Case Report: Atypical Lesions as a Sign of Cutaneous Dissemination of Visceral Leishmaniasis in a Human Immunodeficiency Virus–Positive Patient Simultaneously Infected by Two Viscerotropie Leishmania Species

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Abstract. Leishmaniasis is considered an emerging opportunistic disease in human immunodeficiency virus (HIV)–infected patients who have considerably variable clinical presentation. We report a patient with visceral leishmaniasis who had unexpected clinical aspects (atypical cutaneous lesions appearing after long-term evidence of visceral parasites). The patient had hepatosplenomegaly in the absence of fever, but was otherwise generally healthy. The HIV viral load was low despite severe immunossupression (low lymphocyte proliferation and low level of interferon-γ, concomitant with a high lymphocyte activation status). Surprisingly, two Leishmania strains were isolated from his bone marrow (typical L. infantum sequence MON-1, type A) and skin (L. donovani MON-2 sequence); this second strain had not been previously identified in Brazil. The association of visceral leishmaniasis and HIV/acquired immunodeficiency syndrome is a largely unknown disease, particularly in areas in which leishmaniasis is not endemic. Such atypical cases indicate that this disease can be undiagnosed in clinical settings.

INTRODUCTION

Visceral leishmaniasis (VL) is endemic to different regions of Brazil and is, similar to that in other parts of the world, predominantly a childhood disease. In adult cases, co-infection with human immunodeficiency virus (HIV) emerges as an important predisposing factor for reactivation.1 Fever, hepatosplenomegaly, and weight loss are the main symptoms of VL, and the clinical picture is similar in patients infected with HIV and those not infected with HIV. However, certain features are more frequently described for patients co-infected with Leishmania and HIV. These features include several relapses after appropriate therapy, atypical parasite locations, and skin involvement.2

At this time, the patient had pancytopenia, and levels of CD4+ T lymphocytes were maintained. The viral load, as evaluated elsewere by the same HIV/RNA technique, showed a low level of plasmatic virus (540 copies/mL). In 2004, the patient was admitted to the Instituto de Pesquisa Evandro Chagas/FIOCRUZ for evaluation of cuta-neous lesions. On physical examination, the patient appeared emaciated and chronically ill. However, his vital signs were

Written informed consent was obtained from the patient for publication of this case report. A 39-year-old man from Brazil, originally from Rio Grande do Norte and a resident of the Rio de Janeiro State, was diagnosed with pulmonary tuberculosis and HIV infection in 1998. The patient had traveled throughout different countries in South America but had never traveled outside South America. Treatment for tuberculosis and antiretroviral therapy (azidothymidine and lamivudine [AZT/3TC]) were administered.

In 2002, highly active antiretroviral therapy was initiated. This treatment was composed of two nucleoside analogs, reverse transcriptase inhibitors (lamivudine and tenofovir) and protease inhibitors (lopinavir and ritonavir). After this treatment, the patient reported mild abdominal pain, occasional nausea, and gastric fullness. A physical examination showed hepatosplenomegaly, and a hepatic histopathologic analysis by using serologic tests identified infection with hepatitis C virus. The HIV viral load was < 80 copies/mL, as quantified by a nucleic acid sequence–based amplification assay (Organon Teknika, Durham, NC). However, CD4+ T cell counts were not available.

After one year of the above-mentioned treatment schedule for infection with HIV, the immunologic profile remained similar; it showed identical levels of viral load (< 80 copies/mL) and a CD4+ T cell count of 33 cells/mm3. In 2004, the abdominal volume was increased, and abdominal computerized tomography showed homogenous hepatosplenomegaly. At this time, the patient had pancytopenia, and levels of CD4+ T lymphocytes were maintained. The viral load, as evaluated elsewhere by the same HIV/RNA technique, showed a low level of plasmatic virus (540 copies/mL). In 2005, the patient had asymptomatic, erythematous lumps on the trunk and face, but despite low CD4+ T lymphocyte cell counts, HIV-1 RNA was not detected.

In 2006, the patient was admitted to the Instituto de Pesquisa Evandro Chagas/FIOCRUZ for evaluation of cutane-ous lesions. On physical examination, the patient appeared emaciated and chronically ill. However, his vital signs were
normal. The liver was palpable 8 cm below the costal edge, and the spleen was palpable at the umbilical level. There was no fever. Cervical, axillary, and inguinal lymphadenopathy were detected. Cutaneous lesions, i.e., elevated, erythematous papules and plaques were noted on the trunk, cheeks, and chin (Figure 1A and B). Blood cell counts were as follows: erythrocytes, 2.9 × 10^6 cells/μL; leukocytes, 2.4 × 10^9 cells/μL; hemoglobin, 8.2 g/dL; and platelets, 12.6 × 10^9 cells/μL. The creatinine level was 3.3 mg/dL (291.7 μmol/L), and blood urea nitrogen level was 57 mg/dL (20.4 mmol/L).

Fragments of trunk lesions and bone marrow aspirate were submitted for histopathologic analysis, mycological culture, parasite isolation by using Novy-MacNeal-Nicolle culture medium, and polymerase chain reaction detection of Leishmania DNA. Histopathologic analysis of the skin showed normal epidermis. However, in the mid- and deep dermis, great masses of granulomatous inflammatory infiltrate and numerous amastigotes within macrophages and in the intercellular spaces were noted (Figure 1C and D). Histopathologic analysis of bone marrow showed numerous amastigotes. Culture in NNN medium was positive for Leishmania. Silver impregnation, Wade staining, and mycological culture were negative for fungal elements. Re-examination of the hepatic histopathologic sample from 2002 showed numerous amastigotes (Figure 2), indicating that visceral leishmaniasis had had a long-term clinical course (at least five years) before the skin dissemination.

Results for restriction fragment length polymorphism assays specific for the hsp70 Leishmania gene were positive for the skin and bone marrow samples. Parasites from these samples were identified as L. donovani complex. Two Leishmania isolates were obtained and characterized by enzyme electrophoresis: one from the cutaneous lesion (IOC/L2930-MHOM/BR/2007/JFF) and one from bone marrow (IOC/L3020-MHOM/BR/2007/JFF_BM). IOC/L2930 was characterized as L. infantum IOC/Z1. Strain IOC/L3020 differed from IOC/L2930 in the 6-phosphogluconate dehydrogenase profile. The internal transcribed spacer (ITS)–5.8S–ITS2 ribosomal DNA region of both strains was sequenced. IOC/L3020 has a typical L. infantum sequence (MON-1, type A), and IOC/L2930 had an L. donovani MON-2 sequence (Genbank accession numbers FN398343 and FN398344 for IOC/L3020 and IOC/L2930, respectively). Multilocus microsatellite typing confirmed this data.

When cutaneous leishmaniasis was diagnosed, Montenegro’s skin test was negative, and results of immunofluorescent assay was positive at a dilution of 1/720. The CD4+ T absolute counts were < 50 cells/mm³, and the plasmatic viral load was undetectable as measured by bDNA technology (Versant® HIV RNA 3.0 assay; Siemens, Tarrytown, NY (Figure 3A)). After a cumulative dose of 3 g of amphotericin B, cutaneous lesions were reduced to mild erythema. However, the hepatosplenomegaly persisted with no improvement. Bone marrow histopathologic analysis showed no parasites, and culture for Leishmania was also negative.

Three months after anti-Leishmania therapy, the patient had fever and a new erythematous papule on the skin (Figure 4A). Histopathologic analysis showed a granulomatous reaction and several amastigotes. The patient was retreated with pentavalent antimony at a dose of 20 mg of Sb³/kg/day for 3 weeks. Four weeks after the end of the antimonial therapy, only mild erythema and purpuric lesions on the legs were observed (Figure 4B). We considered several differential diagnoses for the purpuric lesions: papular-purpuric gloves and socks syndrome, B19 parvovirus infection, early manifestations of Kaposi’s sarcoma, a coagulation defect linked to low platelet counts or lack of certain coagulation factors, or initial lesions of bacillary angiomatosis. However, the patient refused further investigations, and a precise diagnosis could not be ascertained.

A qualitative analysis of cellular immune responses was performed during the clinical follow-up as described elsewhere. High levels of T cell activation were observed during the active phase of leishmaniasis (first episode and relapse) (Figure 3B), as assessed by the expression of CD38 in CD8+ T cells and HLA-DR in CD3+ T cells. The degree of activation decreased after therapy and was stably maintained during the remission phases, especially for HLA-DR (Figure 3C). Lymphocyte activation in response to Leishmania or HIV antigens (p-24 protein) in vitro was also assessed by lymphocyte proliferative response (LPR) assays and interferon-γ (IFN-γ) production. The LPR in response to parasite or viral antigens was negative (stimulation index < minimal limit of 2.5)
at any time during follow-up, although the LPR was positive for the mitogen concanavalin A. Interferon-γ (790 pg/mL) was detected in response to leishmanial antigens during the first episode of leishmaniasis, but no difference was noted between cultures with and without stimuli. However, the IFN-γ production decreased progressively during clinical evolution, decreasing to levels of 15 pg/mL.

**DISCUSSION**

Patients co-infected with VL and HIV/acquired immunodeficiency syndrome (AIDS) may have distinct atypical skin involvement, such as cutaneous lesions. The pathogenesis of cutaneous lesions in an HIV-positive patient with VL is not clear since amastigotes can also be observed in different types of skin lesions of HIV patients, such as Kaposi’s sarcoma, herpes infections, and bacillary angiomatosis, and in healthy skin. Classically, cutaneous lesions associated with VL represent the well-known post-kala-azar dermal leishmaniasis (PKDL) which is an aftermath of visceral disease and is seen only after cure of systemic manifestations. Cutaneous lesions of PKDL include hypochromic or erythematous macules and infiltrated plaques and nodules. Photosensitivity is present during PKDL, and there is a predominance of lesions on the face and upper thorax. However, some cases of African kala-azar have cutaneous lesions and parasites in lymph nodes and bone marrow, a clinical presentation known as para-kala-azar dermal leishmaniasis.

In our patient, the distribution of cutaneous lesions and their visual aspect were compatible with that of classical PKDL. The patient also showed features of African para-kala-azar dermal leishmaniasis, i.e., concomitance of cutaneous and visceral active disease and parasites present in the liver, bone marrow and skin. *Leishmania* spp. can be isolated from blood. In the present case, based on the skin histopathologic analysis, which showed normal epidermis and great masses of infiltrate with many parasites in the deep and mid-dermis (Figure 1C and D), we hypothesized that hematologic dissemination could have occurred. Furthermore, the profound degree of immunodeficiency possibly facilitated survival of the parasite within macrophages.

The skin *Leishmania* isolate (IOC/L2930) was distinguished from the reference strain of Brazilian *L. infantum* (IOC/L579) and from the bone marrow isolate (IOC/L3020) by the mobility of 6-phosphogluconate dehydrogenase. This enzyme analysis suggested that the patient had a *Leishmania* mixed infection, which was confirmed by DNA sequence analysis. ITS rDNA sequence analysis and multilocus microsatellite typing indicated that the cutaneous lesion parasite was probably *L. donovani* MON-2, the most common zymodeme reported from India. To our knowledge, MON-2 has never been isolated from cutaneous lesions. However, this zymodeme is closely related to MON-18 and MON-37, both of which are associated with cutaneous leishmaniasis in the Old World. The immunologic status of the patient probably contributed to the clinical manifestations observed.

Until now, *L. infantum* IOC/Z1 (i.e., MON-1) was the only species from the *L. donovani* complex associated with leishmaniasis in Brazil. Identification of *L. donovani* in this patient suggests that this parasite is circulating in Brazil or in other VL-endemic areas in South America as the patient traveled across different countries in South America without causing disease or being identified. The immunocompromised status probably favored the pathologic damage caused by this species.
Visceral leishmaniasis and HIV/AIDS induce lymphocyte depletion, polyclonal activation, and cytokine disregulations, and showed a synergy in their immunodeficiency mechanisms that aggravates both diseases.\textsuperscript{27} This fact could explain the maintenance of CD4\textsuperscript{+} T lymphocyte counts < 100 cells/mm\textsuperscript{3} despite the administration of anti-leishmanial therapy and successful highly active antiretroviral therapy (undetectable viral load during follow-up).

Although immunologic discordance\textsuperscript{28} could have occurred in this patient, we cannot exclude that lymphopenia was a consequence of VL pathogenesis.\textsuperscript{29} It is intriguing that this patient was evaluated over a long period and showed a high Leishmania infection load plus severe immunosuppression without affecting the viral load.

This case of coinfection with VL and HIV with long-term evolution of liver parasites is surprising, given the known severity of VL in HIV/AIDS patients.\textsuperscript{3} The absence of fever in the patient and his good general clinical status could have contributed toward refuting the VL hypothesis. However, the newly revised guidelines in Brazil consider that hepatomegaly or splenomegaly with or without fever and cytopenia in AIDS patients must be investigated for VL.\textsuperscript{30} The patient had three unexpected features: cutaneous lesions after dissemination of long-term VL parasites, infection by two viscerotrophic strains, one of them not previously identified in Brazil, and low HIV viral load despite the severe immunosuppression status. The association between VL and HIV/AIDS is a largely unknown disease entity, especially in areas in which leishmaniasis is not endemic. The present case suggests that many such cases may pass undiagnosed in clinical practice, and that greater clinical awareness is needed for accurate diagnosis.

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