

First case report of atypical disseminated cutaneous leishmaniasis in an opium abuser in Iran

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ABSTRACT

Leishmaniasis is a worldwide tropical infectious disease caused by different species of intracellular protozoa parasites of the genus *Leishmania*. Herein, we report a 78-year-old man with unusual diffuse cutaneous leishmaniasis (DCL) who had a history of opium abuse and chronic obstructive pulmonary disease (COPD). He had multiple papular, crusted and severely ulcerated lesions extended to his arm and chest. Direct smears and skin punch biopsy of the lesions were suggestive of leishmaniasis. Parasite DNA was amplified from ulcers, and identified as *Leishmania major* by PCR-RFLP, confirmed by sequencing analyses. The aim of the current study was to bring to attention this atypical form of disease in CL endemic countries. Thus, this is the first case of DCL in an opium abuser with COPD due to *L. major* in Northeastern Iran indicating that atypical and extensive forms of CL (DCL) owing to *L. major* are increasing in Iran.

KEYWORDS: DCL. Opium. *L. major*. Leishmaniasis. COPD. PCR-RFLP. Iran

INTRODUCTION

Leishmaniasis, as a cosmopolitan infectious disease, is caused by protozoa parasites from the Kinetoplastida *Leishmania* spp., which are transmitted by the female sandfly and affects almost 12 million people worldwide^{1,2}. The infection outcome varies according to the *Leishmania* species from severe cutaneous lesions to erosive mucosal ulcers with severe dermal disfigurements, to a life threatening visceral disease, depending directly on the interaction between parasites and the host immunity^{3,4}.

Iran is the main endemic focus of cutaneous leishmaniasis (CL)^{5,6} in the Middle East and the main etiological agents of CL in the country are three species: *L. major*, *L. tropica* and less frequently *L. infantum*^{1,5,7,8}. Different forms of CL have been reported in Iran¹ including post kala-azar dermal leishmaniasis (PKDL)⁹, mucocutaneous leishmaniasis^{10,11} and recently diffuse cutaneous leishmaniasis (DCL)¹². DCL, a rare and unusual form of CL, is characterized by the absence of cellular immunity, high number of parasites inside host cells, uncontrolled progression of disease and no response to antileishmanial therapy.

This is the first report of an atypical case of DCL caused by *L. major* in an opium abuser with chronic obstructive pulmonary disease (COPD) in the Northeastern region of Iran who had a good response to antileishmanial therapy.

CASE REPORT

A 78-year-old male patient was referred to the Imam Reza Hospital, North

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Khorasan University of Medical Sciences, Bojnurd, Iran, for evaluation of disseminated skin lesions over the arm and hemithorax. As shown in [Figure 1](#), he presented with one-month history of multiple skin lesions, which started with a small furuncle on the left forelimb which progressed to other parts of the arm and hemithorax. He was originally resident in Esfarayen city in the Northeastern region of Iran, which is one of the new endemic regions of cutaneous leishmaniasis (CL). The patient declared that prior to the dissemination of the lesion, he had edema and lymphedema over the arm and hemithorax. Physical examination was remarkable for mild peripheral edema and lymphedema, but in radiological and laboratory tests, there were no abnormalities. At the time of physical examination, mild peripheral edema and lymphedema, but no systemic symptoms such as fever, hepatosplenomegaly were observed; however, he had a 10-year history of opium smoking abuse, as well as Chronic Obstructive Pulmonary Disease (COPD) which had previously been treated with Salbutamol (100 µg/4 times (puffs) per day) and Fluticasone/Salmeterol Spray (250/50 µg twice a day). The patient was hospitalized with an initial suspicion of diffuse bacterial infection due to lymphedema, opium smoking and history of respiratory disease. Physical examination showed that he had multiple, disseminated and wet-looking skin lesions which were papular, crusted and severely ulcerated all over the left arm and hemithorax ([Figure 1](#)). Furthermore, the patient's lesions spread to his palm and all over the left finger nails which are very rare manifestations. It should be noted that three days before referring to the hospital, the patient bandaged up the lesions with plastic covers; therefore, all covered parts turned into creamy, wet-looking and severely inflammatory lesions which looked like second-degree burn wounds.

A hypothesis of leishmaniasis was made, and further



Figure 1 - Atypical disseminated cutaneous leishmaniasis (DCL) caused by *Leishmania major* in a patient with opium abuse and chronic obstructive pulmonary disease before antileishmanial therapy. The patient had multiple papular, crusted, severely ulcerated and creamy, wet-looking lesions that spread to his left arm and chest

evaluation by direct examination of the skin of the wrist and forearm using lesion scraping by a vaccinostyle, under sterile conditions was performed (Giemsa staining) revealing abundant intracellular *Leishmania* amastigotes inside and outside host cells (3+ parasitemia grade: 1–10 parasites/microscopic field) ([Figure 2A](#)). In addition, for histopathological examination, punch biopsy of lesions of the wrist and forearm were performed (Hematoxylin and Eosin staining: H&E). Histopathological smears of wrist and forearm skin lesions revealed mild hyperkeratotic epidermis based on dermis infiltrated by numerous mixed inflammatory cells including abundant macrophages at dermis filled with amastigote forms of *Leishmania* ([Figure 2B](#)). Direct microscopy skin smears for acid-fast bacilli were negative. According to positive Giemsa-stained slide and skin biopsy, diagnosis of diffuse cutaneous leishmaniasis (DCL) was made Bone marrow aspiration showed no detectable *Leishmania* parasites among

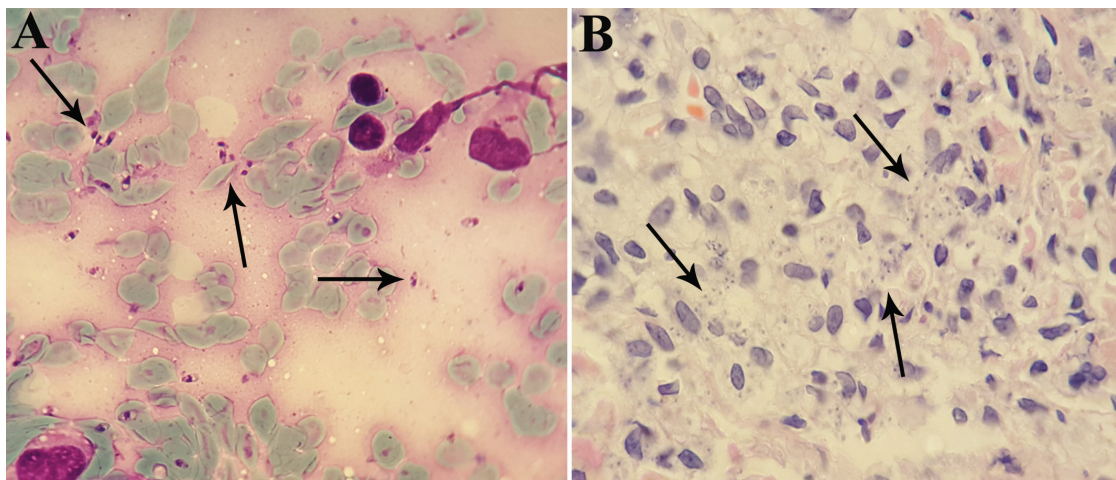


Figure 2 - A) Abundant intracellular amastigote forms of *Leishmania major* (arrows) in the Giemsa-stained smear; B) in the skin biopsy stained with Hematoxylin and Eosin, prepared from an ulcerated area of the left wrist and forearm of the patient with disseminated cutaneous lesions (original magnification: $\times 1,000$)

myelogenous elements. All routine laboratory tests of the patient were normal. Viral markers (HIVAb, HBSAg, HCVAb), and anti-Brucella Ab (Wright, Coombs Wright and 2-mercaptoethanol) were negative. In addition, serous fluid from the patient's skin lesion were cultured in Schneider's *Drosophila* medium (Sigma, Darmstadt, Germany), supplemented with 5% heat-inactivated fetal bovine serum (HI-FBS) (Sigma, Germany). Isolated parasites were subcultured and monitored regularly to check promastigotes growth. In this experiment, 2×10^6 parasites/mL at the logarithmic (2 days) growth phases were counted and used for DNA extraction.

Total genomic DNA of *Leishmania* parasites was extracted from cultured parasites and also from paraffin-embedded blocks of the patient's skin biopsy using the GF-1 DNA extraction kit (GF-1, Vivantis, Canada), and *Leishmania* ITS1 was amplified by polymerase chain reaction (PCR). For identification of *Leishmania* species by PCR-RFLP, the PCR product was digested using *HaeIII* (*BsuRI*) enzyme^{5,13}; *L. major* parasites were identified as the causative agents of DCL.

The patient's first PCR product was subjected to sequence analysis using the forward and reverse primers according to the manufacturer's instructions (Bioneer, South Korea). GenBank was searched for similar sequences and a significant similarity was found with other *Leishmania* species. The patient's ITS1 sequence was similar to the Iranian strain of *L. major* (AB04/HU64) (99%).

The patient was initially treated with a bilayer wet dressing that was coated with silver sulfadiazine and petroleum salve/ointment along with vancomycin (1 g/kg/per 12 h) and ceftriaxone (1 g/kg/per 12 h) as broad spectrum antibiotics. At the same time, he received antileishmanial therapy including: meglumine antimoniate (Glucantime) 20 mg/kg/day for 10 days. Since he showed no significant improvement with Glucantime and developed pancytopenia as a complication of therapy, his treatment was discontinued at day 10. Consequently, he was placed on amphotericin B (3 mg/kg/per day for 10 days) and showed progressive and marked improvement of cutaneous lesions (Figure 3).

During hospitalization and successful treatment, he developed a severe lung infection and disseminated bronchiectasis probably due to his history of opium abuse and COPD; therefore, he was admitted to ICU and unfortunately died because of a systemic bacterial superinfection.

DISCUSSION

This is an unusual report of DCL caused by *L. major* in the Northeastern region of Iran in a patient with



Figure 3 - Patient with atypical disseminated cutaneous leishmaniasis (DCL) showing good response to treatment with amphotericin B and regression of his cutaneous lesions

10 years history of opium abuse and respiratory disorder. He presented with an atypical form of CL with multiple, disseminated and creamy wet-looking skin lesions, which were papular, crusted and severally ulcerated all over the left arm and hemithorax.

DCL has been described in many countries, with a significant increase in the number of severe cases in highly endemic areas in the New World, and more recently in the Old World. It is characterized by an initial injury of the superficial and then the deep dermis followed by a high number of cutaneous polymorphic lesions with distinct features in the patient's body surface, with or without mucosal involvement and usually having no effective response to treatment^{14,15}.

In the current case, the patient who was suffering from severe DCL described the initial appearance of a single furuncle exactly on the sand fly bite site of his left hand.

Few months later, due to opium abuse and COPD, the patient's immune system was reduced; therefore, parasites could disseminate to other parts and lesions spread to the entire remote surface of the left arm and hemithorax, turning into disfiguring and extensive lesions within a few weeks.

Pathogenesis of *Leishmania* infection depends directly upon the interaction between host cells and the *Leishmania* parasite involving immunological mechanisms¹⁶. Nevertheless, in DCL, situations are very different and the exact immunopathogenesis has not been well established so far. DCL has been characterized in several underlying conditions such as malignant diseases, organ transplantation, HIV positive patients, and importantly in opium abusers, due to diminished immune responses^{17,18}. Some authors have shown that DCL is associated with a defective cellular immunity to *Leishmania* genus, parasites allowing their uncontrolled replication within host cells. In our DCL case, the patient was an opium smoking abuser which led to COPD; however, the association of opiates, infection, and their mechanism of action, are complex and multifactorial¹⁸. Opium as a narcotic drug is more prevalent in different parts of Iran, especially in Northeastern areas¹⁹. In chronic abusers of narcotics as in our case, the replication of T lymphocytes diminished significantly, so that exposure of host macrophages cells to opiates significantly reduced the production of distinct T-helper 1 (Th1) cytokines by a downregulation of their coding genes²⁰. Briefly, the cell mediated immunity (Th1) responses deviate into humoral immunity (Th2) via the cAMP dependent pathway, which eventually blocks Th1 cytokines expression such as TNF- α , IFN- γ , IL-10 and IL-5, raising Th2 cytokines expression^{19,20}. Therefore, lower expressions of the Th1 cytokines cause the dissemination of the *Leishmania* parasites^{14,21}. Moreover, some studies showed that alkaloid opioids induce immunosuppression and depress the cell function of natural killer (NK) and phagocytic cells; therefore causing dissemination of parasites to other parts on body^{19,22}.

Although in the current study, *L. major* was identified as the causative species of DCL which is the most frequent species associated with CL in Iran, it has been rarely reported as the causative agent of DCL in both, immunosuppressed and non-immunosuppressed patients. The most frequently described agents of DCL in the country are *L. tropica* and *L. infantum*¹⁷.

Most cases of the DCL in Iran were reported in immunocompromised individuals such as HIV/AIDS patients; however, rare cases of the disease were described in patients with no immunosuppressive disorders¹⁷. In a study in Iran, Hajjarian *et al.*¹² reported four cases of DCL with no immunosuppression history in their medical notes and they did not respond to antileishmanial drugs.

These authors suggested a potential role of *Leishmania spp.* genome characteristics for causing DCL in healthy patients. Therefore, we should not undervalue the potential of parasites genomes and their virulent hybrids in the dissemination of parasites to other parts of the body surfaces¹². It seems that the number of DCL cases in patients with and without immunosuppression in Iran is rapidly increasing.

Although it may be considered that there several limitations in the current study, further investigation must be conducted in all endemic parts of Iran to follow up the patients with atypical forms of CL such as DCL, and the parasites should be sequenced to identify the *Leishmania* strains in the country.

CONCLUSIONS

Although there has been a rise in the number of DCL cases in recent decades in Iran, this is a rare case report of DCL in a patient with history of opium abuse and COPD, parameters that have been poorly described in the literature. The precise identification of DCL causative species by molecular techniques is vital for prevention, treatment and control of the disease and also for epidemiological studies. It is possible that some DCL cases have been missed as a result of the poor reporting system and lack of diagnostic facilities; therefore, it is highly suggested that health workers and physicians are educated to ensure correct diagnosis and management of atypical and rare CL forms in both immunosuppressed and non-immunosuppressed patients. Moreover, we strongly suggest the use of molecular techniques for identification of *Leishmania spp.* and the epidemiology of these parasites in all parts of the country and differentiation of DCL from other forms of cutaneous lesions.

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ETHICAL APPROVAL

The current case report was approved by the local Ethics Committee of North Khorasan University of Medical Sciences, Bojnurd, Iran and a written consent was obtained from the patient.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest in relation to this work.

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