Case Report: Severe Visceral Leishmaniasis in a Patient with HIV Coinfection Undergoing Treatment for Erythema Nodosum Leprosum

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Abstract. We report a case of visceral leishmaniasis (VL)/HIV coinfection in a patient undergoing regular antiretroviral therapy and treatment with thalidomide for erythema nodosum leprosum. He presented at a health service with high fever, chills, asthenia, pale skin, lower limb edema, hepatomegaly, and splenomegaly. Visceral leishmaniasis was confirmed by direct examination, and serological and molecular tests. Serum levels of Th1/Th2 cytokines were measured. The patient began treatment with liposomal amphotericin B, with good clinical response; however, VL recurred 6 months later. Treatment was reinitiated, maintaining secondary prophylaxis with liposomal amphotericin B. The patient showed clinical improvement with important recovery of CD4⁺ T-lymphocyte count.

INTRODUCTION

Visceral leishmaniasis (VL) is an infectious parasitic disease with worldwide distribution; 90% of cases are concentrated in India, Sudan, Nepal, and Brazil. The WHO estimates that there are between 200,000 and 400,000 cases of VL and 20,000–40,000 deaths due to VL annually.¹

It is estimated that approximately one-third of people infected with HIV around the world live in areas at a high risk of transmission of VL, and an increase in the number of cases of VL–HIV coinfection has been reported in 35 countries.^{2,3} In Brazil, changes in the distribution of cases of HIV and VL, related both to an increase in the number of cases of HIV in rural areas in the interior of the country and to the urbanization of cases of VL, have elevated the risk of coinfection.^{2,4}

The municipality of Petrolina, located in the state of Pernambuco, in the Northeast region of Brazil, has undergone an intense process of immigration in recent years, motivated by the expansion of fruit production in the region. In conjunction with a lack of health infrastructure and basic sanitation, this disordered growth has contributed to an increase in the incidence of diverse neglected infectious diseases in the region, such as VL and leprosy.^{5,6} Co-occurrence of these diseases, coupled with immunosuppression caused by HIV, has contributed to the appearance of atypical clinical pictures that require cautious investigation.

In this case report, we describe a severe case of VL in a patient with HIV infection who was also undergoing treatment for erythema nodosum secondary to lepromatous leprosy.

CASE REPORT

On October 11, 2017, a 40-year-old male patient was attended at the municipal health service in Petrolina, Pernambuco, Brazil, with 30-day-history of high fever, chills, asthenia, and weight loss. Clinical examination revealed pale skin, lower limb edema, and increased abdominal volume

due to hepatomegaly and splenomegaly. Laboratory tests revealed pancytopenia and renal dysfunction. The patient had been followed up by the service since 2014, for the treatment of HIV infection, with a regular use of tenofovir/lamivudine/efavirenz. At the time, his examinations showed a CD4⁺ T-lymphocyte count of 14 cells/mm³ and a viral load of < 40 copies. The patient also had erythema nodosum leprosum, and he had been undergoing treatment with thalidomide 100 mg daily and prednisone 20 mg daily since 2015.

The patient was hospitalized, and diagnosis proceeded with chest radiography, which showed no changes; abdominal ultrasound, showing hepatomegaly and splenomegaly; negative blood cultures; and negative urine culture. Microscopic examination of bone marrow aspirate revealed the presence of numerous amastigote structures compatible with *Leishmania*; on the other hand, immunochromatographic test (rK39) showed a negative result. Then, samples were sent to the Aggeu Magalhães Institute/Fundação Oswaldo Cruz (FIOCRUZ) (Recife, PE) for serological and molecular analyses, which demonstrated positive ELISA (rK39) for antileishmanial antibodies, positive direct agglutination test (DAT) for VL (1:51,200), positive urinary antigen for VL (Katex) (+++), and positive real-time PCR (qPCR).

Quantification of serum levels of the cytokines, such as interferon-gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-4 (IL-4), and interleukin-2 (IL-2), was performed by multiplex fluorescent bead immunoassay (CBA, BD-Biosciences, San Jose, CA). IL-10, and IL-6 levels were 10.58 pg/mL, and 1.32 pg/mL, respectively. IFN- γ , TNF- α , IL-4, and IL-2 were not detected (Figure 1).

The patient began treatment with liposomal amphotericin B 4 mg/kg daily for 5 days, showing good clinical response, with no fever on the second day of treatment. Physical examination during treatment revealed reduction in the size of the liver and spleen. The patient also showed laboratory improvement, with progressive increase in leukocytes and platelets. The patient was subsequently discharged for outpatient follow-up. During this period, the patient did not undergo secondary prophylaxis with liposomal amphotericin B 4 mg/kg every 15 days, and VL recurred approximately 6 months after the first treatment, with the patient again presenting fever, chills, asthenia, weight loss, and worsened hepatosplenomegaly.

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FIGURE 1. Serum levels (pg/mL) of IFN-γ, TNF-α, IL-10, IL-6, IL-4, and IL-2 in a patient with visceral leishmaniasis/HIV coinfection before and after treatment with liposomal amphotericin B.

The patient was hospitalized again in April 2018; a new bone marrow aspirate was performed, revealing the presence of numerous amastigote structures compatible with *Leishmania*. Liposomal amphotericin B 4 mg/kg daily was reinitiated for 5 days, and the patient once more evolved with good clinical and laboratorial response.

Six months after the last treatment for VL, the patient was clinically cured, undergoing secondary prophylaxis with liposomal amphotericin B 4 mg/kg every 15 days, to prevent recurrence of the disease. The patient currently shows important recovery in CD4⁺ T-lymphocyte count (144 cells/mm³), maintaining undetectable viral load.

Two years after the first treatment, the patient presents undetectable levels of IFN-γ, TNF-α, IL-10, IL-4, and IL-2. Serum levels of IL-6 are almost undetectable (1.17 pg/mL).

A summary of the patient's time line is shown in Figure 2.

DISCUSSION

We have reported a case of VL–HIV coinfection in a hyper-endemic area for VL and leprosy; the patient was undergoing ART and receiving immunosuppressive medications for the treatment of erythema nodosum secondary to multi-bacillary leprosy.

FIGURE 2. Patient time line showing diagnosis time, CD4 counts, and therapy scheme. ART = antiretroviral therapy; DAT = direct agglutination test; MB-PCT = multi-bacillary polychemotherapy; TDF/3TC/EFV = tenofovir/lamivudine/efavirenz; VL = visceral leishmaniasis. This figure appears in color at www.ajtmh.org.

In the reported case, the patient presented a typical clinical picture of VL with the presence of high parasite load by direct examination. In patients with VL-HIV coinfection, clinical presentation may be similar to that in immunocompetent patients. However, as patients with coinfection have some specific immunological characteristics, their clinical manifestations may differ from those of patients who are not infected with HIV, making diagnosis challenging, because of similarities with other opportunistic infections.⁷ In addition, elevated parasite load is a common finding in coinfecting patients, due to the compromised immune system (reduced monocyte and macrophage function) and stimulation of viral replication.^{8,9}

Serological tests for antibodies normally show low performance for VL/HIV coinfecting patients. Freire et al.¹⁰ demonstrated that, among serological tests available in Brazil for diagnosing VL, DAT showed the highest sensitivity in individuals with coinfection, and immunochromatographic assay (IT LEISH, rk39) showed sensitivity of only 63.2%. *Leishmania* urine antigen tests using KAtex (Kalon Biological Ltd., Guildford, UK) and detection of parasitic DNA using molecular biology have shown good performance both in patients with VL alone and in those with VL-HIV coinfection.^{11–13} In this case report, only immunochromatographic assay (rk39), which is recommended by the Brazilian Ministry of Health for diagnosis of VL, showed a negative result. This underscores the importance of implementing, in addition to immunochromatographic assay, other tests with higher sensitivity for diagnosing VL in patients with coinfection.

It is well known that cytokines play a role in pathogenesis and hosts' resistance of VL. In this case report, serum levels of IFN- γ , TNF- α , IL-4, and IL-2 were undetectable before and after treatment. The patient did, on the other hand, express IL-10 and IL-6 before treatment, but only IL-6 remained detectable after treatment. Our results are in line with a previous study that found significantly high serum levels of IL-10, IL-6, and IFN- γ , whereas TNF- α , IL-2, and IL-4 were minimal in Indian patients with VL. On treatment, significant decrement in IL-10 and IL-6 levels was observed; however, the level of IL-6 remained significantly elevated in comparison with that of controls.¹⁴ In addition, Caldas et al.¹⁵ observed that IL-10 and IFN- γ circulating levels dropped dramatically 30 days after treatment.

It is important to highlight that the results found in the present case report cannot be directly compared with previous studies in patients with VL only. Here, we have a patient with VL and AIDS undergoing immunosuppressive treatment for erythema nodosum leprosum. Therefore, this could explain, at least in part, the absence of expression of other cytokines, such as IFN- γ , which is produced mainly by activated CD4 and CD8 T cells.¹⁶ In addition, lack of IFN- γ may be related to the presence of elevated levels of IL-10, its main suppressor.¹⁷

The lack of IFN- γ may have contributed to the disease's progression in the case described here. IFN- γ is a key cytokine involved in host protection; it is responsible for skewing Th1 response and stimulates the macrophages to produce reactive oxygen species and nitric oxide for oxidative killing of intracellular amastigotes.¹⁷ Accordingly, a recent study showed that asymptomatic immune responders for *Leishmania* had high levels of IFN- γ among HIV+ individuals, reinforcing the role of this cytokine in the resolution of the infection.¹⁸

In leishmaniasis pathogenesis, IL-10 plays a substantial role by causing the downregulation of Th1 response, macrophage activation, and antigen presentation by dendritic cells.¹⁹

Furthermore, IL-10 inhibits the leishmanicidal functions of macrophages by diminishing the production of reactive nitrogen intermediates by macrophages, IFN- γ by T and natural killer cells, and IL-12 mediated activation of macrophages.^{17,20} In a murine model of VL, overexpression of IL-10 during the initial phase of infection led to higher disease susceptibility due to decreased multifunctional CD4 T cells.²¹ In addition, the severity of VL in human has been associated with increased levels of IL-10 in serum as well as tissue lesions.^{22,23} Similarly, IL-6 has been associated with disease severity and death in patients with VL.^{24,25}

In individuals with VL-HIV coinfection, the chance of recurrence of VL is five times greater than in individuals without coinfection.⁹ The high rate of recurrence may be associated with the inability to control replication of parasites due to an ineffective immune response mediated by *Leishmania*-specific lymphocytes, resulting from immunodeficiency caused by HIV.²⁶ In the reported case, the patient showed clinical worsening 6 months after treatment of VL, as he did not follow up with secondary prophylaxis. The establishment of secondary prophylaxis in these patients would, thus, be of fundamental importance.

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