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Case Report: First Coinfection Report of Mixed *Leishmania infantum*/*Leishmania major* and Human Immunodeficiency Virus–Acquired Immune Deficiency Syndrome: Report of a Case of Disseminated Cutaneous Leishmaniasis in Iran

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Abstract. Visceral leishmaniasis, a neglected tropical disease, is the third most common opportunistic disease in immunosuppressed patients, such as those affected by the human immunodeficiency virus (HIV)/acquired immune deficiency syndrome. Although the reports have been characterized as *Leishmania*/HIV coinfections, the occurrence of a mixed infection by two *Leishmania* species in HIV-positive patients is rare. Here, we present an atypical case of disseminated cutaneous leishmaniasis (DCL) in a 26-year-old HIV-positive man. The diagnosis of DCL was established using skin biopsy and histopathology examinations and confirmed by molecular techniques. This is the first case of a *Leishmania*/HIV coinfection due to a mixed infection of *Leishmania infantum*/*Leishmania major* in Iran.

INTRODUCTION

Leishmaniasis is an infectious disease prevalent worldwide that is caused by intracellular protozoan *Leishmania* parasites and which shows various clinical features.[1,2] Iran is one of the main countries facing the incidence of endemic leishmaniasis; almost all provinces are affected by the disease.[3] Leishmaniasis exists in two main forms in Iran: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL)[1,4,5]; However, other forms of the disease, such as post-kala-azar dermal leishmaniasis,[6] have been reported too.

The *Leishmania*/human immunodeficiency virus (HIV) coinfection has been reported recently in Iran.[7] In immunosuppressed individuals, leishmaniasis is an opportunistic infection that presents specific features, such diffused/disseminated skin lesions. Immunosuppression is a major risk factor for clinical manifestations, and it is able to modify the disease presentation, including the treatment responses.[8,9] Because the endemic areas of *Leishmania*/HIV infections overlap in Iran and there has been an increase in both the infections in recent decades, the occurrence of a mixed *Leishmania* infection in immunocompromised individuals will most likely increase.[7,8,10]

This is a first-of-its-kind report of an atypical case of disseminated CL (DCL) caused by mixed *Leishmania infantum* and *Leishmania major* in a patient with *Leishmania*/HIV coinfection with vacuolar myelopathy and oropharyngeal candidiasis in northeastern Iran, which was unresponsive to the anti-*Leishmania* therapy.

CASE REPORT

A 26-year-old man with a history of HIV/acquired immune deficiency syndrome (AIDS) infection and vacuolar myelopathy was referred to the Imam Reza hospital in the North Khorasan University of Medical Science for the evaluation of multiple and diffused skin lesions on the face, abdomen, and trunk. He was originally a resident of the Zard village near the Mane and Samalghan cities in north-eastern Iran, which forms the main endemic focus with regard to leishmaniasis. The patient had a 14-month history of being infected by HIV with vacuolar myelopathy. He was diagnosed in the year 2014 with a $CD4^+$ cell count of 19/mm^3 and treated with highly active antiretroviral therapy including lamivudine, zidovudine, and efavirenz for about 12 months. Plasma HIV viral load was not done. Moreover, he was treated with azithromycin and cotrimoxazole for *Mycobacterium avium* complex pulmonary disease and *Pneumocystis*. He had no history of intravenous drug abuse and extramarital sex. His 15-year-old wife was HIV-positive as well.

In the physical examination, there were diffused, multiple, light purple to reddish-brown papulonodular skin lesions on the face, neck, trunk, and upper extremities (Figure 1). There was no pain and no pruritus in the skin lesions. The initial lesions started 1 month earlier as a big nodule in the lower part of the chest, which generalized over a few weeks to other parts of the body. Simultaneously, he developed severe oropharyngeal candidiasis. He had mild splenomegaly but no hepatomegaly, lymphadenopathy, or fever. The laboratory results of the patient's blood tests were recorded with precision. Some of the important items are presented in Table 1.

Further evaluation was done by a direct examination of the skin biopsy. The Leishman bodies were studied using Giemsa-stained slides of scrapings from lesions on the elbow, left arm, and abdomen (4+ parasitaemic grade: 1–10 parasites/microscopic field) (Figure 2A). The histopathology of biopsies that was taken from the skin lesions on the elbow elucidated large nodular clusters of macrophages at the dermis; their cytoplasm was filled with numerous *Leishmania* amastigotes, accompanied by stromal fibrosis, and showed no lymphocytic reaction (Figure 2B). Based on the skin biopsy, the diagnosis of DCL was made. Bone marrow (BM) aspiration showed active BM with myeloid hyperplasia but no detectable *Leishmania* among the myelogenous elements.

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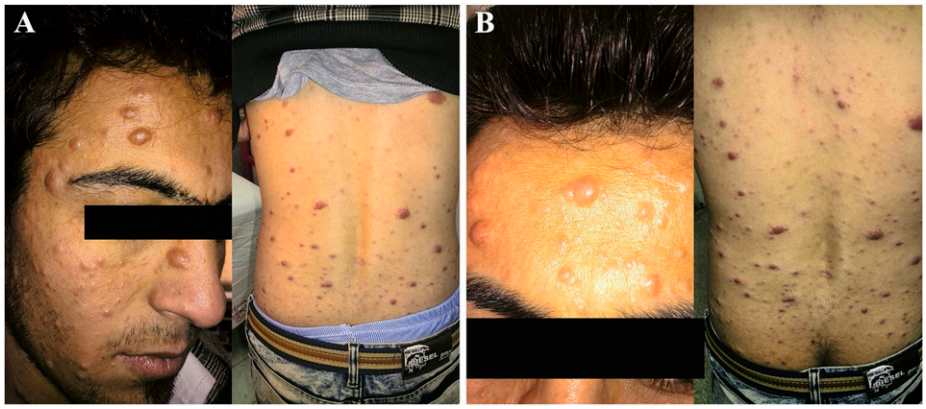

FIGURE 1. Clinical features of the patient with *Leishmania infantum*/*Leishmania major*/human immunodeficiency virus coinfection on the face and back before (**A**) and after treatment (**B**). It shows the disseminated multiple light purple to reddish-brown papulonodular skin lesions. This figure appears in color at www.ajtmh.org.

Leishmania DNA was extracted from the Giemsa-stained slides and leishmanial ITS1 was amplified by polymorphism polymerase chain reaction (PCR). For the final species identification of *Leishmania*, the PCR product was digested using the *HaeIII* restriction enzyme by the restriction fragment length polymorphism PCR (PCR-RFLP) method; *L. infantum* and *L. major* were identified as the causative agents of DCL (Figure 3).[11,12] Briefly, ITS1 amplification and enzymatic digestion ITS1 were amplified using specific primers: LITSR (forward: 5′-CTGGATCATTTTCCGATG-3′) and L5.8S (reverse: 5′-TGATACCACTTATCGCACTT-3′) (Figure 3). A PCR-ready premix (Roche, Germany) was used for amplification in 25 µL total reactions comprising 10 µL premix, 2 µL forward and reverse primers (10 pmol), 1 µL DNA template, and 13 µL double-distilled water. The Iranian reference strains of *Leishmania tropica* (Acc. # EF653267), *L. major* (Acc. # JN860745), and *L. infantum* (Acc. # EU810776), which were previously identified based on isoenzyme analyses were included as positive controls in all the PCR assays. An RFLP analysis was deployed in identifying the *Leishmania* species. Next, 10 µL of the PCR product was added to 2 µL of the enzyme buffer, 1 µL of the FastDigest *HaeIII* (*BsuRI*) enzyme (Fermentas, Life Sciences, Germany), and 17 µL of double-distilled water. The mixture was incubated at 37°C for 5–10 minutes, as recommended by the manufacturer's protocol. The separation of the digestion products was discerned by the use of 3% agarose gels and visualized after effective ethidium bromide staining (Figure 3).

The patient received glucantime (20 mg/kg/day). The treatment was stopped on the seventh day because of the development of pancytopenia, which worsened the DCL lesions in all parts of his body (Figure 1). He was then treated with amphotericin B (3 mg/kg/day) for 10 days. Unfortunately, 10 days after the treatment, his DCL had worsened again and he died of a septic shock. A written consent signed by his family was taken for the publication of this case report.

DISCUSSION

This is the first report where a complicated case of a patient coinfected with *L. infantum*/*L. major*, HIV/AIDS, and candidiasis with myelopathy has been documented in Iran. The patient's clinical manifestations were consistent with DCL as he had a severe immunocompromised status with a very low level of $CD4^+$ T-cells.

Leishmania/HIV coinfection has been reported in 34 countries globally. VL-HIV co-infection was initially characterized in southern Europe but is being increasingly recognized in Brazil and East Africa.[10,13] HIV/AIDS is one of the major emerging diseases in Iran that occurs sporadically. Leishmaniasis is an endemic disease in the country and one of the main opportunistic diseases in HIV-positive individuals.[10,14] A limited

TABLE 1
Laboratory results of DCL/HIV patient due to mixed infection of *Leishmania infantum* and *Leishmania major* in Iran

Test	Result	Unit	Normal	Test	Result	Unit	Normal
WBC	2.06	10^3/µL	4–10	HIV Ab	High/positive	Qualitative	–
Neutrophils	44.1	%	37–72	RPR	Negative	Qualitative	–
Lymphocytes	34.5	%	20–50	HTLV1 and 2	Negative	Qualitative	–
Monocytes	17	%	0–14	HBS Ag	Negative	Qualitative	–
Eosinophil	4.4	%	0–6	HCVAb	Negative	Qualitative	–
Platelets	214	10^3/µL	150–450	Toxoplasmosis	Negative	Qualitative	–
RBC	3.28	10^6/µL	4–5.9	Blood smear	Normal	–	–
$CD4^+$ T cells	19	cells/mm^3	500–1,000	Blood culture	Normal	–	–
ESR 1 hours	55	mm/hr	20 mm/hr	Urine culture	Normal	–	–
AST	25	U/L	Up to 40	Brucellosis Ab	Negative	–	–
ALT	7	U/L	Up to 40	CRP	Negative	–	–
ALP	213	U/L	98–280	RF (Latex)	Negative	–	–

ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; CRP = C-reactive protein; DCL = disseminated cutaneous leishmaniasis; ESR = The erythrocyte sedimentation rate; HIV = human immunodeficiency virus; RBC = red blood cell; RPR = rapid plasma reagin; WBC = white blood cell.

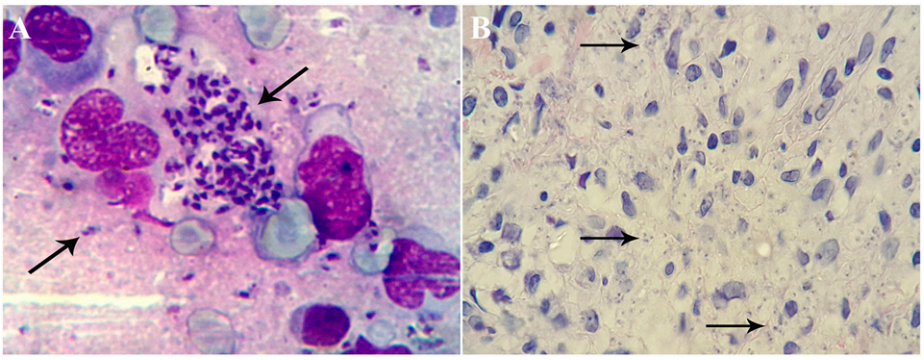

FIGURE 2. *Leishmania* bodies (arrow) in the Giemsa-stained smear (**A**) and skin biopsy stained with hematoxylin and eosin (**B**) prepared from disseminated cutaneous lesions of the patient (×1,000). This figure appears in color at www.ajtmh.org.

number of coinfections due to *Leishmania*/HIV have been described in Iran and most of the cases were VL/HIV due to *L. infantum*.[8,14] Rare cases of CL/HIV due to *L. tropica* and *L. major* were reported as well.[7]

The patient had a single localized lesion on his chest that disseminated and caused the DCL. The reason for his DCL lesions can be explained by the reduction of the $CD4^+$ T-cell levels to below 200/mm^3. *Leishmania* has a tendency to disseminate to various parts of the HIV patient's body. It may have had a role as a cofactor in the HIV pathogenesis because *Leishmania* has main-surface molecules such as lipo phosphoglycan that can induce the transcription of the HIV genome in the T-cells.[15] Furthermore, both *Leishmania*/HIV deplete the T-cells in infected patients.[13,15]

In HIV-positive patients, the clinical symptoms of *Leishmania* can be atypical, which includes visceralization, DC lesions, resistance to drugs, and severe side effects to medications.[13] Dermotropic species (*L. major*/*L. tropica*) can visceralize and viscerotropic species (*L. infantum*) can diffuse from the viscera to the skin, thereby causing severe DCL. *Leishmania tropica*/*Leishmania major* and *L. infantum* may cause DCL in HIV-positive patients.[7,14] In the present case, *L. infantum* and *L. major* were identified as the causative agents of DCL in the patient. *Leishmania major* is a dermotropic species and located only in cutaneous lesions but *L. infantum* can be located in the cutaneous and the visceral surface of DCL patients. In this case, we did not find any parasite from BM and peripheral blood, and the patient had no hepatomegaly; therefore, in our case, both the species of parasites were dermotropic. In cases of severe immunosuppression in individuals, the immune system of the patient fails to activate the cell-mediated response accomplished by the Th1 and Th2 $CD4^+$ lymphocytes, which is responsible for *Leishmania* clearance.[15] Impaired immunity in HIV-positive patients results in the reactivation of the latent parasite, whereas *Leishmania* infection can promote virus replication and raise the progression to AIDS.[15] Although our patient received antiretroviral and anti-*Leishmania* treatment, the lesions failed to show a response to the therapy besides worsening the clinical symptoms.

The limitation in this study is that we reported solely one of the registered official cases of *Leishmania*/HIV coinfection in

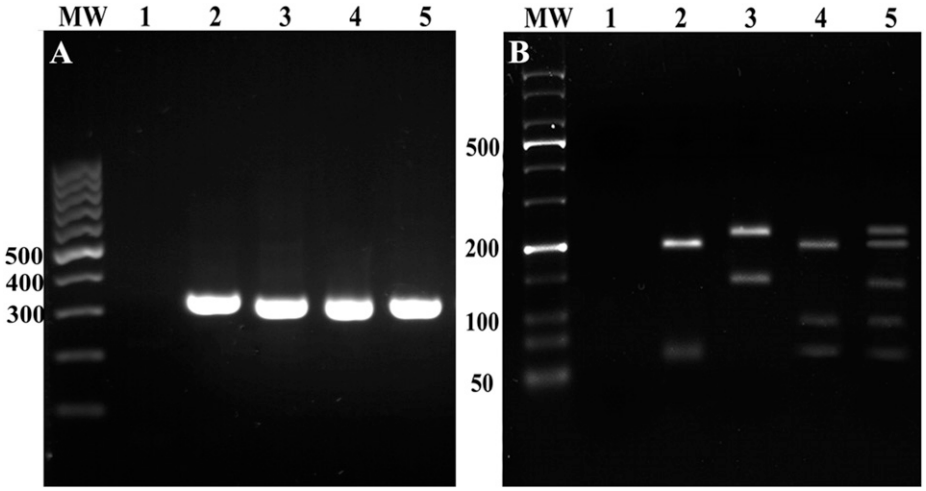

FIGURE 3. Polymerase chain reaction (PCR) results (**A**) of ITS1-rDNA of *Leishmania* spp. and disseminated cutaneous leishmaniasis (DCL) patient. Molecular weight (MW), 100-bp size marker (Jena Bioscience, Jena, Germany). Lane 1 represents the negative control; Lanes 2, 3, and 4 represent *Leishmania tropica*, *Leishmania major*, and *Leishmania infantum* positive reference stocks, respectively (fragment between 300 and 360 bp as *Leishmania* genus). Lane 5 is a positive sample of the patient due to *Leishmania* on the left arm. Restriction fragment length polymorphism PCR analysis (**B**) of 350 bp of ITS1-rDNA of *Leishmania* spp. by using the *HaeIII* restriction enzyme. MW, 50-bp size marker (Jena Bioscience). Lane 1 represents the negative control; Lanes 2, 3, and 4 represent *L. tropica* (fragments of 200 and 60 bp), *L. major* (fragments of 220 and 140 bp), and *L. infantum* (fragments of 200, 80, and 60 bp) positive reference stocks, respectively. Lane 5 is positive in the samples from patients due to *L. infantum*/*L. major* on the left arm.

Iran. The authors believe that the newly emerging coinfection of *Leishmania*/HIV has been seriously underestimated and its epidemiology has not been widely verified; therefore, it needs further study in the country.

Leishmania infantum/*Leishmania major* can be the causative species of severe DCL cases in HIV-positive patients. It should be kept in mind that HIV/*Leishmania* causes the depletion of T-helper cells; thus, *Leishmania*/HIV coinfections intensify the speed of immunosuppression in infected patients. It must be noted that an immediate and effective treatment of both HIV infection and leishmaniasis is obligatory in these cases.

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