologically intact parasites. This may pose a problem particularly in the chronic phase of CL where parasite levels in skin lesions are very low. In contrast, the molecular approach is both sensitive and specific (Ali, *et al.*, 2018). In this study we set up a well-documented, genus-specific PCR to detect *Leishmania* species in clinical cutaneous samples and compared this method with classical methods.

Sixty skin samples of suspected patients were enrolled in our study: 31(51.6) males and 29(48.4) females. The highest infection (70 %) appeared by using Nested-PCR method while the lowest infection (51.6%) appeared by Giemsa-smeared method. The high prevalence (31.6%) in age groups (1≤ -10) and (11-20) years old. In Iraq, cutaneous leishmaniasis (Baghdad boil) caused by two species *L. major* zoonotic disease and *L.tropica* anthroponotic disease (Al-Jeboori, & Evans, 1980). The present study revealed that prevalence of *L. major* (63.3%) were higher than

L.tropica (6.7 %) in the studied areas. Similar to the findings were recorded of some other studies (Qader, *et al.*, 2009; Alimoradi, *et al.*, 2009).

Most of the CL cases were had single lesions 23(38.3%) for *L. major* and 3(5%) for *L.tropica* and most affected part of the body was hand and legs. This can be due to the fact that some ulcers do not necessarily lead to the appearance of scars for several possible reasons, i.e. immune system interference or early healing of the ulcers, spontaneously or due to treatment. These results with agreements with other studies in Iraq (Khalifa, & Rafid, 2004) and other countries (Talari, *et al.*, 2006; Hojat, *et al.*, 2012).

The rate of the disease although not significant is more seen in males 31(51.6%) than females 29(48.4%) which is similar to previous reports and most likely because of the more exposure to sand fly bites in males than females (Al-Ani, *et al.*, 2012; Kumar, *et al.*, 2007).

CONCLUSIONS:

L.major is the main species responsible of cutaneous leishmaniasis in areas of Wasit Province mid-southern of Iraq and Nested-PCR can be used for diagnosis and identification of *Leishmania* species.

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