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CASE REPORT

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Visceral leishmaniasis caused by *Leishmania (Leishmania) amazonensis* associated with Hodgkin's lymphoma

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ABSTRACT

Visceral leishmaniasis (VL) is mainly caused by *Leishmania (Leishmania) donovani* and *Leishmania (L.) infantum*; however, other *Leishmania* species have been associated with VL. We report a case of a patient simultaneously diagnosed with VL caused by *Leishmania (L.) amazonensis* and Hodgkin's lymphoma. After treatment with liposomal amphotericin B and chemotherapy, the patient presented a clinical cure. This case report reinforces the hypothesis that other *Leishmania* species can cause visceral lesions mainly related to immunosuppression.

KEYWORDS: Leishmaniasis. Immunosuppression. Malignance. *Leishmania (Leishmania) amazonensis.*

INTRODUCTION

Leishmaniasis (L) is caused by the protozoan of the genus *Leishmania* (Kinetoplastida, Trypanosomatidae), transmitted by infected female sandflies of *Phlebotomus* and *Lutzomyia* genus. Approximately twenty *Leishmania* species considered pathogenic for humans and can cause tegumentary or visceral leishmaniasis (VL). *L. (L.) donovani* and *L. (L.) infantum* are the main species that can cause VL in the Old and New World respectively[1]. However, reports have described other species of *Leishmania* causing VL[2]. In this context, *L. (L.) amazonensis*, the etiologic agent of the localized or diffuse cutaneous form in Brazil[3], has also been implicated in the etiology of VL[4]. The disease can be manifested as an asymptomatic infection or as severe or fatal when caused by *L. infantum*. Common symptoms are weight loss, fever, enlarged spleen and liver, and pancytopenia. In immunosuppressed patients, VL can manifest as an opportunistic disease due to a recent infection or as reactivation of a latent infection[5]. In addition, *Leishmania* can survive in the lymph nodes for a long time after clinical cure and may manifest later, under immunosuppressed conditions, even after the patient is outside the area of autochthonous transmission[6]. Hodgkin's lymphoma is composed of normal cells and Hodgkin/Reed-Sternberg cells, which can expand regulatory T cells, inhibiting CD8 T cells and repolarizing tumor-associated macrophages[7], leading to immunosuppression status and favoring the replication of infectious agents. Here we report a clinical case presenting VL, caused by *L. (L.) amazonensis* associated with Hodgkin's lymphoma, in a patient outside the endemic transmission area of *Leishmania*.

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CASE REPORT

A 65-year-old male, builder, born in the state of Paraiba (Northeast Brazil), but who had lived in the city of Sao Paulo since he was 9 years old, was admitted to the hospital in February 2016 complaining of diffuse abdominal pain for one year, weight loss of 14 kg and fever in the last 4 months. The fever occurred once or twice a day, more frequently in the morning and night, with profuse sweating and shivers. He had no history of previous diseases although he had smoked for several years. He claimed not to have traveled outside the city of Sao Paulo. He lived in his hometown until he was 7 years old, moved to the state of Pernambuco between the ages of 7 and 9, and had been living in Sao Paulo ever since. He denied having had contact with unpasteurized milk or dairy products, domestic animals, rodents, or farm animals.

At the assessment, his physical examination was unremarkable except for swollen lymph nodes in several body regions; the largest measured 3 cm and was located in the left inguinal region. His laboratory tests revealed an elevated C-reactive protein (CRP), normocytic normochromic anemia and elevated canalicular enzymes (CRP: 99.9 mg/L; sodium 130 mEq/L; hemoglobin 11.1 g/dL; hematocrit 32.4%; leucocytes 5300 cells/mm^3; neutrophils 3260 cells/mm^3; eosinophils 270 cells/mm^3; basophils 10 cells/mm^3; lymphocytes 910 cells/mm^3; monocytes 850 cells/mm^3; gamma-glutamyl transferase 570U/L; alkaline phosphatase 574 U/L; and beta-2 microglobulin 3.3 mcg/mL). All other blood tests were normal, including blood cultures, electrolytes, and kidney and liver functions. The following serology tests were all negative: HIV, syphilis, hepatitis C and B, toxoplasmosis, CMV, EBV, rk39 rapid test, enzyme-linked immunosorbent assay, and indirect immunofluorescence for *L. (L.) major*-like antigen. The transthoracic echocardiography was normal. The positron emission tomography revealed an increased glycolytic metabolism in several lymph nodes, some of them forming conglomerates, both above and below the diaphragm, as well as increased glycolytic metabolism in the spleen, liver and bones, suggestive of lymphoproliferative disorder (Figures 1A and 1B). The bone marrow aspiration identified the presence of *Leishmania* spp. in direct microscopy, which was confirmed by a positive polymerase chain reaction and culture of the aspirate, and further identified as *L. (L.) amazonensis.* The biopsy of the inguinal lymph node revealed a high-grade nodular sclerosing Hodgkin's lymphoma (Figures 2A and 2B) and positive immunohistochemistry for *Leishmania* spp. (Figure 2C).

The *Leishmania* species were characterized by two different methods: 1) *Leishmania* promastigotes were identified by multilocus enzyme electrophoresis (MLEE) at the Oswaldo Cruz Institute (Fiocruz-RJ), using two loci enzymatic systems: 6-phosphogluconate dehydrogenase and nucleoside hydrolase. The strain was identified as *L. (L.) amazonensis*. 2) To confirm *Leishmania* species, the DNA extracted from *Leishmania* promastigotes was analyzed by high resolution melting (HRM) analysis targeting Hsp70[8]. According to the dissociation profile by HRM, the *Leishmania* strain was characterized as being *L. (L.) amazonensis* (Figures 3A and 3B).

The patient was treated with liposomal amphotericin B in a total dose of 20 mg/kg with partial fever improvement. After discharge, he was submitted to six cycles of chemotherapy with Adriamycin, Bleomycin, Vinblastine and Dacarbazine. In combination with chemotherapy, he received prophylaxis with liposomal amphotericin B, 3 to 5 mg/kg every 21 days. On his last recorded visit, in July 2017, he was in clinical remission.

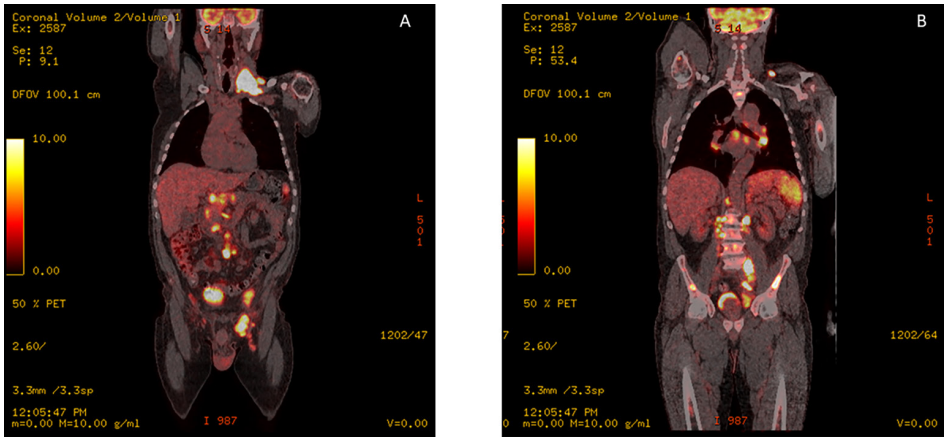

Figure 1 - Positron emission tomography (PET-CT): A and B) Increased glycolytic metabolism in several lymph nodes, some of them forming conglomerates, above and below the diaphragm. Increased glycolytic metabolism in the spleen, liver and bones, suggestive of lymphoproliferative disorder.

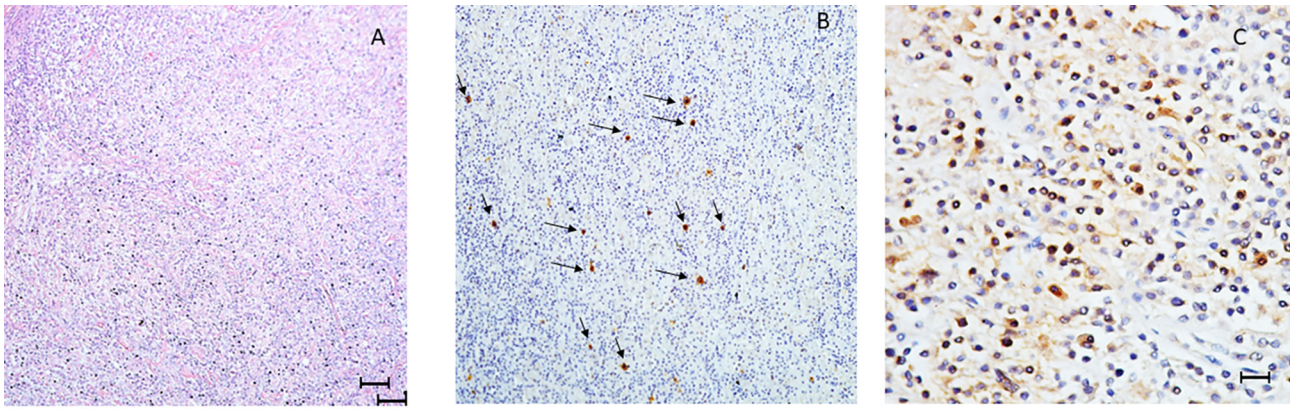

Figure 2 - Photomicrographs of lymph node section: A) Histological panoramic view of the left inguinal lymph node stained with hematoxylin eosin showing architectural modifications and cellular proliferation with poorly defined nodules; B) Immunohistochemistry stain: CD15+ (B), CD30+ e PAX-5+, CD20 (-), CD3 (-). Nodular sclerosing Hodgkin's lymphoma x 100; C) Positive immunohistochemistry reaction for *Leishmania* spp. antigens mononuclear cells x 400.

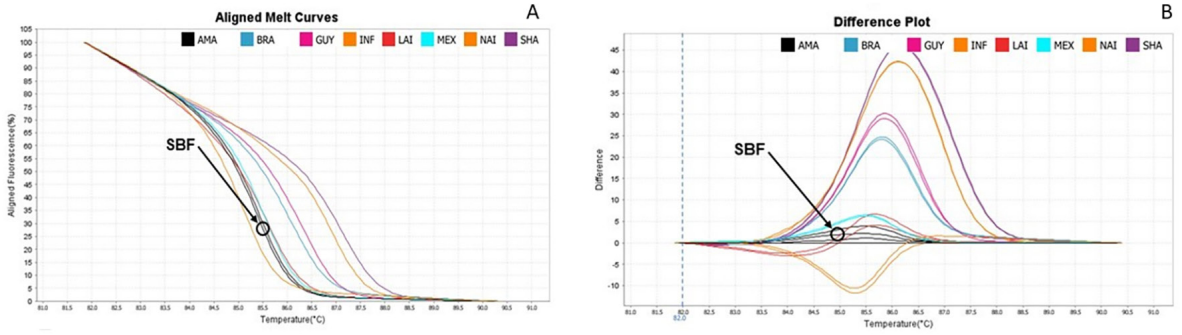

Figure 3 - Plotting of Hsp70-HRM assays. Melting profiles of *Leishmania* reference strains and samples from the patient. Panels show the melting profiles with organized data in normalized melting curves (A) and normalized difference curves (B). (INF): *L. (L.) infantum chagasi*; (AMA): *L. (L.) amazonensis*; (LAI): *L. (Viannia) lainsoni*; (BRA): *L. (V.) braziliensis*; (GUY): *L. (V.) guyanensis*; (NAI*): L. (V.) naiffi*; (SHA*): L. (V.) shawi*; (SBF): patient.

DISCUSSION

This case is interesting because of several aspects, such as the atypical VL clinical presentation; the association between VL and lymphoma with both being detected in the same lymph node; the identification of a *Leishmania* species, usually associated with cutaneous leishmaniasis, manifested as a visceral disease; and the successful treatment and prophylaxis of an immunosuppressed patient with VL. Considering the patient epidemiological data, and knowing that *Leishmania* parasites can remain viable in healthy patients even decades after the initial infection[6], we suppose that he probably had a reactivation of a latent infection that might have been acquired when he lived decades earlier in an endemic zone.

Another important point in this case report is the negative rk39 rapid test. This test is used in the diagnosis of VL caused by *L. infantum*, presenting specificity of 98% and sensibility from 92 to 96%, in an immunocompetent patient[9]. In our clinical case, we considered the rk39 test to be negative, as the clinical manifestation of VL was caused by *L. amazonensis*, which does not show cross-reactivity with rk39, because it is a recombinant antigen of *L. infantum*.

The *L. amazonensis* is classically associated with cutaneous leishmaniasis (CL). Most times it is manifested as a single lesion and rarely as diffuse cutaneous leishmaniasis (DCL)[3]. Barral *et al.*[4] described the association of *L. amazonensis* with a wide spectrum of clinical presentations, including mucocutaneous leishmaniasis (MCL), CL, DCL, VL and Post-kala-azar dermal leishmaniasis. Except for this study, there are few other reports of VL associated with *L. amazonensis* in humans even in areas with a high prevalence of *L. amazonensis*[10,11].

Immunosuppression has also been described in association with VL. The clinical presentation of VL is similar for both immunocompetent and immunosuppressed patients. However, for immunosuppressed people, the incidence of symptomatic disease, therapeutic failures and recurrence of disease is higher[5]. Due to the similarity of the clinical manifestations of lymphoma and VL, a more

accurate diagnosis of both diseases is difficult. Furthermore, the atypical manifestations of VL are described and mainly characterized by diffuse enlargement of lymph nodes and associated with lymphomas. The similarity of symptoms between hematological disorders and VL, as well as the atypical presentations of VL, associated with lymphomas make its diagnosis difficult[12,13].

In the city of Sao Paulo there is no transmission of leishmaniasis mainly caused by *L. amazonensis*. The atypical clinical presentation is probably explained by the underlying immunosuppression caused by the lymphoma as well as an overlap of symptoms from both diseases. Cases like this are particularly challenging and a high degree of clinical suspicion, supported by epidemiological information, is needed for a proper diagnosis. The association between leishmaniasis and neoplasms is still poorly understood. The concomitant finding of *Leishmania* and neoplasms in the same tissues, as well as the development of neoplasm in tissues previously affected by *Leishmania,* have equally been described[14,15].

A prophylactic maintenance treatment for immunosuppressed patients with VL is recommended, especially in people living with HIV-AIDS[5], even though it is based on a few studies with a low level of evidence. The prophylactic treatment for other immunosuppressive conditions is not well established, but it does have a theoretical basis considering a higher risk of recurrence in these populations, and is therefore currently adopted in our department. However, we observed an improved clinical status in our patient and we did not identify VL reactivation during the secondary prophylaxis, even when the patient was undergoing chemotherapy for lymphoma treatment. Thus, even without ample evidence in the literature, in a case report like this, consisting of association with lymphoma, we suggest that the indication for secondary prophylaxis for VL (regardless of the *Leishmania* species involved) during chemotherapy, should be carefully evaluated , with the aim to avoid recurrence.

CONCLUSION

This case reinforces the hypothesis that *L. amazonensis* could be associated with VL. The identification of *Leishmania* and lymphoma in the same tissue is intriguing, however further studies are needed to explore this association. The finding of a lymphoma and *Leishmania* spp. in the same tissue raises speculation around an association between both diseases. The possibility of atypical VL presentation in immunosuppressed patients strengthens the need for a high level of clinical suspicion of VL in patients coming from endemic areas.

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