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Original Research Paper

Value of different diagnostic assay for detection of *Leishmania* parasite in dermal clinical samples

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Abstract: Cutaneous leishmaniasis (CL) is a widespread disease in many countries, including Iraq. The current study aimed to assess in Salah Al-Din Governorate, for comparison various methods for diagnosis the infection with *Leishmania* parasite which causing CL and comparing their sensitivity and efficiency. **Methods:** A total of 102 samples were collected from patients attended to the Tikrit teaching hospital in Salah Al-Din Governorate through the period from September 2024 till March 2025. The patients from both sexes, ages ≤ 2-75 years. Initially, clinical diagnosis was performed by a specialist physician, followed by taking blood from the central and edge of lesion for staining smears with Giemsa. Also, a third portion of aspirated blood was preserved for molecular analysis. **Results:** The results showed a 100% success rate for clinical diagnosis, while microscopic diagnosis, considered the gold standard, achieved a higher success rate (97.32%). A lower percentage of infection was recorded using molecular diagnosis for ITS1 gene (73.53%). The results of the current study confirm that the molecular method should be combined with clinical diagnosis and microscopic examination to increase the accuracy of leishmaniasis diagnosis. **Conclusion:** The present study recommends future research using different DNA extraction methods for ITS1 gene since some of samples were negative for this gene. However, the study recommends future comparative research to evaluate the efficiency of the kDNA gene for identification the species of *Leishmania* causing CL in endemic areas.

Keywords: *Leishmania*, diagnosis, ITS1 gene

1. Introduction

Leishmaniasis is a vector-borne infection caused by the protozoan parasite of the genus *Leishmania*, which transmit to human by female sandflies (*Phlebotomus* and *Lutzomyia*) [1].

The World Health Organization (WHO) has designated leishmaniasis as neglected tropical disease, with the number of cases increasing annually in endemic areas [2]. More than 12 million people are affected by leishmaniasis, with approximately two million new infections each year [3]. Typically, each *Leishmania* species is associated with a recognized clinical form in identified geographical settings. Old-World cutaneous leishmaniasis (CL) is caused by either *L. tropica* or *L. major*, or *L. infantum* [4]. In addition to the species mentioned above, there are several other species of CL in humans, such as *L. mexicana* and *L. amazonensis* in the Americas, as well as *L. aethiopica* in various other locations, reflecting the diversity of species causing CL in different geographical regions [5].

Cutaneous leishmaniasis is the most common form of leishmaniasis, presenting in several clinical forms that vary depending on the parasite species and the host immune response. These include the localized cutaneous form and the diffuse cutaneous form where the lesions appear at the skin site of sandfly bite. In addition to mucocutaneous form which may occur when the infection extends to the mucous membranes of the nose or mouth. The clinical manifestation of CL may

resemble other skin diseases, making clinical diagnosis challenging and requiring laboratory diagnosis [6]. Therefore, diagnosis of infection with CL is necessary for accurate diagnosis and treatment, as well as for successful patient management [7].

There are several methods available for diagnosis the CL, including microscopy, histopathology, culturing and molecular methods. All of these methods are with varying levels of accuracy [8]. Diagnosis is primarily depending on the clinical features of the cutaneous lesion and microscopically to detect the *leishmania* amastigote in lesions smears staining with Giemsa [9]. These above methods have relatively low sensitivity, especially in cases where the parasite count is low or in samples where amastigote are difficult to distinguish under the microscope. Studies have shown that microscopic examination detects a lower percentage of cases compared to molecular techniques, leading to false-negative results and delays in diagnosis. This highlights the need for more accurate methods in suspected cases [10]. Molecular diagnostic methods, such as polymerase chain reaction (PCR), are emerging as highly sensitive and accurate diagnostic tools compared to traditional methods for cutaneous leishmaniasis. They can detect the parasite's DNA even in samples with low parasite counts, reducing the possibility of false negatives and increasing diagnostic accuracy. Numerous studies reported that PCR yields positive results in many cases where microscopic findings are negative, making it a reliable tool for confirming infection and initiating appropriate treatment more quickly [11, 12]. Several targeted regions in nuclear DNA, such as the Internal Transcribed Spacer 1 (ITS1), or mitochondrial DNA, such as kinetoplast DNA (kDNA) mini circle fragments, have been amplified using conventional PCR based methods [13]. Since, the diagnostic methods are differing in their sensitivity to detecting *Leishmania* parasite, therefore, this study aims to compare the diagnostic efficacy of clinically, microscopy and molecular test (using ITS1 gene) in confirm the presence of parasite in CL suspected patients.

2. Methodology

Sample Collection and blood staining smear

Through the period from September 2024 till March 2025, the present study was conducted on one hundred and two patients whose attended to Tikrit teaching hospital. The patients were from both sex, ages ≤ 2-75 years) and suffering from skin lesions. The skin lesions were clinically diagnosed by a specialist dermatologist. Blood smears were taken from lesion under sterile conditions from the border and central sites of the lesions. All lesions were tested for molecular investigation. Two scraping samples were collected for each patient. The first sample was used for smears which staining with Giemsa and examined microscopically, the second sample was stored in phosphate buffered saline (PBS) at -20 °C for further molecular test [14].

For making a blood smears, the lesion cleaning with 70 %immersed cotton five times in an outward -alcohol circular motion. Using a sterile lancet or sterile surgical ong supblade, 2mm ler facial incisions were made on the margins of the lesion and pressure was maintained with a finger to achieve hemostasis. From each patient's, four touch smears of blood smears were collected, air dried, fixed in absolute methanol, and Giemsa stained to shown the amastigote phase in macrophages [15].

Traditional PCR

DNA was extracted from each clinical sample (which preserved at -20 °C), using Mini kDNA Kit (Geneaid, Taiwan) according to the manufacturer's instructions. The extracted DNA was kept at -20 °C until PCR processing. In present study, a set of primer for amplification the genes are used [16]. Since, the amplification of the ITS1 gene was carried out using the LITSR (forward) (5'-CTG GAT CAT TTT CCG ATG-3') and L5.8S (reverse) (5'-TGA TAC CAC TTA TCG CAC TT-3').

3. Results and discussion

Cutaneous leishmaniasis diagnosed by using three different methods, which revealed varying detection rates, as showing in Table (3).

The prevalence of infection with CL was 100% clinically (Figure 1). Whereas, the prevalence of infection with CL was 97.32% t using blood smears. The smear showed the presence of the amastigote phase inside macrophages (Figure 2A), and sometimes its presence was observed outside these cells (Figure 2B). For molecular test, the results of the gel electrophoresis showed that the percentage of samples which are positive for the ITS1

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gene was 73.53% and the size of band at 330-350 bp, which confirms the presence of the *Leishmania* sp. parasite. The gene used showed a characteristic band of the expected size when transferred on an agarose gel (Figure 3).

Table 1: Prevalence of infection with CL using different diagnostic methods.

Groups	Total no. of examined lesions	no. of positive lesions	Prevalence of infection (%)
Diagnosis clinically	112	112	100
Blood smears	112	109	97.32
Molecular test (ITS1)	102	75	73.53

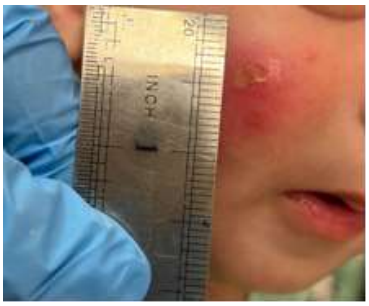

Fig.1. Patients suffer from one lesion on the cheek.

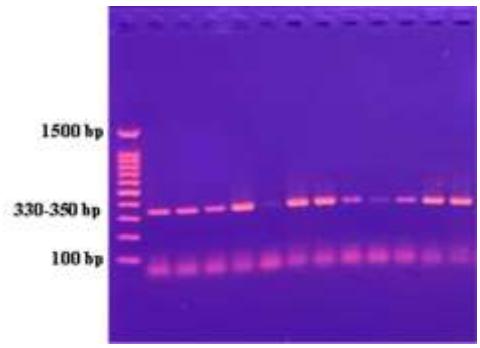

Fig.3. Agarose gel (1.5%) of the PCR products for ITS1 gene of Leishmania sp. in clinical CL isolates.

Discussion

Leishmaniasis remains a significant health burden in endemic areas and requires early and accurate diagnosis to improve treatment outcomes and disease control. The primary diagnostic tool in endemic areas is a thorough clinical examination, which helps clinicians suspect the disease based on characteristic clinical symptoms such as chronic skin lesions. This is essential before proceeding to laboratory diagnosis [17].

Microscopic examination is considered the gold standard method for detecting the parasite by visualizing amastigotes in stained smears of skin biopsies or lesion fluid. This method has been relied upon for many years due to its simplicity and low cost when an excellent examination and well-equipped laboratory are available. However, the sensitivity of microscopic examination depends on the parasite density in the sample and the examiner's skill, and sensitivity can be low in cases with a low parasite load or in less-than-ideal samples [18]. However, the similarity of forms of leishmaniasis lesions with other skin diseases may make clinical diagnosis difficult without laboratory confirmation [19]. Despite the value of microscopic examination, molecular techniques have become vital tools in the modern diagnosis of leishmaniasis, due to their high sensitivity and ability to detect even when the numbers of parasites are very small, as well as their ability to identify the species which helps

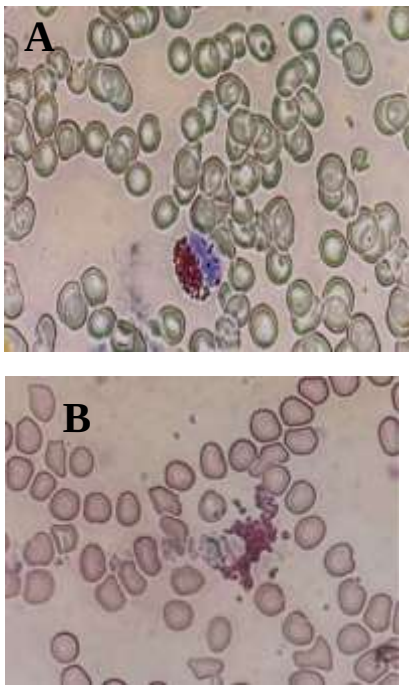

Fig.2. Leishmania parasite appear in blood staining smear; amastigote inside (A) and outside (B) macrophage.

in making accurate treatment decisions and understanding the dynamics of the epidemic spread [20].

In the current study, molecular approaches were used to confirm *Leishmania* parasite infection using the ITS1 region. In present study, initially we are used kDNA

gene as a target for detection *Leishmania* DNA in clinical lesions samples but the results of gel electrophoresis reflect that there are no matching bands at 120bp (data not appear in present study). kDNA is a common target in molecular diagnostics because of its high copy number in the cell, which theoretically makes it possible to achieve higher detection sensitivity using polymerase chain reaction [21]. However, in present study (as in some field studies), kDNA did not show satisfactory migration results during gel analysis, which may be attributed to factors related to primer efficiency, the quality of the isolated DNA, or the complexities of kinetoblast structure in some parasite isolates, affecting the quality of amplification and interpretation. The results of kDNA target analysis vary in studies depending on the protocols and the regional patterns of the parasite. Since, kDNA is one of the most important molecular targets used in the diagnosis of *Leishmania* parasite due to its high sensitivity in detecting parasitic DNA compared to other methods. However, variations in DNA extraction methods and sample types can affect the quality of results and necessitate the development of improved protocols to obtain more consistent and satisfactory results [22]. Conversely, the ITS1 target performed exceptionally well and sensitively in identifying the parasite in present study, exhibiting clear and easily readable amplification, thus confirming its ability to accurately detect the parasite and allowing for comparison of results with other diagnostic methods. This is because the ITS1 region, although present in fewer copies than kDNA, is relatively stable and sufficiently heterogeneous to reliably identify the species. The ITS1 gene is an important molecular target in the diagnosis of leishmaniasis due to its high ability to detect parasitic DNA [23]. In the present study, the sensitivity of the blood smear in diagnosis of CL was higher compared with molecular test (using ITS1 gene), due to using of four replicates of blood smears for each lesion sample as recommended in previous studies [24, 25], since CL is endemic disease in Iraq including Salah Al-Din province [25].

The results in present study recommended to use ITS1 gene and kDNA gene with different extraction methods for detection the species of *Leishmania* and its genotyping.

Conclusion

The combining of traditional diagnostic methods such as clinical and microscopic examination with molecular techniques is essential and provides the best diagnostic approach for detection the cutaneous leishmaniasis in endemic regions

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Author's Contributions

The first author made the execution, acquisition of data, analysis and interpretation. The second author made the conception, study design. The authors together made a significant contribution in the reported work.

Ethics

Ethical approval was performed from the Committee on Human Research and Ethics from Salah Al-Din general hospital and using the publication of Tikrit University.

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