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Original article

Diagnostic Performance of Microscopy, Culture, Histopathology, and PCR in Cutaneous Leishmaniasis: Evidence from an Endemic Region in Libya

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Cutaneous Leishmaniasis, Diagnostic Method, Demographic Criteria.

ABSTRACT

Cutaneous leishmaniasis is a neglected tropical disease with a significant public health impact in many endemic regions. Its diagnosis traditionally relies on clinical evaluation supported by microscopic examination of amastigotes obtained from the edges of skin lesions. Although this approach is simple, inexpensive, and quick, it has low sensitivity. Other conventional methods, such as culture and histopathology, provide additional diagnostic information but also have limitations in sensitivity, specificity, and practicality. In recent years, molecular techniques, especially polymerase chain reaction (PCR), have changed the diagnostic landscape of infectious diseases, including cutaneous leishmaniasis. PCR offers high sensitivity and specificity and can identify Leishmania species. However, its use is limited by high costs, longer processing times, and the need for specialized laboratory facilities and trained personnel. Given these challenges, this study aimed to evaluate and compare the diagnostic performance and practical applicability of four diagnostic methods for cutaneous leishmaniasis: slit-skin smear microscopy, culture, histopathological examination, and polymerase chain reaction (PCR). PCR was used as the reference standard to assess the diagnostic performance of slit-skin smear microscopy, culture, and histopathology by determining their sensitivity and specificity. A comparative diagnostic study was conducted between June 2024 and January 2025 at Tripoli Central Hospital and the National Center for Disease Control (NCDC), Libya. Patients of all ages and both genders with clinically suspected cutaneous leishmaniasis and no prior treatment were enrolled. Clinical and demographic data were collected, and lesion samples were examined using slit-skin smear microscopy, histopathology, parasite culture, and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The sensitivity of each diagnostic method was calculated using PCR-RFLP as the reference standard. Among 42 patients, PCR-RFLP demonstrated the highest sensitivity, detecting Leishmania in (41 sample) 97.6% of cases; (L. major, 92.7 %; L. tropica, 7.3%), followed by slit-skin smear (88%), histopathology (76.1%), and culture (42.8%). Females (59%) were more affected than males (41%), and children aged 0–9 years were the largest group. Most cases originated from Kikla and Gerian, with the majority of patients lasting about 8 weeks before seeking medical advice. The face and extremities were the most commonly affected sites, with ulcerated nodules being the predominant clinical presentation type, and PCR proved to be the most reliable diagnostic tool. PCR demonstrated superior sensitivity and reliability compared to conventional methods, underscoring its value as the most effective diagnostic tool for cutaneous leishmaniasis, particularly for species identification and confirmation in endemic regions.

Introduction

Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus Leishmania, transmitted to humans by bites from infected female sand flies [1]. It remains a significant global health concern, with an estimated 1–2 million new cases annually, including about 600,000 to 1 million cases of cutaneous leishmaniasis (CL) and as many as 12 million people worldwide living with the disease. More than 350 million individuals are at risk, particularly in tropical and subtropical regions [2]. The disease presents a spectrum of clinical manifestations that vary by Leishmania species and host immune response: (1) Localized cutaneous leishmaniasis (CL), characterized by cutaneous ulcers mainly on exposed parts of the body. (2) Mucocutaneous leishmaniasis (MCL) involving the mucosa and underlying connective tissues. (3) Visceral leishmaniasis (VL) affects internal organs and the reticuloendothelial system [1,3]. From a European perspective, Leishmania parasites are broadly categorized into two major groups.

Old-World species are primarily found across the Mediterranean basin, the Middle East, the Horn of Africa, and the Indian subcontinent, and New-World species, by contrast, are found in Central and South America [4]. Diagnosis relies on a

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combination of clinical presentation, epidemiological context, and laboratory confirmation. A wide range of diagnostic techniques exists, each with varying accuracy [5]. These include direct parasitological approaches, such as microscopy, histopathology, and parasite culture, as well as indirect methods, including serological assays and molecular diagnostics [5,6]. In practice, the choice of diagnostic method is often influenced more by the facility's infrastructure and resource availability than by the test's sensitivity or specificity.

Traditional methods, such as slit-skin smear microscopy, remain widely used in resource-limited, endemic regions because they are rapid and cost- effective. However, microscopic examinations have variable sensitivity (ranging from 42% to 70%), primarily depending on the technician's skill and parasite density. Gadri et al. (2025) applied YOLOv8 to detect Leishmania parasite bodies in microscopic images, achieving an accuracy of 97% across the entire test dataset [7]. Culture and histopathology also have limited sensitivity and are resource-intensive [8,9,10]. In contrast, PCR offers significantly higher sensitivity and specificity, enabling the detection of low parasite levels. For example, kDNA-PCR has demonstrated sensitivity of up to 99–100% and nearly 100% specificity; some studies report that nested PCR sensitivity reaches 97%, outperforming microscopic and culture techniques [8, 9]. The effectiveness of existing treatments varies among different Leishmania species, making accurate species identification essential for guiding appropriate therapy and improving patient outcomes. Libya is a significant focus for Old World cutaneous leishmaniasis (CL), with L. major as the dominant species responsible for zoonotic transmission. L. tropica and L. infantum have also been identified in specific regions. A study reported 13,625 cases from 2019 to 2022 (Saad 2025).

Many studies have found that PCR was reliable for diagnosis, supporting a shift toward molecular methods in endemic settings [11,12]. Most diagnostic results from previous local studies focus on epidemiological prevalence and distribution, clinical presentation and associated infections, and species Identification. Globally, many studies have been initiating the application of advanced, integrated methods, molecular tools, and AI-assisted techniques that show promise. To sum up, studies from Libya on the diagnostic investigation of cutaneous leishmaniasis (CL)are showing: (1) Lack of comprehensive diagnostic comparisons. (2) Limited assessment of diagnostic accuracy. (3) Geographical and contextual limitations. (4) Neglect of advanced and combined methods, as most studies have used individual diagnostic methods, such as microscopy, culture, and histopathology.

To address these gaps, this study applies a comparative diagnostic approach across five methods, providing evidence to inform the selection of the most suitable tests for specific patient populations. It compares microscopic and PCR-based diagnostics for CL in northwestern Libya by conducting a comprehensive comparative analysis of four diagnostic methods: Microscopy, Culture, Histopathology, and PCR, within the same group, aiming to assess their relative efficiency, accuracy, and suitability for routine diagnosis of cutaneous leishmaniasis in Libya.

Materials and Methods

The study sample comprises participants visiting the Leishmaniasis Clinic in the Dermatology Department at Tripoli Central Hospital (TCH) and the corresponding Leishmaniasis Clinic at the Libyan National Center for Disease Control (NCDC) in the Northwestern Region of Libya, which encompasses 16 endemic areas. Cases were collected from June 2024 to January 2025.

Study Materials

The study material comprises data from a structured questionnaire, results of multiple tests, and observed clinical lesions, including papules, nodules, and ulcerated nodules. The questionnaire was used to collect general patient information, lesion history, and clinical examination findings. The study included 42 patients with cutaneous leishmaniasis (CL). (Figure 1) Shows the study methodology.

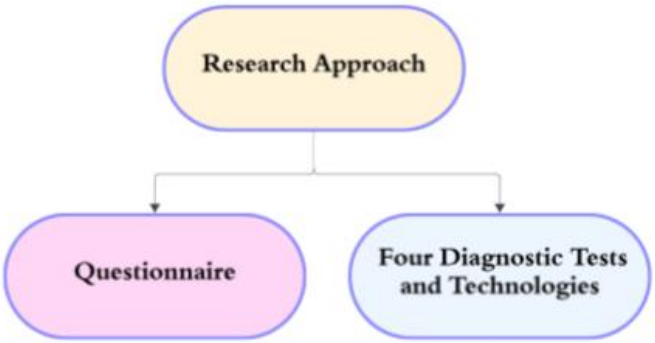

Figure1. Study approach

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Study Method
The study employed a comparative diagnostic approach to evaluate the performance of four tests for detecting the same condition, focusing on their relative accuracy and diagnostic effectiveness. The study process included obtaining ethical approval, collecting samples, conducting clinical evaluations, performing diagnostic tests, analyzing the data, and ultimately identifying the study's limitations.

Ethical Approval
Patients gave informed consent prior to participation. The study was approved by institutional review boards, which ensured confidentiality was maintained.

Sample Collection
The inclusion criteria encompassed patients of all ages and genders with suspected cutaneous leishmaniasis, recruited from Tripoli Central Hospital and the National Center for Disease Control between June 2024 and January 2025, who had no prior treatment or systemic illness. The duration of lesions before diagnosis ranged from two weeks to five months.

Clinical Evaluation
Each patient underwent a lesion assessment (type, location, and duration), and demographic and clinical data were recorded using a structured questionnaire.

Diagnostic Testing
Four diagnostic methods were employed on lesion samples, including slit-skin smear microscopy, histopathology, parasite culture, and PCR-RFLP for species identification.

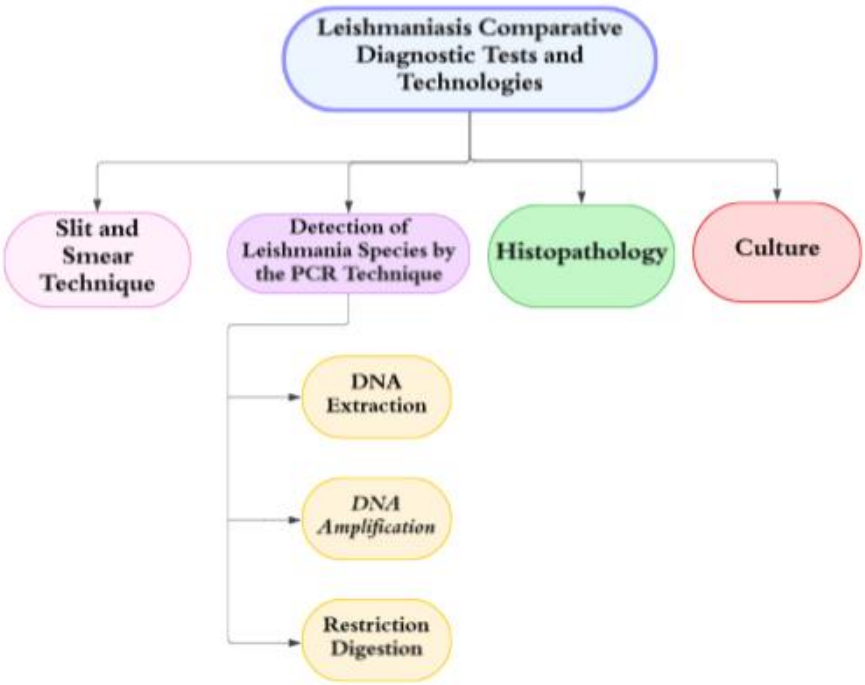

Figure2. Diagnostic tests: The four approaches are used for the Leishmaniasis Comparative Diagnostic Tests and Technologies

Data Analysis
The sensitivity of each diagnostic method was calculated and compared, using PCR-RFLP as the reference standard.

Limitations
Constraints included limited molecular resources, a small sample size, and the absence of treatment follow-up data.

Leishmaniasis Comparative Diagnostic Tests and Technologies
Comparative diagnostic tests and technologies for leishmaniasis assess which technique can effectively identify the condition by comparing results with other methods to determine accuracy and the potential to replace or supplement

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current clinical practices. These comparative diagnostic technologies include the Slit and Smear Technique, PCR Detection of Leishmania Species, and DNA Amplification.

Slit and Smear Technique

The lesions were sterilized with 70% ethanol. Small incisions were made along the lesion margin using a sterile disposable surgical scalpel blade, size 15 (Vimodone, Italy), to obtain skin samples. The blood supply to the marginal area of the lesions was cut by blanching with the thumb and index fingers of one hand, while the other hand made a small incision in the cleaned margin of the lesion with the tip of the blade. The blade was held at a 90-degree angle and scraped along the cut edge of the incision to remove and collect skin tissue, which was then smeared onto a clean glass microscope slide. After the smears had dried completely, they were fixed in 100% methanol and then dried again. At least two specimens were prepared for each case. One was stained with Giemsa stain for microscopic examination [8]. The other was kept for PCR analysis. The stained slides were examined for the presence of amastigote bodies under a light microscope with an oil immersion lens (100X objective).

Detection of Leishmania Species by the PCR Technique

This technique involves three stages: DNA extraction, DNA amplification, and restriction digestion.

DNA Extraction

Parasite DNA was directly extracted from slides using the protocol provided with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The purity of the sample mixture was measured using a NanoDrop Lite spectrophotometer (Thermo Scientific).

DNA Amplification

Extracted DNA from each sample was screened for Leishmania spp. by PCR using the primer pair ITS1F (5' GCAGCTGGATCATTTTCC 3') and ITS2R4 (5' ATATGCAGAAGAGAGGAGGC 3'), which amplifies a 390-bp fragment of the ITS1 gene [13]. All PCR reactions (50 μL final volume) were performed by adding 2.5 μL of DNA template to 25 μL of Hot Start Taq Master Mix (QIAGEN, Hilden, Germany), 5 μL of 10x (2 μM of each primer), and 17.5 μL of RNase–free water. DNA from Leishmania species obtained from the Pasteur Institute, Tunisia, was used as a positive control to confirm the successful amplification of the correct target genes. A negative control (RNase–free water) was also included. PCR cycling in a thermocycler began with denaturation at 94°C for three minutes, followed by 37 cycles. Each cycle consisted of three steps: 30 seconds at 94°C (denaturation), 30 seconds at 58°C (annealing), and 90 seconds at 72°C (extension). After the final cycle, the extension step was extended by an additional 10 minutes, and the reaction was then kept at 4° C [13]. The PCR products were visualized using UV transillumination with Gel Red™ dye (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD, USA) after electrophoresis on a 1.5% agarose gel (Sigma, St. Louis) in 1X TAE buffer for 45 minutes at 150 V. The size of the amplified DNA was determined by comparison with a 100 bp ladder (Sigma–Aldrich, USA). The PCR products were visualized using UV transillumination with Gel Red dye (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD, USA) after running on a 1.5% agarose gel (Sigma, St. Louis) in 1X TAE buffer for 45 minutes at 150 V. The size of the amplified DNA was determined by comparison with a 100-bp ladder (Sigma–Aldrich, USA).

Restriction Digestion

RFLP was performed as outlined by Marfurt et al. (2003).[14] Ten of the PCR products were digested in a 20-reaction mixture containing 10 U of Hae III (Sigma, St. Louis) and two µL of the appropriate reaction buffer in a 37°C water bath overnight. All 20 digests were used for electrophoresis on a 2.5% agarose gel. Fragment sizes were estimated by visualizing the products using UV transillumination with Gel Red™ dye (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD, USA) on a 2% agarose gel (Sigma, St. Louis) in 1X TAE buffer for 45 minutes at 150 V after electrophoresis. The size of the amplified product was determined by comparing it with a 100-bp ladder (Sigma–Aldrich, USA). DNA from L. major, L. tropica, and L. infantum obtained from the Pasteur Institute, Tunisia, was used as positive controls to confirm that the correct target genes were successfully amplified. A negative control (RNase–free water) was also included.

Histopathology

Skin biopsies were taken from the margin of the lesion (avoiding ulcerated and heavily crusted areas) after injecting 2% lidocaine as an anesthetic. The samples were obtained with a 2-mm disposable punch (Whatman International, Germany). The tissue was fixed in 10% formalin, processed routinely, embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin.[15] The volume of fixative was at least 15-20 times that of the tissue. Formalin fixation lasted less than 48 hours. The tissue was then dehydrated in ethanol, incubated twice in xylene (30 minutes each), and embedded in paraffin. The tissue was sectioned at 5µm on a microtome, placed on clean slides, and then deparaffinized. Finally, the

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sections were rehydrated and then stained with hematoxylin and eosin. All biopsies were examined for the presence or absence of the following morphological changes:

a. Epidermal alterations, including hyperkeratosis, acanthosis, spongiosis, parakeratosis, granulosis, and atrophy.
b. Dermal alterations, including inflammatory cell infiltration, macrophages (histiocytes), lymphocytes, plasma cells, and giant cells in the dermis, and either unorganized or organized granulomas.

The histopathological spectrum in CL has been categorized into different stages. In this study, we follow the classification of both de Magalhaes et al (1986) [16] and Gonzalez et al (2018) [17] that characterizes the histopathology of CL and MCL Leishmania.

Culture

Clinical specimens were obtained from the active margins of suspected cutaneous leishmaniasis lesions after they were disinfected with 70% ethanol. Tissue samples were collected using sterile scalpels, targeting viable, inflamed areas while avoiding necrotic tissue. Immediately following collection, the clinical specimens were directly inoculated into sterile culture tubes containing biphasic Novy-MacNeal-Nicolle (NNN) medium. The NNN medium consisted of a solid blood agar base (mixture of rabbit blood and saline agar) overlaid with Schneider's Drosophila medium supplemented with 10–20% heat-inactivated fetal bovine serum (FBS). Approximately 0.1–0.2 mL of the sample was introduced into the slant portion of the medium under aseptic conditions within a laminar flow hood. The inoculated tubes were incubated at 24°C ± 1°C and observed microscopically at 2–3-day intervals for up to 21 days. A drop of the overlay medium was examined under light microscopy at 400× and 1000× magnification for the presence of motile promastigotes, which are indicative of successful parasite growth.

Statistical Analysis

The data were organized and analyzed using SPSS Statistics 22. Descriptive statistics were performed using frequency distributions and percentages, and a cross-tabulation analysis was conducted to examine the relationship between histopathological staging and clinical appearance. A correlation test was also used to examine the relationship between these variables. A P-value of less than 0.05 was regarded as statistically significant.

Results

The 42 patients in this study comprised 17 males (41%) and 25 females (59%), with ages ranging from 0 to 65 years. The study found that 37 (88%) were positive on scanned slit skin smear slides for amastigotes, and 41 (97.6%) were successfully amplified and identified by PCR-RFLP: 3 (7.3%) as L. tropica and 38 (92.7%) as L. *Major*. 32 (76.1%) were positive for amastigote detection on histopathological examination, and 18 (42.8%) were positive on culture.

Epidemiology of Data Collected

(Figure 3) shows that the prevalence of CL was higher among females (n = 25, 59% of samples) than among males (n = 17, 41%), with a female-to-male ratio of 1.41:1. The age at onset ranged from 0 to 65 years. Children aged 0-9 years were the most affected age group; all patients were Libyan (Figure 4). Figure 5 indicates that the cases mostly originated from mountain areas (66.7%), whereas 13.3% originated from valleys. Kikla and Gerian were the most common areas of residence, each with the same number of cases.

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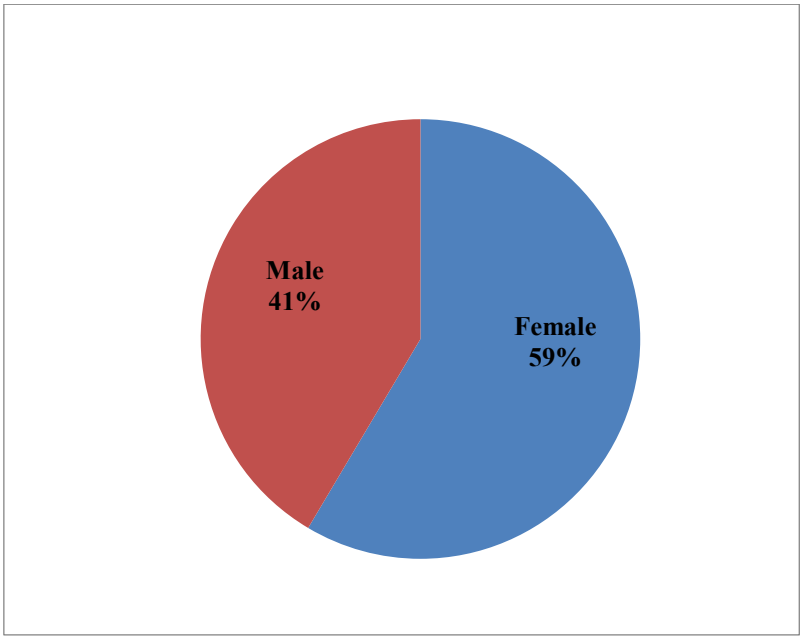

Figure 3. Distribution of patients by gender

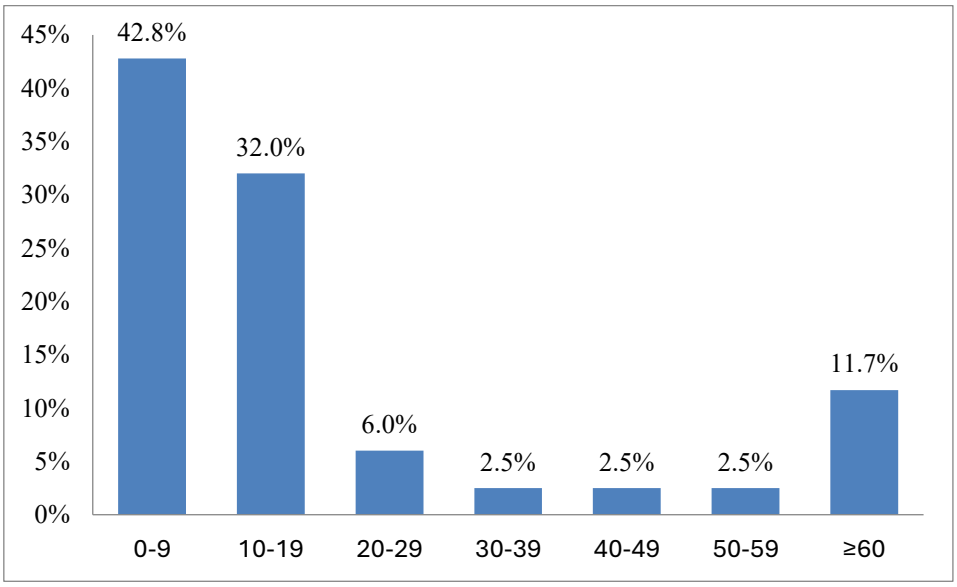

Figure 4. Age group distribution of patients

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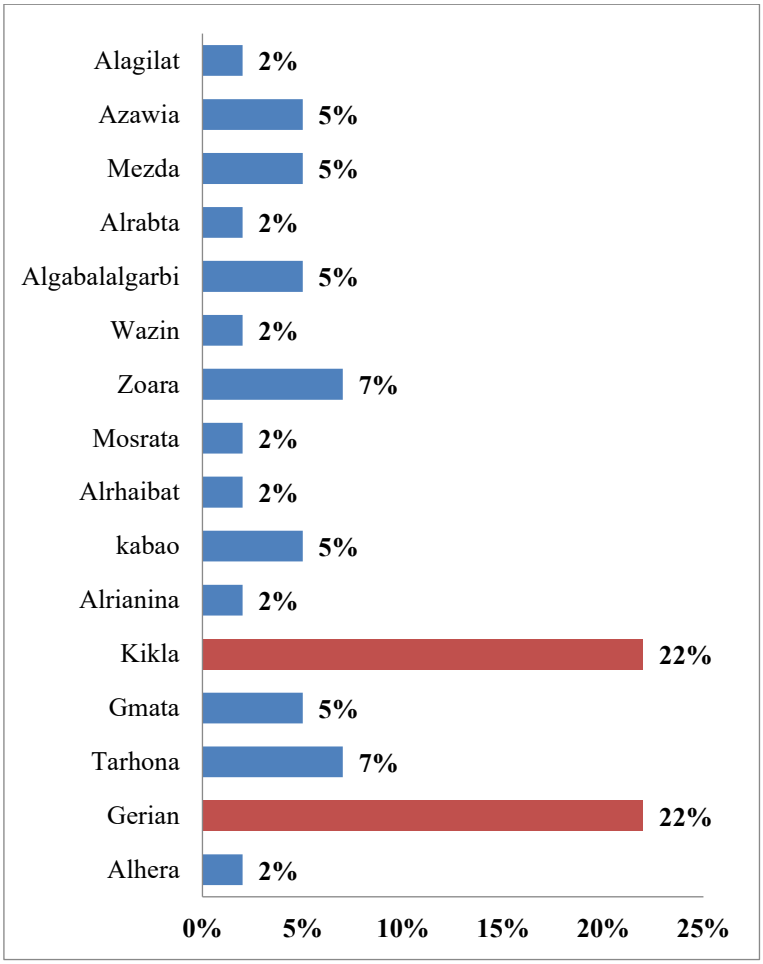

Figure 5. Geographic distribution of patients by place of residence

Figure 6 shows that 49% of patients sought medical advice when the lesion had persisted for approximately 8 weeks, while 12% of patients waited for 4-5 months to seek treatment. (Figure 7) highlights the infected parts of the body. Face and extremities were the majority. (Figure 8) illustrates the clinical presentation.

The nodulo-ulcerative form of Leishmania major was the predominant clinical presentation, accounting for 60% of patients with multiple lesions and 40% of those with single lesions, making it the most common lesion type overall. Nodular lesions represented the second most frequent presentation of L. major, occurring in 31% of patients with multiple lesions and 20% of those with single lesions. In contrast, Leishmania tropica was more commonly associated with single lesions. Nodular lesions caused by L. tropica were observed in 20% of single-lesion cases compared with only 3% of multiple-lesion cases, while the noduloulcerative form accounted for 10% and 3% of single and multiple lesions, respectively. Less common clinical variants included the zosteriform form of L. major, which was detected exclusively among patients with multiple lesions (3%), and the paronychia-like form, which was observed only in patients presenting with single lesions (10%) and was absent among those with multiple lesions. Figure 9 shows some samples of clinical presentation.

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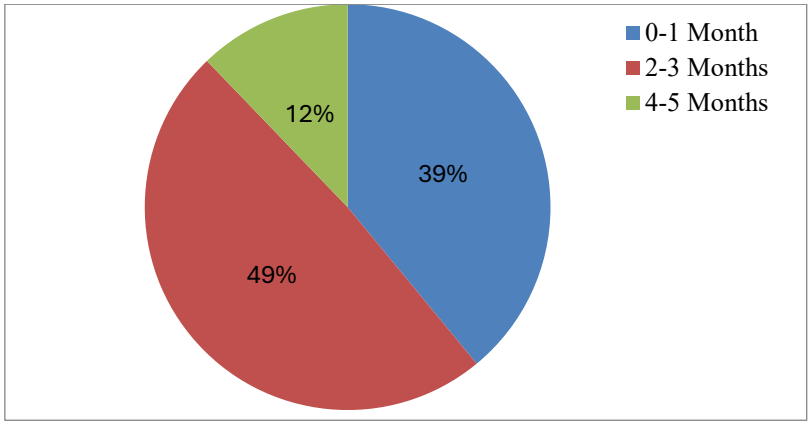

Figure 6. Duration of lesion prior to diagnosis. As shown, the duration ranged from 0 to 5 months

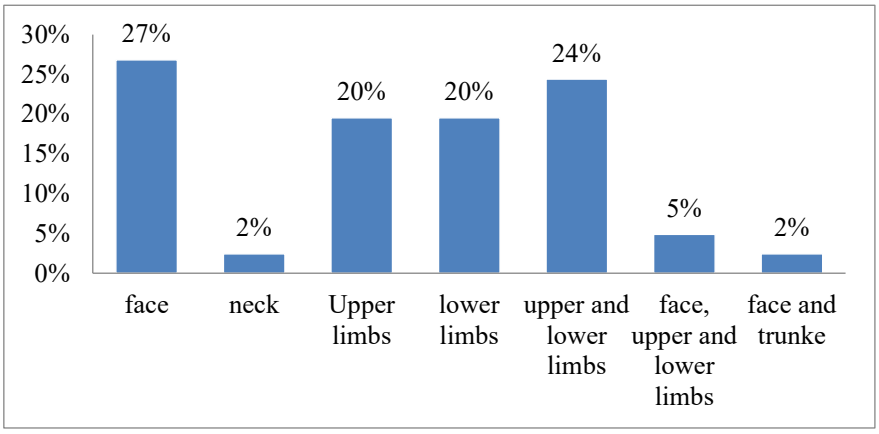

Figure7. Anatomical distribution of lesions

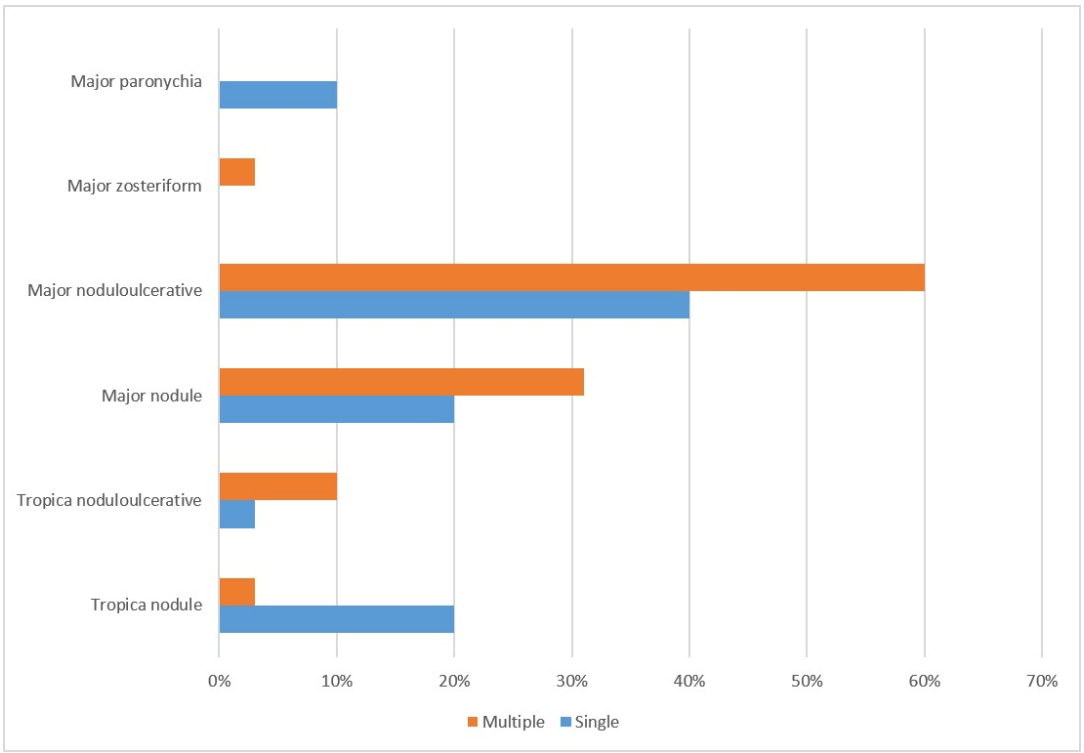

Figure 8. Clinical presentation of lesions

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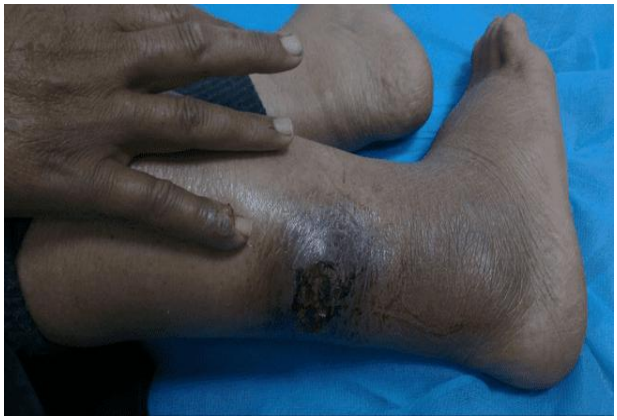

Figure 9. Crusted Ulcerated Nodule on leg (picture of some studied sample)

Figure 10 shows the diagnostic methods used in this study. PCR was the most effective and reliable diagnostic tool compared to conventional methods. The results indicate that PCR was the most sensitive diagnostic technique for detecting Leishmania infection, outperforming conventional methods such as microscopy, histopathology, and culture. Although slit-skin smear microscopy showed relatively high sensitivity and remains a rapid and inexpensive diagnostic tool, its performance was inferior to PCR. Histopathology demonstrated moderate diagnostic accuracy, while culture had the lowest detection rate, likely due to the stringent growth requirements of the parasite and the potential loss of organism viability during specimen handling

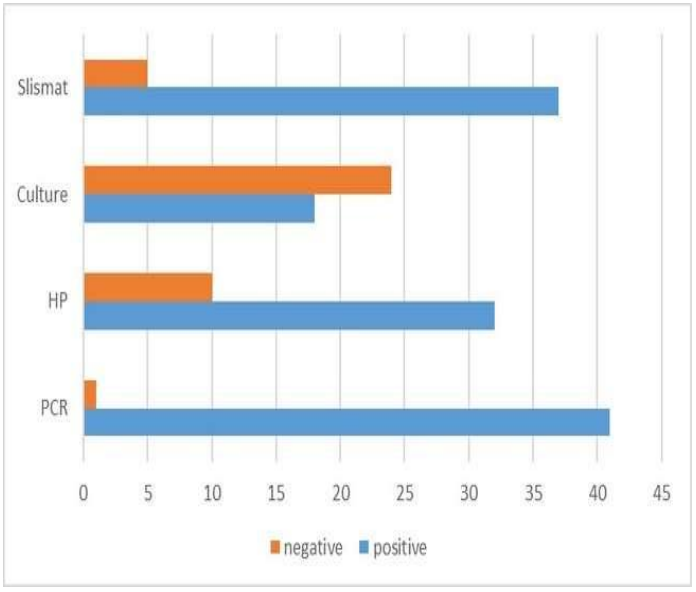

Figure10. Diagnostic methods used for confirmation of cutaneous leishmaniasis

Results of diagnostic tests
Slit Skin Smear Test
Figure 11 shows the positive sample for visualized parasite in Giemsa-stained slit skin smear examination.

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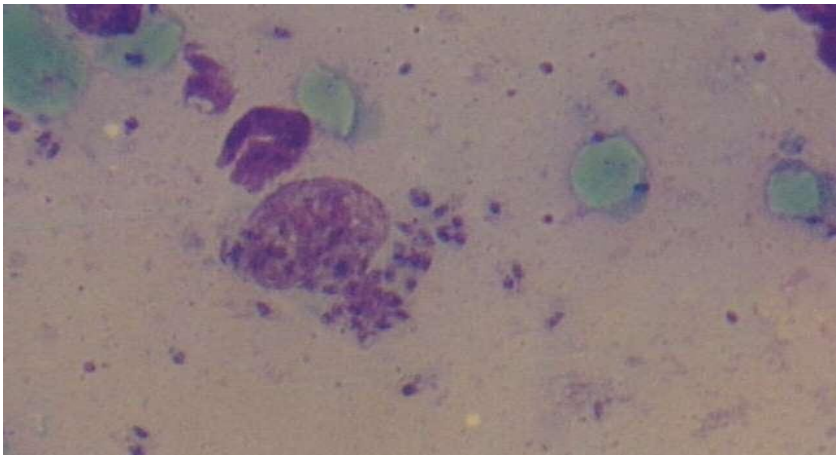

Figure 11. Intra- and extracellular Leishmania amastigotes as shown in a Giemsa-stained slit-skin smear

PCR Test

Molecular identification of parasites using the ITS1-PCR approach targeting the ITS1 region of the rDNA was successful in 41 Leishmania patients (97.6%), and 1 was negative. ITS1-PCR produced a 390-bp amplicon (Figure 12).

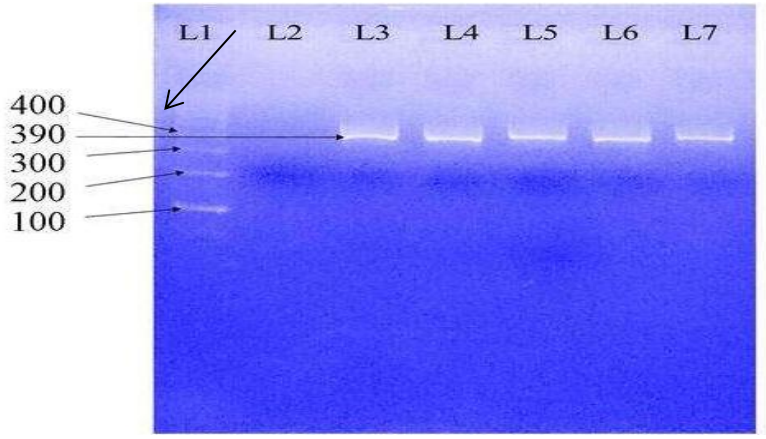

Figure 12. Red gel–stained agarose gel electrophoresis showing the representative PCR amplification of Leishmania spp. DNA Amplification of ITS1 (Lane 1 is a 100 bp molecular leader marker, Lane 2 is a negative control, Lane 3 is a positive control, Lanes 4,5,6,7 Leishmania primary samples)

The restriction fragment length polymorphism (RFLP) profiles of the ITS1-PCR products, generated with the endonuclease HaeIII in the PCR assay (Figure 8), revealed a single Leishmania species as the causative agent. *Major*

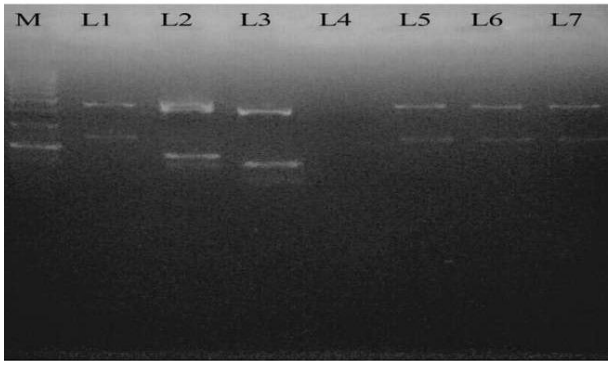

Figure 13. Red gel–stained agarose gel electrophoresis showing the restriction fragment length polymorphism profile of the internal transcription spacer-1 polymerase chain reaction product using HaeIII restriction enzyme Leishmania major in lanes 5, 6, 7 (M is 100 bp molecular marker, lane 1 L. l.major control, lane 2 tropica, 3 L. infantum control, and lane four negative control)

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Histopathological Test

Histopathological examination of the skin biopsies revealed that amastigotes (Figure14) were demonstrated in the dermis in 32 (76.1%) cases. The parasites were demonstrated in the upper dermal layer and were more evident in the early stage of the lesion.

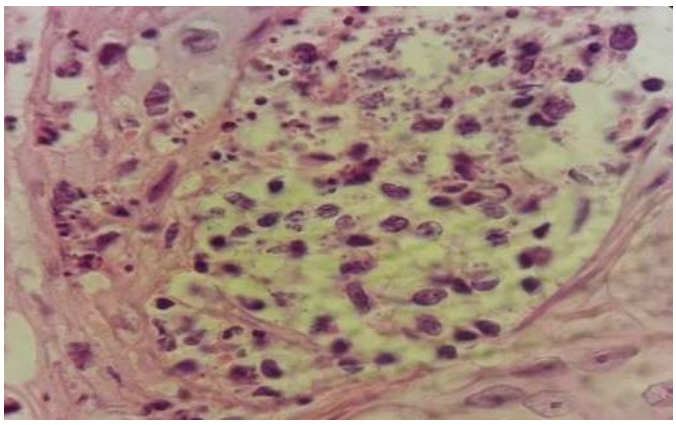

Figure 14. Leishman-Donovan bodies within the macrophages and extracellular (Hematoxylin and Eosin)

Culture

Promastigotes were obtained from cultures grown in Novy–MacNeal–Nicolle (NNN) medium and stained with Giemsa stain to visualize their morphology under light microscopy. (Figure 15).

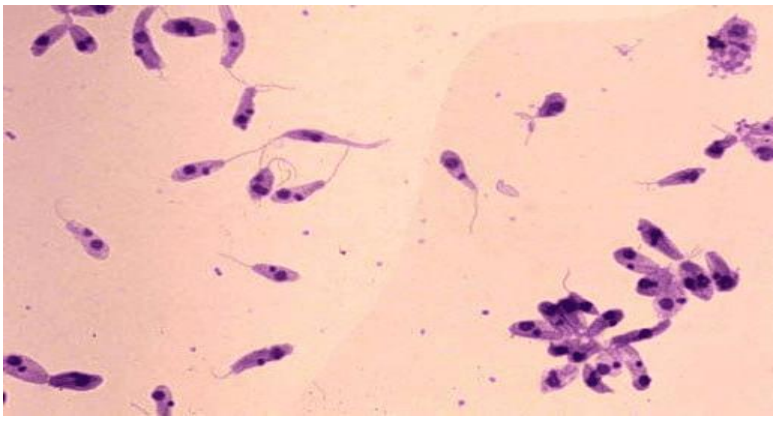

Figure 15. Giemsa-stained smear from culture isolate showing numerous* major Leishmania *promastigotes observed under light microscopy (1000×)

The promastigotes are elongated with a single flagellum and a kinetoplast, characteristic of *Leishmania* species.

Discussion

This study highlights the predominance of *Leishmania major* as the primary causative agent of cutaneous leishmaniasis (CL) in northwestern Libya; this finding is consistent with reports and studies from other regions of Libya and neighboring countries. [12,18] A higher burden was observed among children under 9 years of age, consistent with the findings of Kaula A. Saad et al. [11]. However, our study showed a gender distribution, with females more frequently affected than males. This contrasts with the findings of [11,12,19], where males predominated, possibly due to greater male access to healthcare facilities. Another study has suggested some evidence that supports the idea that the sex hormones may play a role in modulating the cellular immune response to *Leishmania* infection [20].

Our study finds that most of the frequent cases were in the face and extremities, which may explain the higher number of females than males who went to see medical advice. Most lesions were multiple and located on the extremities, consistent with the observations of Saad et al. [11], most cases are from rural areas without taking the right measures to prevent the sandfly bite. The typical clinical presentation was an ulcerated nodule with crusting, although atypical lesions were also noted, such as zosteriform and paroychiform; these findings agree with those of Remadi et al. and Layouni et al., who reported both typical and atypical forms of CL. [21,22] Regarding the time to diagnosis, most patients sought medical attention within 2–4 months of lesion onset, whereas 39% delayed seeking medical care. Most lesions were located on the

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face and extremities, which may explain why many patients sought medical attention relatively early. Lesions in these visible areas are of particular cosmetic concern and are associated with a higher risk of permanent disfiguring scars. In addition, increased public awareness of these potential cosmetic consequences may have encouraged earlier healthcare-seeking behavior. The majority of cases were concentrated in 16 municipalities in the western region, indicating that the disease burden is geographically clustered in this area. [11,12,23–20].

Among diagnostic methods, PCR-RFLP demonstrated the highest sensitivity and reliability, outperforming conventional techniques, including slit-skin smear, histopathology, and culture, which is consistent with the previous studies [8–10]. The superior detection rate observed with PCR (41 of 42 cases) may be explained by the predominance of early lesions, as most patients presented within three months of symptom onset. Early cutaneous leishmaniasis is generally associated with a higher parasite load, which enhances the sensitivity of molecular detection methods such as PCR. [23] Slit-skin smear microscopy achieved a detection rate of 88% in the present study, surpassing previously reported rates of up to 76% [8–10]. This finding may be explained by the predominance of early-stage lesions, which are generally associated with higher parasite loads, as well as the collection of samples by experienced practitioners using appropriate sampling techniques from the active edge of the lesion. Both factors are likely to have contributed to the enhanced diagnostic yield of microscopic examination.

Similarly, Gadri et al. (2025) [7] demonstrated that the application of the YOLOv8 deep-learning model for automated detection of *Leishmania* amastigotes achieved a detection accuracy of 94%, highlighting the potential of artificial intelligence to enhance the performance of microscopic diagnosis. Nevertheless, although microscopy remains a rapid, inexpensive, and widely accessible diagnostic method in endemic regions, its inherent limitations in sensitivity and inability to reliably identify *Leishmania* species support the complementary use of molecular techniques, particularly PCR, for species identification and the evaluation of diagnostically challenging or equivocal cases.

Table1. Comparison of Diagnostic Methods for Cutaneous Leishmaniasis Using PCR-RFLP as the Reference Standard

Methods	Number of cases	Percentage Referenced to PCR	Result Time	Disadvantage
PCR	41	100%	1-2 days	-Expensive - Requires specialized laboratory -Issues with availability in endemic areas
Slit and Smear	38	90%	1-2 hours	- Requires an experienced microscope - Cannot identify species
Histopathology	32	78%	2-6 days	- Parasites may be scarce - Invasive - Species identification is not possible
Culture	18	44%	3-21 days	-Time-consuming (days–weeks) - Requires sterile conditions - Risk of contamination

In this table we using PCR-RFLP as the reference-standard reference method, the study found that molecular diagnostics provided the highest accuracy, with PCR-RFLP achieving 100% sensitivity. The sensitivities of microscopy (slit skin smear), histopathology, and culture were 90%, 78%, and 44%, respectively. These findings highlight the superior diagnostic value of PCR-RFLP over conventional methods for detecting cutaneous leishmaniasis. Overall, strengthening diagnostic capacity through the integration of PCR-based methods, alongside enhanced clinical awareness, is essential for timely detection, accurate species identification, and improved management of cutaneous leishmaniasis in endemic areas.

Conclusion

The study demonstrates that molecular diagnostic methods, particularly PCR-RFLP, exhibit superior sensitivity and specificity compared to traditional approaches, with one referenced sensitivity study reporting 100% accuracy in diagnostic cases. In contrast, conventional techniques such as microscopy (Slit and Smear), histopathology, and culture yielded accuracies of approximately 90%, 78%, and 44%, respectively, underscoring the limitations of these methods when used in isolation. Nevertheless, microscopy through Slit and Smear remains a valuable diagnostic tool due to its simplicity and accessibility. Importantly, its diagnostic accuracy can be significantly enhanced through the incorporation of advanced computational models, such as the YOLOv8 framework, which offers promising potential as an adjunctive tool in clinical diagnostics.

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Conflict of interest. Nil

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