

Liver biopsy for visceral leishmaniasis diagnosis in pregnancy: report of 2 cases

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Abstract

Visceral leishmaniasis (VL) or kala-azar is a zoonosis caused by intracellular protozoa of the *Leishmania* genus and is transmitted to humans by the bite of phlebotomine sandflies. It particularly affects cells in the phagocytic mononuclear system, accompanied by disturbances of cellular and humoral immunity. VL is potentially fatal and is characterized by fever, hepatosplenomegaly, diarrhea, epistaxis, jaundice, anemia, leucopenia, thrombocytopenia, hypoalbuminemia and hyperglobulinemia. Diagnostic suspicion is based on epidemiological, clinical and laboratory data and is

confirmed by detecting the parasite in infected tissue. Splenic aspiration is the most sensitive method, followed by bone marrow aspiration (BMA) by sternal puncture, liver biopsy and lymph node aspiration; but, due to safety concerns, BMA is the most recommended method. VL is included as a target disease by players in drug research and development. Severe liver dysfunction associated with VL is uncommon. We report two VL cases in pregnant women from Bauru, Sao Paulo state, Brazil, considered an endemic area. The first of them developed hepatic failure due to fulminant hepatitis. In both cases, BMA was unable to find the protozoan; thus, liver biopsy was the only means of making the diagnosis.

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Key words: Visceral Leishmaniasis; Infection in pregnancy; Liver biopsy; Bone marrow aspirate; Differential diagnosis

Core tip: Visceral leishmaniasis, which has many severe presentations, is an endemic disease found in many countries around the world, especially in South America, where Brazil is the most affected country. We herein present two cases of this disease affecting women during pregnancy, when the diagnosis and management can be very difficult. In both patients, the usual method of diagnosis failed so liver biopsy was the only option to make the correct diagnosis. Therefore, liver biopsy may be considered in special situations when severe visceral leishmaniasis is suspected, as in the cases herein presented.

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INTRODUCTION

Visceral leishmaniasis (VL) or kala-azar is a zoonosis caused by intracellular protozoa of the *Leishmania* genus. The disease is transmitted to humans by the bite of phlebotomine sandflies and *Lutzomyia longipalpis* is one of the major transmitting agents. In Latin America, 90% of VL cases occur in Brazil, where the disease is found in 19 states and affects approximately 1600 cities^[1,2]. The main reservoirs in wild and household environments are foxes and dogs, respectively^[1,2]. The disease affects the phagocytic mononuclear system, leading to disturbances of cellular and humoral immunity^[3,4]. The city of Bauru, 325 km from the state capital, is considered an endemic area for VL^[5]. The disease affects individuals at any age and children under 10 years are commonly involved in endemic areas^[6,7]. In contrast, VL is rare among pregnant women and there are few studies in these patients. The most common manifestations are fever, hepatosplenomegaly, diarrhea, epistaxis, jaundice, anemia, leucopenia, thrombocytopenia, hypoalbuminemia and hyperglobulinemia^[1]. Diagnostic suspicion is based on epidemiological, clinical and laboratory data. In endemic regions, the diagnosis is confirmed by detecting the parasite in infected tissue and the first recommended option is bone marrow aspiration (BMA) by sternal puncture. Given that gestational VL is rare in South America, severe liver dysfunction associated with this diagnosis is uncommon^[1]. Herein, we report two VL cases in pregnant women from Bauru, Sao Paulo state, Brazil, the first of which developed fulminant hepatic failure. In both cases, BMA was negative and liver biopsy was necessary to make the diagnosis.

CASE REPORT

Case 1

A 26 year old pregnant woman had a preterm vaginal delivery after 5 d of daily fever associated with hypogastric abdominal pain and foul lochia. She was initially treated with cephalothin and later with ampicillin, amikacin and metronidazole, prescribed due to suspected puerperal infection. She developed jaundice, choluria, hepatosplenomegaly and increased liver enzymes (Table 1). Imipenem and vancomycin were then introduced. Abdominal ultrasonography (US) and computed tomography showed no alterations in the liver or biliary tract. Transvaginal ultrasonography also showed no alterations. Serology for viral hepatitis, autoantibodies, ceruloplasmin, iron profiles, blood cultures and urocultures were negative. The patient had pancytopenia which was investigated by BMA but no specific alterations were found. Due to progressive liver failure, percutaneous liver biopsy

was performed and the liver histology showed acute hepatitis, with intense mixed portal inflammation and structures compatible with amastigotes (Figure 1). The patient was treated immediately with liposomal amphotericin B, at a total dose of 20 mg/kg body weight, for 5 d. At that time, anemia, thrombocytopenia and abdominal pain worsened. Subsequent US identified a clot retained in the abdomen which was removed by exploratory laparotomy. Hemotherapeutic support was then initiated, including concentrations of red blood cells and platelets, as well as fresh plasma, cryoprecipitates and neutrophil colony-stimulating factors. The patient became unconscious, with increased serum ammonia and bilirubin levels concomitant with a progressive reduction in aminotransferases, thus characterizing hepatic failure due to fulminant hepatitis. Despite all efforts, she died on the 32nd puerperal day.

Case 2

A 31 year old woman in the 12th week of pregnancy arrived at the hospital with epigastric and right flank pain, progressive jaundice and choluria that had persisted for the previous 12 d. Physical examination showed jaundice without visceromegaly or fever. Laboratory tests suggested cholestasis and hepatocellular lesions. Serology for viral hepatitis was negative and no signs of biliary obstruction were found at abdominal US. BMA was performed but no specific findings were observed. Percutaneous liver biopsy was indicated and the histological analysis showed acute hepatitis and the presence of amastigotes, compatible with VL. Liposomal amphotericin B infusions were immediately initiated (at the 12nd gestational week) and this time the treatment was followed by clinical and laboratory improvement (Table 2).

DISCUSSION

There are no estimates of VL in pregnant women, particularly due to the small number of publications on such cases. In South America, VL in pregnant women is considered to be rare and the first Brazilian case was reported in 1993^[8,9]. In case 1, clinical, laboratory and epidemiological data led to suspicion of VL because the patient had been residing in an endemic city (Bauru) and presented with associated symptoms of daily fever and acute hepatitis. Despite the possibility of trans-infectious hepatitis, the major causes of acute hepatitis were investigated, including viral diseases (hepatitis A, B and C, herpes simplex, cytomegalovirus, varicella, dengue fever, Epstein-Barr), which represent 40% of jaundice causes in pregnancy. These viral diseases do not usually affect the natural course of VL, except for hepatitis E and herpes simplex virus (HSV), which may lead to acute liver failure and fetal loss. Although HSV is considered to be a rare hepatitis agent, it can cause severe hepatitis in immunosuppressed individuals, neonates, pregnant women and transplant patients, with a mortality rate of up to 50% or even 60%^[10,11]. The patient showed no

Table 1 Biochemical test profile, blood and coagulation tests of the 1st case

	Post-partum (d)				Normal range
	19	24	29	32	
Biochemical test profile					
GGT (U/L)	577	289	47	58	15-73
Alkaline Phosphatase (U/L)	1081	924	121	103	36-126
Aspartate transaminase (U/L)	338	3879	1212	429	30-110
Alanine transferase (U/L)	94	727	279	110	21-75
Albumin (g/dL)	2.3	2	1	1.5	3.5-5
INR	1.34	2.55	2.51	1.68	< 1.25
Total bilirubin (mg/dL)	13	15	10	16	0.2-1.3
Indirect bilirubin (mg/dL)	3	4	2	2	0-1.1
Direct bilirubin (mg/dL)	10	11	8	14	0-0.3
Blood and coagulation tests					
Platelets ($\times 10^3/\text{mm}^3$)	155	132	55	22	140-440
Leukocytes ($\times 10^3/\text{mm}^3$)	7.3	5	2.5	0.9	4-11
Hemoglobin (g/dL)	10	9.7	4.4	10.5	12-16
Factor V (%)	-	70.2	40.7	64.8	50-150
Factor VII (%)	-	43.5	36	35.2	50-150
Fibrinogen (mg/dL)	-	164	90	193	146-380

GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio.

Table 2 Main laboratory tests of the 2nd case during LV treatment

	Gestational week				Normal range
	12	13	14	22	
GGT (U/L)	102	94	83	9	15-73
Alkaline Phosphatase (U/L)	116	106	109	45	36-126
Aspartate transaminase (U/L)	1711	2041	1905	16	30-110
Alanine transferase (U/L)	2198	2237	1809	15	21-75
Albumin (g/dL)	3.3	3.4	3.5	-	3.5-5
INR	1.29	1.51	1.69	1.2	< 1.25
Total bilirubin (mg/dL)	14.5	19.6	24.6	0.6	0.2-1.3
Indirect bilirubin (mg/dL)	1.6	2	2.4	0.4	0-1.1
Direct bilirubin (mg/dL)	10.7	15	16.3	0	0-0.3

GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio. LV treatment was initiated at the 12th gestational week.

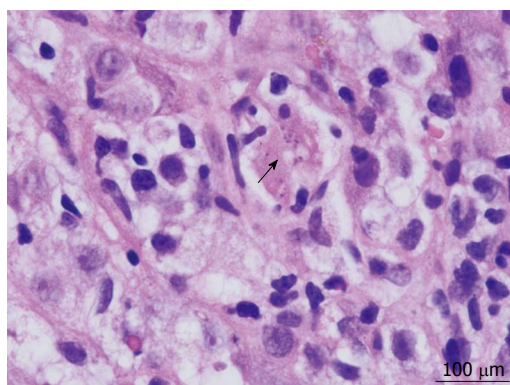


Figure 1 Pathological findings (hematoxylin/eosin staining $\times 1000$ magnification-immersion oil). Liver biopsy. Structures compatible with amastigotes (arrow).

improvement after the use of broad spectrum antibiotics. Therefore, other possible hepatic diseases were

also investigated: autoimmune hepatitis (AIH), Wilson's disease, as well as other lymphoproliferative, metastatic and metabolic diseases. With regards to the liver diseases associated with pregnancy, the most common are viral and drug-induced hepatitis, pre-eclampsia, acute hepatic steatosis in pregnancy (AHSP) and intrahepatic cholestasis in pregnancy (IHCP)^[11-14]. The HELLP Syndrome (Hemolysis, Elevated Liver enzymes and Low Platelets) is a serious complication that most frequently occurs in the 3rd trimester but can also occur in the puerperium in 25% of cases^[15]. However, the first case had no signs of hemolysis or the presence of schizocytes in the peripheral blood. AHSP occurs between the 30th and 40th pregnancy weeks and begins with the slow onset of anorexia, indisposition and cephalgia, followed by vomiting, abdominal pain, fever and, at a later phase, jaundice^[16-18]. In 2% of cases, AHSP causes acute hepatic failure, requiring urgent liver transplantation^[19]. However, patients with AHSP tend to show clinical and laboratory

improvement after delivery, which did not happen in the case described. IHCP manifests in the 3rd gestational trimester with jaundice and pruritus in up to 70% of cases, but liver function deterioration is rare^[12,18,20-22]. AIH affects young patients and due to the conditions of physiological immunosuppression induced by pregnancy, the activity of the disease may be exacerbated after delivery when immunological conditions are reversed. Again, case 1 did not meet the criteria established by the International AIH Study Group^[17] so a diagnosis of AIH was rejected. When VL became the major diagnostic suspicion, other exams were considered. Splenic aspiration is the most sensitive method (90%-95%), followed by BMA (80%-90%), liver biopsy and lymph node aspiration, but BMA is the method recommended due to safety concerns^[23]. When BMA is performed, the absence of leishmania in BMA does not preclude a diagnosis of the disease. For this reason, percutaneous liver biopsy was indicated and confirmed the diagnosis. Liver compromise resulting from VL has been reported in approximately 2% to 28% of cases^[24]. Hepatomegaly may occur in up to 90% of cases, followed by slight increase in liver enzymes without severe disorders^[25]. The presence of the parasites in Kupffer cells may be found in up to 40% of cases before treatment^[26]. Severe cases with a bad prognosis have been associated with severe anemia, fever for longer than 60 d, diarrhea and jaundice^[27]. Fulminant liver failure (FLF), which rarely occurs in VL, has been described more often in children^[1]. According to a previous study by Singh *et al.*^[28] of 155 VL cases with liver compromise, moderate liver dysfunction was found in 16% and FLF in 1.6% of cases. Malatesha *et al.*^[29] reported an isolated case of an immunocompetent adult male with FLF by VL who recovered after therapy with amphotericin B. Unfortunately, our first case developed FLF without any response to liposomal amphotericin B. During the hospitalization, she developed multiple organ dysfunction and her 7 year old daughter was also hospitalized with fever and VL, which was successfully treated. If liver biopsy had not been performed to confirm the maternal disease, the child would not have been diagnosed so early. The rapid diagnosis was critical to the successful treatment of this child. Our second case was an oligosymptomatic (without fever or splenomegaly) pregnant woman. The lack of fever and visceromegaly could have delayed the diagnosis but the positive VL epidemiology was fundamental for the diagnostic suspicion. Once again, BMA was not confirmatory. The progressive increase in transaminases and liver dysfunction were the criteria used to indicate the liver biopsy. The American Association for the Study of Liver Diseases does not consider pregnancy to be a contraindication to this procedure^[30]. Therefore, a diagnosis of VL was confirmed by the liver biopsy, allowing immediate treatment and resulting in the favorable outcome in this second case. Serological tests may be particularly useful for diagnosing VL given their high predictive value in the diagnosis of immunocompetent individuals. However, in severe

cases from endemic areas, they are not sufficient to indicate the specific therapy, because the time required for cured individuals to return to a negative serology (anti-leishmania) is not known. Data suggest that a cellular immune response may still be present in subjects cured of the disease. This would explain the persistence of significant *Leishmania sp.* antibody titers in some subjects after treatment^[31]. Thus, a positive test in the absence of clinical manifestations does not authorize the administration of therapy, which is not free of toxic effects.

VL has epidemiological importance in South America, especially in Brazil. Although the disease is rare among pregnant women and rarely causes severe liver dysfunction, both situations can be present in the same patient, requiring early and accurate diagnosis to reduce morbidity and mortality rates. The contribution of liver biopsy as an alternative to BMA in parasite detection was noteworthy in our cases. We conclude that other patients with VL in whom BMA is negative can obtain a correct diagnosis if liver biopsy is performed, as we showed in our last case.

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