RELATO DE CASO

VISCERAL LEISHMANIASIS-HIV COINFECTION: A CASE REPORT

ACESSO LIVRE

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ABSTRACT

Visceral Leishmaniasis (VL) is popularly known as kalazar, a denomination inherited from the Hindu vocabulary, where the disease was known as "black fever" (*kala-azar*). It is the systemic form of the disease caused by the flagellate protozoan of the *Leishmania donovani* complex, that can be found in several parts of the world in the form of three subspecies: *Leishmania chagasi* in Latin America; *Leishmania donovani* in India, China, Iraq and East Africa; *Leishmania infantum* found in North Africa and Mediterranean Europe.(1) The main characteristic of this genus of protozoa is its dimorphism, which can then be found in the form of amastigote - rounded, not flagellate, obligatory intracellular parasite, capable of infecting and causing the disease in men; and promastigota - flagellate, mobile, found within the digestive tract of the vectors. The latter are of the species *Lutzomyia longipalpis*, there are also the species *Lutzomyia cruzi,* found in Mato Grosso do Sul, popularly known in Brazil as mosquito-palha, birigui or tatuquira. These mosquitoes are known for their hematophagous habits during the late afternoon and early evening, they are anthropozoophilic, meaning, they feed of human blood as well as other mammals. In Brazil, in addition to humans, dogs are the main reservoirs of the disease. The infection occurs when the female mosquito injects the protozoa in the promastigote form during the blood meal, then it is phagocytosed by macrophages, becoming not flagellated and rounded forms - amastigotes - inside, capable of performing binary division and then infecting new cells. After reaching the bloodstream the main organic site of infection are the organs of the reticuloendothelial system - liver, spleen and bone marrow.(3) The infectious cycle is maintained when sandflies ingest the amastigote forms found within the monocytes present in the bloodstream.

KEY-WORDS: Acquired Human Immunodeficiency Syndrome Virus; Coinfection; Visceral Leishmaniasis.

INTRODUCTION

Visceral Leishmaniasis (VL) is popularly known as kalazar, a denomination inherited from the Hindu vocabulary, where the disease was known as "black fever" (*kala-azar*). It is the systemic form of the disease caused by the flagellate protozoan of the *Leishmania donovani* complex, that can be found in several parts of the world in the form of three subspecies: *Leishmania chagasi* in Latin America; *Leishmania donovani* in India, China, Iraq and East Africa; *Leishmania infantum* found in North Africa and Mediterranean Europe.[1] The main characteristic of this genus of protozoa is its dimorphism, which can then be found in the form of amastigote - rounded, not flagellate, obligatory intracellular parasite, capable of infecting and causing the disease in men; and promastigota - flagellate, mobile, found within the digestive tract of the vectors. The latter are of the species *Lutzomyia longipalpis*, there are also the species *Lutzomyia cruzi,* found in Mato Grosso do Sul, popularly known in Brazil as mosquito-palha, birigui or tatuquira. [1][2]

These mosquitoes are known for their hematophagous habits during the late afternoon and early evening, they are anthropozoophilic, meaning, they feed of human blood as well as other mammals. In Brazil, in addition to humans, dogs are the main reservoirs of the disease. The infection occurs when the female mosquito injects the protozoa in the promastigote form during the blood meal, then it is phagocytosed by macrophages, becoming not flagellated and rounded forms - amastigotes - inside, capable of performing binary division and then infecting new cells. After reaching the bloodstream the main organic site of infection are the organs of the reticuloendothelial system - liver, spleen and bone marrow.[3] The infectious cycle is maintained when sandflies ingest the amastigote forms found within the monocytes present in the bloodstream. The infectious cycle is maintained when the sandflies ingest the amastigote forms found within the monocytes present in the bloodstream. As they enter the gastrointestinal tract of the insect, the protozoa find a suitable environment to live in the promastigote form. After about 5 days the newly infected mosquitoes are able to transmit the disease.

This is a endemic disease diagnosed in 88 countries, where approximately 90% of the world's cases are concentrated in the region of India, Bangladesh, Sudan and Brazil.[4] In our country are about 3,400 cases per year, reaching 6300 considering underreporting,[5] registered mainly in the states of Minas Gerais, Bahia, Ceará, Piauí, Maranhão, Roraima, Mato Grosso, Goiás, Tocantins and Rio de Janeiro.

The VL in most people presents as the asymptomatic form. The factors that determine the severity of the disease are mainly: age, nutritional status and patient immunogenetics. The incubation period lasts approximately 3 months. The clinical manifestation varies according to the time of disease evolution. Initially occurs: fever, discrete hepatosplenomegaly and skin-mucous pallor, associated or not with cough and diarrhea. If there is no treatment, the patient progresses to the period of state characterized by massive hepatosplenomegaly, persistence of fever, worsening of skin-mucosal pallor and progressive weight loss. In the final period of the disease, it is common to associate with bacterial infections, severe protein-energy malnutrition, epistaxis, cutaneous or digestive bleeds; being bacterial infections are responsible for the majority of deaths.[6]

CASE REPORT

J.I.G.M., 52 years old, male, native of Miracema -TO, divorced, rural worker. Admitted to the Public General Hospital of Palmas (HGPP) by the Infectious Diseases Department, referred due to febrile syndrome with 2 months of evolution, associated with pancytopenia and HIV rapid test positivity. Initially, he sought care in Barrolândia-TO due to daily fever, unmeasured, initially 2 months earlier, predominantly during the afternoon, associated with chills and odorless sweating. He referred in symptomatic questioning: abdominal distension, inappetence, intestinal constipation with an evacuation pattern of 1 bowel movement every 5 days, asthenia, weight loss of 4 kilograms in said period and gingivorrhagia after brushing. He denied known comorbidities or continued use of medications. Social drinker, former smoker with a load of 30 packs a year for 20 years, having ceased about 3 months ago, sexual promiscuity, and denied the use of illicit drugs. He reported, 3 months earlier, cohabitation with 18 people in precarious housing in rural areas, ingesting unfiltered water during this period.

When the patient was admitted to the Infectious Diseases Department, the patient was in a general regular state, lean and pale. In the physical examination, the patient had normal oroscopy, without moniliasis, respiratory and cardiovascular systems within normality, but only the abdominal examination showed hepatomegaly of 12 cm from the right costal border and splenomegaly of 5cm from the left costal border, with a tendency to expand to the epigastric region.

Complementary examinations were requested: chest x-ray, confirming bilateral perihilar infiltrate; USG of abdomen showed homogeneous splenomegaly, bilateral lithiasis and/or renal calcification, with no signs of obstruction. Viral Load (VL) for HIV denotes 16,572 viral copies, CD4 T-cell count of 481; negative serologies for hepatitis A, B and C; non-reactive VDRL; and negative screening for Leishmaniasis with *calazar detect*, serology and aspirated bone marrow.

During the entire hospitalization the patient kept with afternoon fever, and epigastralgia, restrictive burning, intense sialorrhea, worsening of prostration and onset of dry cough began. Serologies were therefore sought for: Chagas, cytomegalovirus, toxoplasmosis, histoplasmosis, aspergillosis, paracocidioidomycosis, cryptococcosis, leptospirosis, brucellosis, HTLV, isolated sputum BAAR, PPD skin test and TRM-TB in blood and urine. With the exception of positive HTLV serology, the other tests requested at the time presented negative results. Computed tomography (CT) of the abdomen revealed a spleen of 21 cm, a slightly enlarged liver, thickened and edematous vesicle wall. Chest CT showed interstitial thickening of the lung, associated with nonspecific air cysts, with mediastinal lymph nodes, but not lymph node enlargement. A new bone marrow puncture was performed

that also did not detect leishmania, in addition to a negative rapid test for malaria. Plasmodium search, a new sample of BAAR and EBV were requested, all without diagnostic findings. Antibodies to smooth muscle and LKM1 also negative. Upper GI endoscopy was requested which indicated esophageal erosions in lower third grade and mild/moderate gastritis.

On the 25th day of hospital stay, lab tests indicate, associated with fever, important neutropenia (480) indicating the onset treament with Cefepima. Supraclavicular and cervical lymph nodes were found on the left. Supraclavicular lymph node biopsy was performed, presenting in anatomopathological result: lymphoid reactive hyperplasia with frequent plasmocytes and histiocytes and tiny structures that suggested Leishmaniasis.

Treatment was instituted for Visceral Leishmaniasis with Liposomal Amphotericin B fulfilling criteria (age over 50 years and hepatic insufficiency). After 14 days of treatment the patient was discharged with significant clinical improvement and guidance for outpatient return and follow up.

DISCUSSION

Clinic

Leishmaniasis can modify the progression of HIV disease and the immunodeficiency caused by the virus is a facilitating factor for the progression of leishmaniasis. There is no defined profile that can be indisputably associated with coinfection, however the severity of clinical manifestations, response to treatment, evolution and prognosis are directly associated with the patient's immunological condition, evaluated by the CD4 + T lymphocyte count. The classic triad of Visceral Leishmaniasis (VL) is also the most common manifestation of this disease in coinfection when symptomatic: hepatosplenomegaly, fever and pancytopenia are observed in 75% of cases.[6]

From the symptons, the infectious diseases team initially thought of VL, but as the rapid test, serology and two direct marrow searches came negative, a more detailed investigation of the patient was initiated. Chagas, tuberculosis and malaria were ruled out by local epidemiology. The febrile pattern led to an investigation of cytomegalovirus and toxoplasmosis. The presentation of respiratory complaints such as cough during hospitalization led to the investigation of histoplasmosis, aspergillosis, paracoccidioidomycosis and cryptococcosis. The social history of housing in a rural area in a cluster of people led to the search for leptospirosis and brucellosis. The gastrointestinal complaints and especially the epigastralgia associated to HIV, caused that the serology for HTLV to be requested, the only one of all those mentioned above that had a positive result. The whole picture and waiting for these results delayed the treatment. After a month of hospitalization, without resolution of the condition, the patient presented supraclavicular lymph node enlargement that led to biopsy due to all the history and diagnostic challenge. It was only after the results of this biopsy that the diagnosis for visceral leishmaniasis could be closed.

Diagnosis

The diagnosis of VL besides the clinic is based on laboratory, immunological and parasitological aspects. Laboratorially it becomes useful the use of the hemogram that can reveal anemia, leukopenia with predominance of lymphocytes and thrombocytopenia. There is an increase in inflammatory tests such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and ferritin. Hypoalbuminemia with inversion of the albumin / globulin ratio is also present. The globulinemia is due to hyperactivation of B cells that leads to the large production of antibodies, which can result in indirect Coombs positivity, antinuclear, anti-DNA, anti-smooth muscle and anticardiolipin antibodies detection.[7] There is activation of the coagulation cascade (increase of D-dimer, decrease of fibrinogen, prolongation of TAP and TTPa), circulation of immunocomplexes, hypocomplementemia and cryoglobulinemia. There may be elevation of aminotransferases, bilirubins, and a slight increase in urea and creatinine.[8]

Immunological diagnosis in Brazil can be done through indirect immunofluorescent antibody test (IFAT) or Enzyme Linked Immmunosorbent Assay (ELISA) serology. Serology is a very useful option in immunocompetent patients and in children, but they are less reliable in HIV patients, more than 40% of VL/HIV patients may present negative results.[9]

Many serological tests are available as the direct agglutination test (DAT) and the rK39 antigen-based test. The sensitivity of the tests varies from 94 to 95% and the specificity from 86 to 91%.[10] One of the major drawbacks of serology is that they can not be used to detect relapses since antibody titers remain elevated even after curing and, besides, may be positive in endemic areas due to exposure to asymptomatic infection.[11]

The gold standard for the diagnosis is the direct demonstration of the parasite through microscopy with culture of the splenic aspirate. However, aspiration of the spleen is associated with hemorrhage and the procedure can only be performed through surgical access. For this reason bone marrow and lymph node aspirates are frequently used for the direct search of parasites.[12] The specificity of direct parasite search is high, but sensitivity ranges from approximately 94 to 99% in the spleen, 53 to 86% in the bone marrow, and 53 to 65% in lymph nodes.[13] Direct parasite search is also the most sensitive method in immunocompromised patients, mainly by aspiration of the spleen.[9] The sensitivity of bone marrow microscopy is estimated to be 81% in VL/HIV patients, but negative findings do not rule out VL, and there are some reports of positivity only after repeated analyzes.[14] The culture can increase sensitivity and allow differentiation between the species, but it is time consuming. There is also the possibility of performing direct microscopy and culture with a peripheral blood sample, it has sensitivity of 50% in patients with HIV.[9]

The molecular method through PCR (amplification of the DNA of the parasite) using both bone marrow and peripheral blood is the most specific and sensitive method for the diagnosis of VL/HIV patients. In addition, when comparing parasitological, serological and molecular methods in VL/HIV patients the sensitivity of PCR in blood samples was similar to that of direct search in bone marrow aspirate. It is also agreed that the use of PCR is useful in monitoring long-term treatment,

thus evaluating the efficacy of drugs and the occurrence of relapses in treated patients, avoiding the repetition of invasive methods. PCR becomes negative soon after the institution of the treatment, the presence of positive results indicates the existence of recurrences. For these reasons, there is a strong recommendation for the use of PCR in leishmaniasis-infected HIV positive patients both in diagnosis and in treatment monitoring.[15]

Technique	Tissue/fluid	Imunosupression	Sensitivity (%)	Invasiveness
Microscopy	Blood	HIV	50	+
	Spleen	HIV	>95	+++
	Bone marrow	HIV	67-94	++
	Lymph node	HIV	53-65	++
Culture	Bone marrow	HIV	70-81.3	++
	Blood	HIV	67	+
IFAT	Serum	HIV	48-75	+
TAD	Serum	HIV	87.8	++
PCR	Blodd	HIV	72-100	+
	Bone marrow	HIV	82-100	++
rK39	Serum	HIV	46.6-93.9	+

Table 1 – Sensitivity of diagnostic methods available to patients VL/HIV. Adapted from van Griensven *et al* 2014.[16]

Diagnostic Test	Sensitivity %	Specificity %
rK39	97 (90-99.5)	90.2 (76.1-97.7)
DAT	97.1 (94.9-98.4)	88.1-98.5
Parasitology pleen	>95	100
Parasitology bone marrow	60-85	100
PCR	92.3 (88,4-94,9)	63.3 (53.9-71.8)
IFAT	86.1	97.2
Elisa	87.4	92.4

Table 2 – Sensitivity and specificity of diagnostic methods available for VL patients. Table created based on the results presented in Medley *et al* 2015[17] and Peixoto *et al* 2015[18].

In the case reported, thinking about leishmaniasis, it was requested the rapid test for the disease, serology by ELISA and also the direct search through aspirate of bone marrow. All the results were negative. A new collection of bone marrow sample was performed, also without the presence of leishmaniasis. By observing the described studies, the methods of higher sensitivity and specificity available in the service were used. Spleen puncture was not performed because of the risk of hemorrhage since the patient had large splenomegaly.

We discuss here the possibility of HIV coinfection having decreased diagnostic sensitivity. Among those performed, there may have been a failure, based on the studies described above, in bone marrow microscopy that has a sensitivity ranging from 60 to 80%, in addition to the ELISA method, which could present failure in 18.6% of cases in cases of coinfection. The rapid test (rK39) presents a lower chance of error, since when there is a concomitant presentation of the two diseases the sensitivity and the specificity reach 90%. The association of all these methods, even in immunocompromised patients, should present a lower chance of error. For this reason, this report aims to incite the questioning about the existing diagnostic methods and the possibility of failure. Thus the clinic maintains its prominence in the diagnosis and the negativity of the diagnostic exams within a classic picture of Visceral Leishmaniasis can not prevent the beginning of the treatment. Delaying the introduction of anti-leishmania drugs may increase the mortality of these patients.

Treatment

In Brazil, pentavalent antimoniate and amphotericin B are available for the treatment of visceral leishmaniasis, and the choice between them is based on criteria such as age, gestation and comorbidities that contraindicate the use of one drug or another. Because of the possibility of use at the outpatient level, antimoniate is preferable, since it does not require hospitalization and the inherent risks. The Amphotericin, used in pregnant women and patients with contraindications to the use of pentavalent antimonials, is considered the current drug of greatest potency against leishmaniasis.[19]

The success of the treatment of the disease involves a series of factors that are related to the host, such as genetics, immune response and clinical presentation of the disease; related to the treatment itself, such as drug quality and duration of therapy; and factors related to the parasite, such as sensitivity to certain drugs.[20]

N-methyl glucamine antimoniate is very effective in the treatment of cutaneous, mucocutaneous and visceral forms of the disease, with a rapid decrease in the clinical and hematological manifestations caused by leishmaniasis. In recent years, however, it has been associated with a series of adverse reactions.[21] It has toxic side effects to the cardiac, renal and hepatic systems, so they are contraindicated in patients suffering from diseases that affect these systems. The use in pregnant women is also not recommended, since the drug's ability to cross the transplacental barrier and affect the fetal nervous system, resulting in severe mental retardation syndromes.[20]

Amphotericin B is the drug of choice in special situations and is available in two formulations by the Brazilian Ministry of Health: amphotericin B deoxycholate and liposomal amphotericin B. Liposomal Amphotericin B is indicated for patients who meet one of the criteria: age greater than 50 years or less than 1 year; cardiac, renal or hepatic impairment; corrected QT interval greater than 450 ms or use of drugs that alter the QT interval; hypersensitivity or therapeutic failure with pentavalent antimonials; HIV coinfection or immunosuppressive diseases; use of immunosuppressive medications; pregnant women.[22] Due to the concomitance of the diseases in our case the patient was treated with liposomal amphotericin B showing significant improvement already in the first days after the institution of the treatment.

In the absence of liposomal amphotericin B, the use of amphotericin B deoxycholate is an alternative. Severe adverse reactions have been reported with amphotericin B, with cases of toxicity, myocarditis, severe hypokalemia, renal dysfunction and death. Use requires hospitalization and patient monitoring. The main and most feared limiting factor for its use is high toxicity.[23]

In the case presented, the patient, even though presenting coinfection with HIV-leishmaniasis, manifested clinically with the classic case of VL: persistent fever, cachexia,

weight loss, hepatosplenomegaly and pancytopenia. There was a diagnostic delay since the rapid test, the serology and the two direct searches through bone marrow puncture that were performed came negative for the disease. Faced with this situation, there was an extensive laboratory investigation to confirm or rule out other infections that may present in this way or be an atypical variant of some epidemiologically common disease in our state such as hepatitis, cytomegalovirus, toxoplasmosis, chagas disease, HTLV, parvovirus B19, paracoccidioidomycosis, histoplasmosis, cryptococcosis, aspergillosis, tuberculosis, leptospirosis, brucellosis, malaria and Epstein-BAAR infection.

Many of these serologies are analyzed out of state, this causes the results to be delayed leading to diagnostic delay, as occurred in the case reported. When the presence of leishmania was confirmed in the lymph node biopsy, the treatment was instituted, but for more than a month the patient was under investigation, with clinical worsening. For this reason, we emphasize that, although reliable and with high sensitivity and specificity, serological tests are not free of failure and, in the face of strong clinical suspicion, treatment should be instituted, especially in states with poor health conditions that do not provide all the laboratory tests quickly so that the diagnosis is soon closed.

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