Comparison between Microscopic Identification and Nested PCR for Detection of Cutaneous Leishmaniasis at Wasit Province

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Abstract

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* . Twenty skin samples were taken from suspected patients with CL and checked for *Leishmania* amastigote, during the period from October 2019 to February 2020 in Al-Karamah teaching hospital of Kut city, Iraq. The highest infection 100% using Giemsa- smeared and 75% using Nested PCR methods. Totally 20 patients aged from (1- <40) years old were included in current study.The prevalence of CL were in males 9(45) and 11(55) in females and high prevalence in age groups (>20) years old. The current study 12(60%) were brought on *Leishmania major* and 4(20%) *Leishmania tropica* using Nested PCR method . In present study the direct smear could be considered a good test for testing the cutaneous Leishmaniasis but Nested PCR assay was more touchy than parasitological technique in diagnosis of *Leishmania* species in skin lesions. *L.major* is the main species responsible of cutaneous leishmaniasis in areas of Wasit Province.

Keywords: *Cutaneous Leishmaniasis, Nested PCR, Giemsa, Human*

Introduction

Leishmaniasis is a disease spread through the bites of a female sandfly and triggered through different varieties of leishmaniasis, which is expressed in three main clinical types: cutaneous, mucous and visceral leishmaniasis [(1–4)]. Cutaneous leishmaniasis exists in at least two shapes; Amastigote elliptical and non-flagellated, 3-5 µm long and promastigote was a cutaneous type contained in the host sand fly [(5)]. Cutaneous Leishmaniasis cases were more abundant in winter, with a peak in February, the rate of infection then started to decline from April and reaches its lowest in July and August [(6)].

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Cutaneous Leshimaniasis, commonly known as called Baghdad boil , is a very old disease in Iraq, it is a less severe of disease which manifests self-healing ulcers. *Leishmania major* and *Leishmania tropica* are causative agents of Cutaneous Leishmaniasis in Iraq [(7)]. According to species of parasite and immune response of the patients, the symptoms differ in regions, that beginning as erythematous papule, increase in size producing a nodule, ulcerate and crusts [(8,9)]. The zoonotic type is caused by *Leishmania major* and anthroponotic type is caused by *Leishmania tropica* [(10,11)]. Other modes of transmission such as parenteral, congenital, sexual, occupational exposures, and person to-person transmission could also theoretically occur[(12)].

Nested PCR approach was applied for the detection and identification of the *Leishmania* speies according to the Noyes *et al.*, (1998) method [(12)]. Nested PCR is a one of the best parts of the parasite genome for sequencing

to identify different *Leishmania* species [13,14]. The current study aimed to compare between microscopic identification and Nested PCR methods for detection of cutaneous leishmaniasis at Wasit province.

Materials and Methods

Population study

This study was carried out during the period from October 2019 to February 2020 in Al-Karamah teaching hospital of Kut city, Iraq. A total of 20 skin samples were taken from suspected patients with CL. All patients were divided into four age groups. Samples were taken from the skin 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 [15].

DNA Extraction

Genomic DNA was extracted from skin lesions and aspirates using AccuPrep®Genomic DNA extraction kit (Bioneer, Korea) and done according to company instruction. The extracted genomic DNA was checked using Nanodrop spectrophotometer (Thermo, USA), and measured the purity of DNA through reading the absorbance at (260/280 nm) [16].

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* and 560 bp for *L. major* . Amplification reactions visualized in 1.5 % Agarose Gel Electrophoresis, using a 100 bp DNA ladder [17].

Results and Discussion

Table (1): Prevalence of CL According to the Age Groups and Gender

Age groups / Year	Gender		Total (%)
	Male (%)	Female (%)	
>20	5(25)	6(30)	11(55)
20-40	2(10)	3(15)	5(25)
<40	2(10)	2(10)	4(20)
Total (%)	9(45)	11(55)	20(100)

Twenty skin samples of suspected patients were enrolled in our study: 9 (45%) males and 11(55%) females. The high prevalence (55%) in age group (>20) years old and the lowest prevalence(20%) in age group (<40) years old. Which may be due to several factors, such as children's outdoor activities and sleeping outdoors, which increases exposure to sand fly bites during their active hours. The same result has been reported by some other researchers [24, 25 -28]. AL-Janabi, (2001) in Najaf province, who found that the age group

10-15 years old was the most affected age group [31] . While the most rate of disease reported by Azizi *et al*.,(2013) was in age group (10-19) years old assemble for Esfahan region in Iran [32]. The rate of the disease although not significant is more seen in females 11(55%) than males 9(45%) which is do not agreed to previous studies and most likely because of the more exposure to sand fly bites in males than females [29,30].

Table (2): Comparison between Giemsa-smeared and Nested-PCR in Diagnosis of CL

Test	Positive (%)	Negative (%)
Giemsa -smeared Nested-PCR	20(100) 15(75)	0 5 (25)

In this study, the results showed that the rate of positive cases of CL using Giemsa -smeared were 20 cases which constitute (100%). while Nested PCR recorded 15 positive cases constitute (75%) from total samples. The highest infection (100 %) appeared using Giemsa-smeared method while the lowest infection (75%) appeared by Nested-PCR method.

There are many diagnostic tests used to detect the *Leishmania* parasite, microscope examination is the most reliable and conventional method, the parasite is demonstrated in direct smear stained with Giemsa stain or leishman stain to detect the presence of amastigotes [17].

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 [18]. Several reports have shown the high rates of infection with *Leishmania* species and that agreed with our results, our finding is steady with the discoveries of the study conducted by others [19]. Al Samarai and Al Obaidi, (2009) reported that 73% of the cases were certain to Giemsa stain and 43% were sure in societies for Al Hawija locale of Kirkuk region in Iraq [9]. Rahi, (2015) was recording that the rate of infection was 97.8% by utilizing Giemsa technique for Wasit province in Iraq, Rahi reporting the pervasiveness of positive instances of CL was 94% by utilizing smear strategy for Wasit region in Iraq and microscope examination of smears is quick and simple to use for conclusion of CL [20]. In Tuz- Kirkuk province, suggested the diagnosis of CL by staining the aspirated material with Giemsa stain and culturing on semi-solid media, found that 73% of samples were positive for Giemsa stain, while 27% were negative [35].

Most regularly utilized strategies for the immediate identification of the parasite (e.g., minute examination of Giemsa-recolored smears and in vitro development) need affectability as a result of the shortage of *Leishmania* parasites in a few examples or the parasites might be inadequate and are generally extracellular in the slide arrangements, or are hampered by the issue of defilement [21]. These techniques have restricted sensitivities since they require coordinate perception of the parasites and the scarcity of parasites inside the injury is a sign of sores with old age. Our result were about like the outcome reported in Iraq [19]. Among these, currently, the most ordinarily utilized strategy is DNA-based systems, utilizing PCR and particular ground works for species and even strains portrayal [22,23].

Table (3): Distribution of CL cases in relation of Residence

Leishmania sp.	No. of patients in Rural areas	No. of patients in Urban areas	Total (%)
L.major	5	7	12(60)
L.tropica	0	4	4(20)
Total (%)	5(25)	11(55)	16(80)

In Iraq, cutaneous leishmaniasis (Baghdad boil) caused by two species *L. major* zoonotic disease and *L.tropica* anthroponotic disease [20]. The present study revealed that prevalence of *L. major* (60%) were higher than *L.tropica* (20%) in the studied areas. Agreement to the findings were recorded of some other studies[18,33]. The present study also agreed with Rahi *et al*.,(2019) revealed that prevalence of *L. major* (63.3%) were higher than *L.tropica* (6.7 %) [34].

Conclusions

In present study the direct smear could be considered a good test for diagnosis the cutaneous leishmaniasis, the high rate of infection with *Leishmania* species at Wasit province ,but Nested PCR assay was more touchy than parasitological technique in recognition of *Leishmania* parasite in skin lesions. *L.major* is the main species responsible of cutaneous leishmaniasis in areas of Wasit Province.

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