Research in Molecular Medicine

Atypical Cutaneous Leishmaniasis With Negative Direct Smear: Two Case Reports Confirmed by Molecular Diagnosis

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ABSTRACT

Background: Cutaneous leishmaniasis (CL) is a chronic parasitic disease caused by protozoa of the genus *Leishmania*, primarily transmitted to humans by bites of infected female sandflies.

Materials and Methods: This report describes two patients from Damghan with cutaneous lesions that were initially misdiagnosed as fungal infections. The lesions were evaluated using direct smear microscopy and a kDNA-based polymerase chain reaction (PCR) assay.

Results: The first patient was a 5-year-old girl with a chronic lesion on her hand. Despite a negative direct smear, kDNA-based PCR confirmed CL. The second patient was a 43-year-old man with a painful ulcerative lesion on his leg. kDNA-based PCR confirmed Leishmania major infection, and the patient improved following treatment with Glucantime.

Conclusion: Atypical CL presents diverse clinical features, especially in children, which complicate diagnosis. Increased awareness and further research are crucial to improving outcomes and refining management strategies.

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Introduction

Cutaneous leishmaniasis (CL) is a chronic parasitic disease caused by protozoa of the genus *Leishmania* [1], primarily transmitted to humans by bites of infected female sandflies [2]. It is the most common form of leishmaniasis and predominantly manifests as localized skin lesions that can include papules, ulcers, or nodules, typically occurring on the exposed surfaces of the body, such as the face, arms, and legs. While CL lesions can resolve spontaneously, they are often associated with disfiguring scarring and considerable psychosocial morbidity. CL is endemic in tropical and subtropical regions, including the Middle East, South America, Africa, and Asia, with hotspots in countries, such as Iran, Afghanistan, and Brazil [3, 4].

The pathology of CL is determined by the species of *Leishmania* and the host response to the infection. Old World CL is primarily associated with *Leishmania major* and *Leishmania tropica* and results in dry or wet ulcers. New World species may present with a greater variety of pathogens and clinical responses. Transmission is facilitated by infection in sandfly vectors, and various mammals, including rodents, dogs, and wild mammals, serve as reservoirs. The clinical presentation of CL can vary widely, ranging from a single self-resolving lesion to multiple chronic ulcers, which may be atypical and challenging to diagnose and treat [5]. Diagnosis is based on clinical suspicion and confirmed by parasitological and molecular methods, such as polymerase chain reaction (PCR) or immunological investigations. Treatment options include pentavalent antimonials, amphotericin B, and emerging topical or local therapies [1-4].

CL continues to be a public health issue as the disease is widespread in endemic areas, particularly in resource-limited settings with reduced access to timely diagnosis and effective treatment. Epidemiological surveillance, vector control, and educating the public are core components of disease management [1, 2]. Lastly, ongoing research aims to better understand host-pathogen relationships, improve diagnostic tests, and develop safer and more effective therapy options. Reporting atypical or unusual cases will be a valuable contribution to the community, aiding the development of public health responses and clinical guidelines. This report describes two unusual cases of CL from an endemic region of Iran, in which the initial diagnosis was complicated by negative microscopic examination.

Case Presentation

Case 1

A 5-year-old female patient from Damghan district, an endemic region in Central part of Iran, presented with an edematous and erythematous skin lesion on her left forearm that had persisted for approximately three months (Figure 1). The initial clinical diagnosis suggested a fungal and bacterial infection. Despite the chronic nature of the lesion, direct microscopic examination for *Leishmania* parasites was negative.

DNA was extracted from Giemsa-stained smears as described before [6]. A molecular assay using kDNA-based PCR was performed for species characterization of *Leishmania*, using LINR4 as the forward primer (Sina-Clon, Iran) (5′-GGGGTTGGTGTAAAATAGGG-3′) and LIN17 as the reverse primer (5′-TTT GAA CGG GAT TTC TG-3′). The reaction was performed in a 25 µL volume with an initial denaturation at 94 ˚C for 5 min, followed by 35 cycles of denaturation at 94 ˚C for 30 s, annealing at 54 ˚C for 30 s, extension at 72 ˚C for 30 s, and a final extension at 72 ˚C for 10 min, as previously described [6, 7]. The PCR products were visualized under UV transillumination on a 1.5% agarose gel. The assay confirmed the presence of *L. major* DNA, establishing the diagnosis of CL.

This case highlights the diagnostic challenges in endemic areas, where conventional direct smear tests may yield false-negative results, especially in chronic or atypical presentations. Molecular methods serve as a crucial adjunct for detecting *Leishmania* infection, particularly when routine microscopy fails to identify the parasite. The patient's residence in an endemic area and the lesion's clinical appearance warranted further investigation beyond initial negative direct tests. Such findings underscore the importance of incorporating sensitive molecular diagnostic techniques into clinical practice for timely and accurate diagnosis, which is essential for guiding appropriate treatment and improving patient outcomes in CL-endemic settings.

Case 2

A 43-year-old man from Damghan district, an endemic region in Central Iran, presented with an ulcerated plaque lesion on his left foot that had persisted for four months (Figure 2). The ulcerated lesion was associated with pain, pruritus, and burning sensation. The patient had consulted a physician twice, and the diagnosis was initially a fungal skin infection. His medical history was



Figure 1. Cutaneous leishmaniasis presenting as an edematous and erythematous skin lesion on the dorsum of the left wrist



Figure 2. Cutaneous leishmaniasis presenting as an ulcerated plaque lesion on the dorsum of the left foot

significant for having hypertension. Clinical examination revealed an ulcer with typical CL features, but direct microscopic examination and smear for *Leishmania* amastigotes were negative. Molecular diagnostic tests confirmed the presence of *L. major* DNA by a kDNA-based PCR assay, as previously described [6, 7]. After confirmation of the diagnosis, he was treated with Glucantime and clinically improved. This case reinforces the importance of considering CL in chronic skin ulcers in endemic regions and highlights the role of molecular diagnostics when conventional microscopic methods fail to detect the parasite.

Discussion

CL causes a substantial public health and dermatological concern in multiple endemic locations, such as Damghan, where the cases of this study originated. The clinical manifestation of CL is often characterized by dermal lesions localized to a few discrete areas appearing as papules or ulcers, and is classically found on exposed areas of the body, such as the face, legs, and hands [2, 4, 8]. CL typically presents as a classic ulcerative nodule or plaque at the site of the sandfly bite. However, a divergent spectrum of presentations, collectively termed atypical CL, presents a significant diagnostic challenge [5, 6]. These manifestations may include erysipeloid, a rare atypical presentation characterized by diffuse erythema and edema that clinically mimics bacterial erysipelas [9]; psoriasiform lesions, which cause erythematous scaly plaques that closely resemble psoriasis [10]; zosteriform lesions, presenting as unilateral, dermatomal, or band-like skin lesions that often lead to confusion with herpes zoster [11]; sporotrichoid lesions, characterized by linear nodular lesions along lymphatic channels and closely resembling sporotrichosis caused by *Sporothrix schenckii*; and lupoid lesions, which represent a chronic form of CL and manifest as infiltrated papules or plaques, typically without ulceration [3, 4].

The etiology of these unusual forms is multifactorial, often attributed to specific *Leishmania* species, the host's unique immune response, such as leishmaniasis recidivans, or immunocompromised states [12].

Considering its appearance in this 5-year-old patient approximately three months ago, the chronicity observed is consistent with the expected natural history of CL, wherein lesions develop, ulcerate, and progress over time—often for months—before eventual resolution, which may include scarring [2-4]. Atypical lesions frequently mimic other dermatological conditions, like lupus vulgaris, cellulitis, or cutaneous lymphoma, leading to misdiagnosis and delayed treatment. Consequently, in endemic regions, a high index of clinical suspicion coupled with confirmatory molecular diagnostic techniques is essential for the accurate identification and effective management of these non-classical cases [13].

The negative direct smear for this patient reflects a common diagnostic limitation. Direct microscopic visualization of amastigotes remains a first-line diagnostic method; however, its sensitivity varies and is reduced

in cases of chronic lesions or low parasite burden. Genomic diagnostics, including PCR—as employed in this case—are more sensitive than direct stains because they can identify *Leishmania* DNA, particularly when the smear test is negative, supporting their use as a confirmatory diagnostic tool in both endemic and non-endemic settings [5, 6].

These cases highlight the need for heightened clinical vigilance and the use of advanced molecular techniques in the management of CL, especially in endemic settings. Molecular confirmation aids not only in the diagnosis but also in species identification, which may guide treatment decisions based on the recognized differences in drug sensitivity, according to the type of *Leishmania* species [14, 15]. Additional case reporting and research are essential to enhance case identification, strengthen regional epidemiology, and improve treatment and diagnostic protocols, and ultimately mitigate morbidity associated with CL.

Conclusion

This case series highlights the diversity in clinical presentation and the challenges of diagnosis that atypical CL presents, especially in pediatric populations. Recognition of these less common presentations is important for timely diagnosis and effective management in both endemic areas and resource-constrained settings. Our findings show that increased clinical awareness is necessary, and further research regarding the pathophysiology may be of benefit in order to optimize therapeutic options. Improved diagnostics and uniform treatment protocols could lead to a decrease in complications among patients and reduce the burden of atypical presentations among vulnerable groups.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors contribution's

Conceptualization and supervision: Hajar Ziaei Hezarjaribi and Mahdi Fakhar; Methodology and investigation: Amirmohammad Fallahi Lima and Rabeeh Tabaripour; Data collection: Amirmohammad Fallahi Lima, Hedieh Hatamipour, and Fatemeh Mesgarian; Data analysis: Amirmohammad Fallahi Lima and Rabeeh Tabaripour; Writing the original draft: Rabeeh Tabaripour; Review and editing: Mahdi Fakhar.

Conflict of interest

The authors declared no conflict of interest.

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