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Case Report

Hoarseness as the Presenting Symptom of Visceral Leishmaniasis with Muco-Cutaneous Lesions: A Case Report

***Hossein MORTAZAVI [1] , Mehdi MOHEBALI [2, 3], Yasaman TASLIMI [4], Pardis SADEGHIPOUR [4], Mahsa ANSARI 1, Kambiz KAMYAB [5], Marjan TALEBI [1], Ali KHAMESIPOUR [6]**

1. *Dept. of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran*
2. *Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran*
4. *Molecular Immunology and Vaccine Research Laboratory, Pasteur Institute of Iran, Tehran, Iran*
5. *Department of Pathology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran*
6. *Skin and Leprosy Research Center, Tehran University of Medical Sciences, Tehran, Iran*

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***Correspondence Email:**
mortazma@sina.tums.ac.ir

Abstract

Herein, a 28-year-old man with hoarseness, skin and oral lesions is presented. At the time of admission, the patient had an erythematous plaque on his chin near his lower lip and an erythematous-violaceous plaque on his palate near the opening of the pharynx and 20 kg weight lost in last one year. The biopsy of his skin lesions by hematoxylin and eosin staining revealed an infiltration of the dermis by lymphoplasma and histiocytic cells with a loose granuloma formation suggestive of leishmaniasis. Biopsy of mucosal lesions revealed Leishman bodies in dermis. PCR was performed on the specimens of skin, bone marrow, mucosa, and saliva, the results were positive. The pathogenic agent was identified as *Leishmania major* by the nested PCR. Serologic tests including direct agglutination test (DAT) and indirect immunofluorescence test (IFAT) were positive with high titers of anti-*L. infantum* antibodies (1:102400 versus 1:800, respectively), indicative of visceral involvement. The patient responded to a combination of miltefosine and meglumine antimoniate (Glucantime®). Visceral involvement due to *L. major* is rarely reported. To the best of our knowledge, probably hoarseness due to *L. major* has not been previously reported from Iran.

Introduction

Leishmaniasis presents different clinical features in Old World and New World. Mucocutaneous leishmaniasis often occurs in New World mostly due to *Leishmania(L) braziliensis* and less commonly *L. panamensis* and *L. amazonensis*(1). There are also several reports of mucocutaneous leishmaniasis in Old World (2-4).

Recently a case of mucocutaneous leishmaniasis due to *L. major* has been reported from Iran (1). In leishmaniasis, laryngeal involvement often occurs in active cigarette smokers and in patients with chronic respiratory diseases (5).

A case of visceral leishmaniasis caused by *L. major* with skin and oral mucosal lesions in a man who was suffering from hoarseness and weight loss is presented herein.

Case Report

A 28-year-old otherwise healthy, non-smoking man with the chief complaint of hoarseness from southeastern part of Iran was referred to our dermatology center in May 2014. He had a few indurated, erythematous plaques on his face (chin and upper lip) and legs, a violaceous plaque on his hard palate, and complained of hoarseness for the past 7 years (Fig. 1 and 2). He also complained of dysphagia to solid diet, and about 20 kilograms weight loss in the last year. Hoarseness was the presenting symptom and lasted for about 5 years during the time that the cutaneous lesions were present.

The study was approved by the local Ethics Committee and an informed consent was taken from the patient.

After his admission to the dermatology ward, the following work up was performed. Routine laboratory tests were normal except for a mild normocytic normochromic anemia, which was justified with chronic disease anemia.

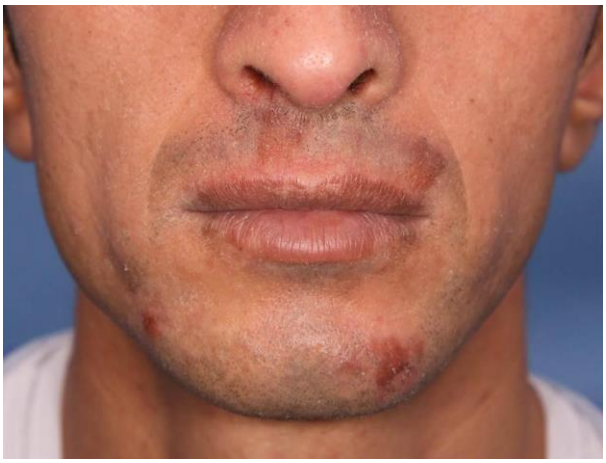

Fig.1: Skin lesions: erythematous plaque on chin and upper lip

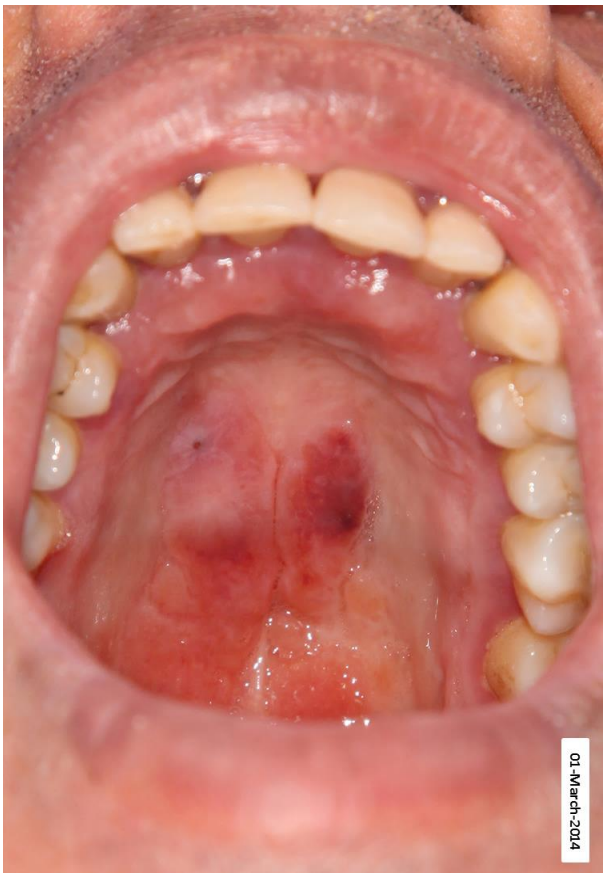

Fig. 2: Oral mucosal lesion: an erythematous plaque on the palate near the opening of the pharynx

Erythrocyte sedimentation rate, complement levels (C3 – C4 – CH50), antinuclear antibody (ANA), and angiotensin converting enzyme (ACE) tests were all within normal limits. Wright, 2-mercaptoethanol (2ME), and Widal tests were done for his constitutional symptoms and low back pain, which all came back negative. VDRL and viral markers (HIVAb – HBSAg – HCVAb) were negative, and a PPD skin test was negative.

Abdominal sonography showed mild hepatomegaly and a 6*8 mm lymph node in retroperitoneal near the superior mesenteric artery origin. Sonography of the neck showed bilateral internal jugular and submandibular lymphadenopathy (9*17 mm – 9*13 mm). A spiral CT scan of the thorax without contrast revealed normal results.

A biopsy from his oral lesion was done with the differential diagnosis of lymphoma, tuberculosis, leishmaniasis, Wegener's granulomatosis, sarcoidosis, squamous cell carcinoma, granular cell tumor and histiocytosis. Biopsies of his face and left leg lesions were also performed. The hematoxylin and eosin stain (H&E) preparation of histopathologic slides of the mucosal and skin lesions showed loose granuloma formation with lymphoplasma cells. In specimen of mucosal biopsy Leishman bodies were seen in the dermis. His cutaneous and mucosal lesions were examined by direct microscopy for acid-fast bacilli and the test was negative. A bone marrow biopsy was performed to detect probable involvement of the bone marrow. Lastly, PCR was performed on the specimens of skin, bone marrow, mucosa and saliva. The result of PCR was positive, and for the determination of pathogenic agent, amplification and sequencing analysis were performed as follow.

Amplification and sequencing analysis

In order to determine the possibility of *Leishmania* infection, nested PCR method was applied. The primers were already designed by Noyes (6). External primers CSB2XF and CSB1XR in the first round of PCR, and internal primers 13Z and LIR in the second round of PCR were utilized (invitrogen, UK). As a positive control, genomic DNAs of standard *L. major* (MRHOM/IR/75/ER) and *L. tropica* (MOHM/IR/09/Khamesipour-Mashhad) were used in parallel. The PCR products were analyzed by 1.5% agarose gel electrophoresis. The result of the nested PCR is shown in Fig. 3.

As it is shown, the nested PCR analysis of the patient sample was *L. major*. For confirmation of the species identification, the 560bp PCR product of kDNAs from the patient as well as standard *L. major* were eluted by Promega Wizard ® SV gel and PCR clean up system (Promega, USA). Then the eluted DNAs were ligated into the TOPO vector PCR2.1 (Invitrogen, USA), transformed in TOP 10' *E. coli*, and plated in LB agar containing 100µg/ml ampicillin. The positive colonies were first isolated and screened with *ECOR*I (Roche, Germany) as the restriction enzyme. After digestion, plasmid DNA was purified (Qiagen, plasmid mini kit) and sequenced with the M13F and M13R as flanking primers using the dideoxy chain termination method on an automated sequencer. The sequence of kDNA of the patient sample was the same as the Iranian strain of *L. major* (MRHOM/IR/75/ER). PCR of skin, mucosa, saliva and bone marrow revealed *L. major*.

With regard to diagnosis of leishmaniasis and probable visceral involvement, the following serologic tests were performed.

The direct agglutination test (DAT) (7) with a remarkable high titer (1:102400) was positive, and the indirect fluorescent antibody test (IFAT) for *Leishmania* infection was also positive (1:800).

Based on the histopathologic findings, the high titer of DAT and IFAT, and the results of PCR, a diagnosis of leishmaniasis with visceral involvement were confirmed. Since miltefosine has been used for visceral involvement and *L. major* infections, the following combination was used to treat the patient (8).

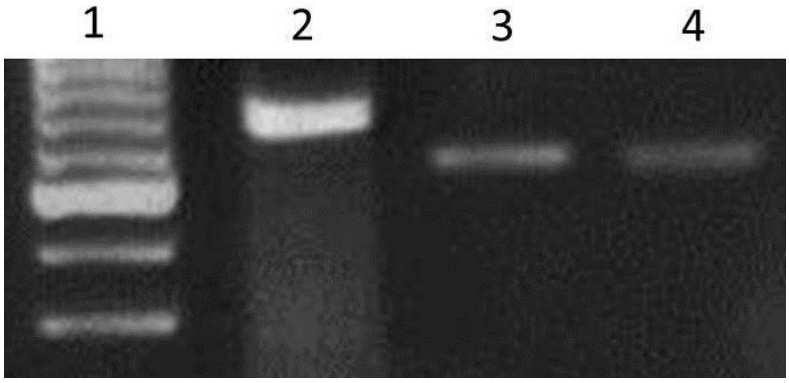

Fig. 3: Nested –PCR amplification of KDNA extracted from skin, bone marrow, mucosa and saliva of the patient which then confirmed by sequence analyses
Lane 1: Molecular weight marker 100bp
Lane 2*: L. tropica*
Lane 3: Patient sample
Lane 4: *L. major*

Miltefosine with the dose of 50mg orally thrice daily was applied in combination with meglumine antimoniate (Glucantime®) 20 mg/kilograms/day for 28 days.

The only side effect of miltefosine was nausea, which was controlled with the ingestion of metoclopramide a half hour before taking miltefosine. The oral and cutaneous lesions, as well his hoarseness, improved to a significant extent.

Discussion

To the best of our knowledge through internet search, the first report of mucosal involvement of *Leishmania* in Iran dated back to 1968 when Ziai et al. reported a case of leishmaniasis with mucosal involvement from Shiraz, Iran (2). Since then there have been several additional reports of mucosal involvement of leishmaniasis from Iran (3, 4). According to a recent report, *L. tropica*, *L. major*, and *L. infantum* may be the causative agents, but *L. major* was the pathogenic agent in most of the patients (3).

The case presented herein with the high titers of anti-Leishmania antibodies using DAT and IFAT were in favor of visceral involvement. DAT, which is positive when the titer is higher than 1/3200, is used for the diagnosis and seroepidemiology of visceral leishmaniasis in endemic areas (9). In the current patient, the DAT showed the highest possible titer value (1/104000). These findings and the involvement of bone marrow confirmed the diagnosis of visceral leishmaniasis in the patient. PCR findings of the cutaneous, bone marrow, mucosa and saliva samples were in favor of *L. major* as a causative agent. Recently, Karamian et al. have reported *L. major* as a causative agent of visceral leishmaniasis in the south of Iran (10).

According to search in the literature, there are a few reports of the coexistence of visceral leishmaniasis with cutaneous lesions in patients with HIV infection (11-13). There is a report from Iran in which a patient showed visceral and cutaneous lesions due to *Leishmania* but, immunological tests in this case were negative and diagnostic splenectomy revealed the *Leishmania* as the causative agent (14).

Our patient also presented with simultaneous visceral and cutaneous leishmaniasis, an HIV test was negative.

The case presented herein was a non-smoker, otherwise healthy man, with visceral leishmaniasis, who had a history of a long period of hoarseness, which can be a symptom of vis-

ceral leishmaniasis in smokers or people with chronic respiratory disease (5).

This patient was presented as a rare case of visceral leishmaniasis with the presenting symptom of hoarseness, *L. major* as the causative agent, and simultaneous muco-cutaneous lesions. Treatment with the combination of miltefosine and meglumine antimoniate was effective and the DAT titer decreased to the level of 1/52000 one month after the patient was discharged from the dermatology ward.

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