

Case Report

First molecular-based detection of mucocutaneous leishmaniasis caused by *Leishmania major* in Iran

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Abstract

Mucocutaneous leishmaniasis, which mostly occurs in the New World, is mainly associated with *Leishmania braziliensis* and to a lesser degree *L. panamensis* and *L. amazonensis* infections. Primary mucosal leishmaniasis is very rare in Iran in spite of high prevalence of cutaneous and visceral leishmaniasis. A nine-year-old boy had cutaneous leishmaniasis for five years involving the left side of his face; he then developed swelling and ulceration of the lip and left side buccal mucosa five months before hospital admission. He had severe swelling of the lower lip and there was ulceration and bleeding of the buccal mucosa. Direct smear revealed leishman bodies and nested PCR confirmed the presence of kinetoplast DNA of *L. major* in the oral mucosal specimen. The patient received amphotericin B deoxycholate 1 mg/kg/day for one month. The lip and face inflammatory reaction disappeared to nearly normal after one month of therapy. The patient was discharged with ketoconazole (5mg/kg/day) for six weeks. To our knowledge, this is the first report of mucocutaneous leishmaniasis caused by *L. major* in Iran.

Key words: mucocutaneous leishmaniasis; *Leishmania major*; PCR; Iran

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Introduction

Leishmaniasis has several diverse clinical manifestations including ulcerative skin lesions, destructive mucosal inflammation, and disseminated visceral infection (also known as kala-azar) [1]. Mucocutaneous leishmaniasis (MCL), characterized by the spread of skin ulcers to surrounding tissues (particularly nose and mouth), mostly occurs in the New World. It is mainly associated with *Leishmania braziliensis* and to a lesser degree *L. panamensis* and *L. amazonensis* infections [1,2].

Cutaneous and visceral types of leishmaniasis are the common types in Iran, whereas primary mucosal disease without skin involvement has been reported in singular cases [3,4]. Cutaneous leishmaniasis (CL) is still a great health problem in many countries of the Eastern Mediterranean region [3]. It is endemic in half of the 28 provinces of Iran [5]. Three *Leishmania* species cause CL in the Old World (*L. major*, *L. tropica* and *L. aethiopica*) [6,7]. Northwest and southern Iran are the primary foci for visceral leishmaniasis (VL), which mainly affects children. *L. infantum* is expected to be the dominant *Leishmania*

strain in Iran [8,9]. *L. tropica* and *L. major*, which are typically dermatotropic, have been confirmed as causative agents for VL in Iran [9-11]. The former has been reported as an etiological agent of VL among US servicemen in the Persian Gulf War in 1990 [12]. Here we report the first case of MCL caused by *L. major* in Iran.

Case report

A nine-year-old boy was admitted to Namazi Hospital, one of the major teaching hospitals affiliated with Shiraz University of Medical Sciences. The patient had become infected with CL on the left side of his face five years previously. Since then he had been followed up by dermatologists and received different medications such as glucantime, rifampin, dapsone, and prednisolone without significant improvement. Cryotherapy was not effective, either. Five months prior to admission to our hospital, the lesion had spread to the mucosal part of the lip. The patient developed swelling and ulceration of the lip and left side buccal mucosa as well as erythema of the left side of the face. On physical examination, redness and

swelling of the left side of the face were detected and scars of the previous CL infection were evident on the left side of the face. There was severe swelling of the lower lip in addition to ulceration and bleeding of the buccal mucosa. No fever or organomegaly was detected and other examinations were normal (Figure 1a).

PCR assay

Nested PCR was used to amplify the variable minicircle kDNA area of *Leishmania*. The parasites were clearly detected in the direct smear samples of the oral lesions and confirmed using PCR with a slight modification [13].

The first-round primers, CSB1XR (ATTTTCGCGATTTTCGCAGAACG) and CSB2XF (CGAGTAGCAGAAACTCCCGTTCA), were used to detect the *Leishmania* genus. A reaction mixture containing 1 mM of MgCl₂, 200 μM of deoxyribonucleotide triphosphate, 2.5 μl of 10X PCR buffer, 1.5 unit of Taq DNA polymerase and 10 pmol of each primer was used in a total reaction volume of 25 μl including 5 μl of the DNA sample. The second round primers, 13Z (ACTGGGGGTTGGTGTAATAATAG) and LIR (TCGCAGAACGCCCT), were used for species detection. In this step, the mentioned reaction mixture was used in a total reaction volume of 30 μl containing 1 μl of the DNA product from the first round. These mixtures were amplified in a programmable thermocycler (Mastercycler, Eppendorf, Hamburg, Germany) for 5 minutes at 94°C (1 cycle) followed by 30 cycles at 94°C for 30 seconds, 55°C for 60 seconds and 72°C for 1.5 minutes followed by a final elongation at 72°C for 5 minutes [14]. The WHO

reference strains of *L. tropica* (MHOM/ IR/89/ARD2), *L. major* (MHOM/ IR/XX/LV114) and *L. infantum* (MCAN/IR/96/LON49) were used as standard DNA. A band of 560 bp indicated that *L. major* kDNA was present in the nested PCR-tested smear [15].

Outcome

The patient received amphotericin B (AmB) deoxycholate (1 mg/kg/day) for one month. The patient's therapy proved effective after one month and the lip and face swelling and redness improved to nearly normal (Figure 1b). The patient was discharged with ketoconazole (5mg/kg/day) for six weeks [15,16].

Slide smears investigated were found positive for amastigotes by microscopy and positive for leishmanial DNA in the nested PCR. The PCR product gave the 560-bp second-round amplicon indicative of *L. major* (Figure 2). No other sizes of amplicon were seen; no sample gave more than one amplicon (indicating a mixed infection); and no amplicons were detected in the negative-control samples.

Discussion

To the best of our knowledge, this is the first case of MCL with an unusual strain of *L. major* reported in Iran and worldwide. Previous studies show that MCL only occurs in the New World and is mainly associated with *L. braziliensis* and to a lesser degree *L. panamensis* and *L. amazonensis* infections. The disease mostly occurs in Latin America and in travellers to that area [2].

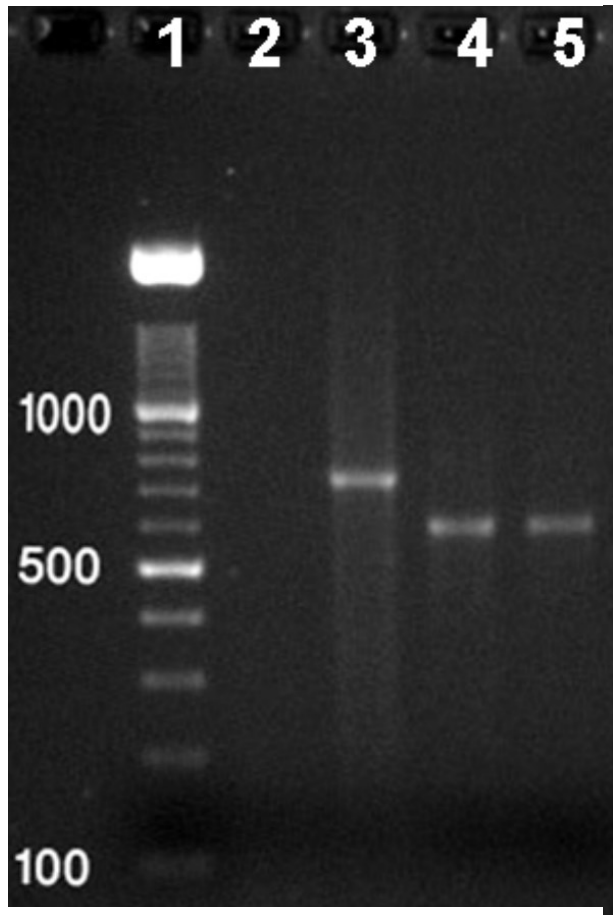
Detection of the kinetoplast DNA of *L. major* by nested PCR in the oral mucosal specimen and clinical course of the illness confirmed the diagnosis of MCL caused by *L. major*. PCR is now the diagnostic method

Figure 1. Mucocutaneous leishmaniasis caused by *Leishmania major*



Redness, swelling of the lower part of the left side of the face, severe swelling of the lower lip, ulceration and bleeding of buccal mucosa, and scars of CL on the left side of the patient's face before (a) and after (b) treatment

Figure 2. Gel electrophoresis results of nested PCR of *Leishmania* parasites isolated from the patient using the primers CSB1XR, CSB2XF, 13Z and LIR



Marker (100-bp ladder) (lanes 1); negative control (lane 2); reference strain of *Leishmania tropica* (750 bp) (Lane 3); reference strain of *L. major* (560 bp) (lane 4); *L. major* isolated from the patient (lane 5)

of choice for parasitological confirmation of cases of MCL. It has a high sensitivity (97.1%) in diagnosis, which is higher than that for all the other diagnostic methods studied and provides a species-specific diagnosis, allowing species-specific treatment [17].

L. major is a causative agent of cutaneous leishmaniasis in the Old World and tends to cause an exudative, "pizza-like" or "wet ulcer". The ulcer usually has a raised outer border, a granulating base, and overlying white purulent exudates. After a typically short incubation period of one week to two months, the ulcer frequently grows to a size up to 6 cm in diameter over a short time. Spontaneous healing typically also occurs quickly, usually within six months [1]. *L. major* has been reported to cause visceral leishmaniasis in southern Iran and also

singular cases of primary mucosal leishmaniasis in Iran and Tunisia [4,11,18,19]. Indeed, it is increasingly reported that the species commonly associated with CL can produce visceral or mucosal lesions, and vice versa.

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