Visceral leishmaniasis – from a case report with retinal haemorrhages

Sónia Carvalho, Sandra Tavares, Manuel Cunha, J. Pereira Pinto, Fernando Guimarães

Abstract

Visceral Leishmaniasis (VL), an infection that occurs worldwide and includes multiple clinical syndromes, is increasingly recognized as an opportunistic infection, associated with immunosuppression conditions, particularly HIV infection. Fever, constitutional symptoms, splenomegaly and pancytopenia are the most characteristic features. Ocular involvement with intra-retinal hemorrhage is very rare.

Diagnosis can be difficult as parasite identification is not always possible, serological tests have suboptimal sensitivity, and molecular biology techniques, like Polymerase Chain Reaction, are often unavailable in clinical practice.

Antimonial drugs have been first choice therapy (except in India), although there is a trend towards Amphotericin in immunocompromised patients.

In this case-report we present a 35-year-old patient, living in an endemic area of the disease, HIV-seronegative, with a longstanding condition of constitutional symptoms, fever, massive splenomegaly and pancytopenia. Attempts to isolate the parasite were of no avail; there was no evidence of another disease, and serological tests results for Leishmania were positive and strongly suggestive of active infection. Just before beginning antimony treatment, the patient mentioned blurred vision and an ophthalmologic examination showed bilateral retinal hemorrhages. Full ocular and clinic laboratorial recovery ensued during the course of treatment.

We report this case due to the unusual ocular lesions in VL and complexity issues concerning the diagnosis. With this paper we also want to remind that in spite of its rarity in developed countries, VL should not be underestimated in a patient with a suggestive clinical condition.

Keywords: visceral Leishmaniasis, Kala-azar, diagnostic difficulties, retinal hemorrhages.

INTRODUCTION

Leishmaniasis is an infection that affects children and adults, encompassing multiple clinical syndromes.[1,2] It is caused by a protozoan of the order *Kinetoplastida,* of the family *Trypanosomatidae,* and of the genus *Leishmania.*[2] Around twenty species have been identified.[3]

Identification of the parasite was first published in London, in May, 1903, by Leishman, who discovered it in the spleen of an Englishman who died in India. Around the same time, Donovan independently described a protozoan in splenic aspirates of a young man, which was later designated *Leishmania donovani*[4] and is responsible for a potentially fatal disease, visceral leishmaniasis (VL).

Internal Medicine Service of the Centro Hospitalar de Trás-os-Montes e Alto Douro - Hospital de Vila Real.

Received for publication on the 26th February 2010
Accepted for publication on the 8th November 2011

Leishmania is an obligate intracellular parasite that infects cells of the reticuloendothelial system and causes a wide range of diseases in humans.[2,5] Transmission to humans occurs via the bite of a vector (the female mosquito of the genus *Lutzomyia* on the American continent or *Phlebotomus* in other geographic regions), with inoculation and regurgitation of metacyclic forms of the parasite. These flagellate promastigotes bind to the macrophage receptors, are phagocytated, and differentiate into non-flagellated intracellular amastigotes.[2] Other rarer means of transmission include parenteral, vertical, and sexual forms of transmission.[3]

In Asia and East Africa, infection by *L. donovani* and *L. tropica* occurs mainly through anthroponotic transmission, by infected humans who constitute the infection host, whereas in the Mediterranean region (*L. infantum*) and in America (*L. chagasi*) the traditional transmission cycle is zoonotic, with dogs and other animals being the source of transmission.[1] However, some zoonotic transmission cycles may be partially anthroponotic or vice-versa, depending upon the environmental context and the epidemiological changes that occur.[5]

Human leishmaniasis is an endemic or emerging

infection in about ninety countries on all continents, with the exception of Australia and Antarctica. The most affected regions are Asia, the Middle East, Southern Europe, and Africa (in the "Old World") and the American continent (in the "New World"), particularly Latin America. There are an estimated twelve million cases worldwide, with one and a half to two million new cases of cutaneous leishmaniasis and five hundred thousand new cases of visceral leishmaniasis (VL) each year.[6] Approximately ninety percent of cases of VL occur in three regions: the Indian subcontinent (India, Bangladesh, and Nepal), Sudan, and Brazil.[2] Geographical distribution of the disease has expanded as a result of increasing urbanization of rural areas (ex. Northeast region of Brazil) and mass migration of populations (Indian subcontinent).[6]

VL or *Kala-azar* usually affects immunocompromised individuals in endemic areas. However, since 1980 it has been increasingly recognized as an opportunistic infection associated with states of immunosuppression, particularly infection by the Human Immunodeficiency Virus (HIV), especially type I.[2] Leishmania/HIV co-infection presents specific epidemiological, clinical, diagnostic, and therapeutic characteristics. Visceral leishmaniasis is the clinical form most often associated with HIV/AIDS infection, especially in the countries of Southern Europe, the region with the majority of reported cases of co-infection.[6] According to the World Health Organization, 1,911 cases were registered in four Southern European countries (Spain, Italy, France, and Portugal) in 2001.[6] It is estimated that between twenty-five and seventy percent of cases in the Mediterranean region occur in adults co-infected with HIV and one and a half to nine percent of patients with AIDS suffer recurrences or primary infections by VL.[7]

Leishmania can produce skin, mucocutaneous (*Espundia*), or visceral (*Kala-azar*) disease.[2] Each species of *Leishmania* tends to produce a specific type of disease, so that the clinical manifestations of leishmaniasis vary by geographic location, according to the distribution of each species around the world.

VL is characterized by persistent fever, asthenia, weight loss, splenomegaly, hepatomegaly, anemia, and in children, retarded growth.[1,2] However, VL encompasses a broad spectrum of clinical manifestations and in reality, a large proportion of infections remain asymptomatic or subclinical.[5,8] When treatment of patients with advanced *Kala-azar* is delayed, death ensues. Even after proper treatment, recurrences can occur, which in those co-infected with HIV, occur during the first year following diagnosis in twenty--five to sixty-one percent of cases. [9]

The ocular manifestations of leishmaniasis, which are rarely manifested early on, are uncommon and include chorioretinitis, thrombosis of the central retinal vein, iritis, papillitis, and keratitis.[10] Rare cases of retinal hemorrhages have also been reported in patients with *Kala-azar*, the first in 1924,[11,12] These hemorrhages were attributed to vascular fragility, low platelet count, prolonged prothrombin time, and even anemia.[12,13]

The diagnosis of VL is sometimes complex because the signals and symptoms often mimic those of other tropical or lymphoproliferative diseases.[14] A definitive and final diagnosis requires the demonstration of the presence of parasites in smears from punctures or biopsies of the spleen or bone marrow, which may be positive in ninety-six percent and sixty to eighty percent of cases, respectively,[3] or in a culture from infected fluids or tissues.[5,14]

As discussed below, specific serological diagnostic methods, like ELISA tests, indirect immunoflourescence, or direct agglutination, have heightened sensitivity and specificity in immunocompetent individuals with VL. However, in those co-infected by HIV, the results of various tests are variable, with sensitivity rates lower than sixty in some patients,15 and higher than eighty percent in others.16,17 The use of Polymerase Chain Reaction (PCR) has emerged due to its heightened sensitivity and specificity for the detection of DNA specific to *Leishmania* in infected tissue. [14,17]

Antigenic tests for diagnosis of the disease, particularly for the rK39 antigen, have been used in countries endemic for the disease, demonstrating high sensitivity and specificity. Recently, the possibility of using rapid immunochromographic urinalysis tests based on rK39 has been evaluated, with promising results.[18]

The time-honored treatment regimen for all forms of leishmaniasis, including VL, is still the first line of defense and consists of daily parenteral administration of pentavalent antimonials,[20] except in areas with significant resistance, such as India, where it can exceed thirty percent.[21] Since the early 1990s, new agents have been tested worldwide, as monotherapy or in combination, in multiple clinical studies.[20] Classic Amphotericin B and Pentamidine

are alternative parenteral forms, traditionally considered to be second line treatments. However, Amphotericin B has been used as the treatment of choice in situations where the benefits outweigh the risks, for example when leishmaniasis resistant to antimonial therapy is suspected, and today, it is the option recommended for patients infected by HIV.[22] It may be used in classic or liposomal form, the latter being more commonly used in developed countries, despite its high cost, due to its lower toxicity and its more advantageous dosage regimen. In India, since 1999, several studies have confirmed the efficacy of Miltefosine as an oral treatment for patients with VL with good response rates in an outpatient setting,[23,24] which is of greater relevance in poor areas with high prevalence of this infection, where the focus for eradication is on the treatment of people as the host of the disease, given the absence of a vaccine. Several small studies have shown positive results using Miltefosine in patients co-infected with HIV, in relapses, and as secondary prophylaxis.[16,25]

Other agents studied for clinical use, including ketoconazole, itraconazole, fluconazole, allopurinol, dapsone, paramomycin and IFN-γ, have not produced enough evidence to warrant their recommendation in clinical practice. [3]

TABLE I

	08/01/2008	14/01/2008	18/01/2008	21/01/2008
Hemoglobin (g/dL)	7,8	7,7	7,5	8,6
Leukocytes/µL	1.700	1.700	1.900	2.700
Neutrophils (%)	34.5	38.2	32.1	54
Erythrocyte Sedimentation Rate (mm)	118	128	—	—
Platelets/ µL	88.000	97.000	123.000	154.000
INR	1,39	—	—	—
Glucose (mg/dL)	106	131	99	103
Creatinine (mg/dL)	0,6	0,7	0,6	0,7
Urea (mg/dL)	27	22	15	30
Sodium (mmol/L)	126	129	136	134
Potassium (mmol/L)	3,9	3,8	4,4	4,1
Total proteins (g/dL)	7,5	8,4	7,2	8,3
Albumin (g/dL)	2,2	2,4	2,0	2,5
ALT (U/L)	20	26	35	44
AST (U/L)	27	45	43	38
Alkaline phosphatase (IU/L)	47	51	56	72
GGT (IU/L)	24	31	36	79
Total bilirubin (mg/dL)	0,4	0,6	0,4	0,5
Direct bilirubin (mg/dL)	0,1	0,1	0,1	0,1
LDH (IU/L)	532	647	483	486
Ferritin (ng/ml)	1.771,7	> 2.000	—	—
CRP (mg/dL)	15,7	—	—	—

CASE REPORT

A male patient, thirty-five years of age, born and residing in Carrazeda de Ansiães, Mirandela, began experiencing anorexia, progressive weight loss, and asthenia in September, 2007, which he attributed to excessive work. At the beginning of December, predominantly nocturnal fever, diaphoresis, and chills were added to these complaints. The fever yielded to antipyretics and was absent during the day. He was treated with unspecified antibiotics at his local Health Center, with no improvement. After several emergency consultations, a hemogram was performed which revealed pancytopenia, for which he was admitted to the Hospital de Mirandela on the 21st December. During this hospitalization, his fever became more persistent, at any time of day, and he lost fifteen kilograms. Laboratory tests confirmed anemia (microcytic hypochromic, with iron kinetics suggestive of a pattern of inflammation), leukopenia, thrombocytopenia, and elevated sedimentation rate. A Computed Tomography scan showed homogeneous splenomegaly. Serology for *Brucella* and *Rickettsiae,*

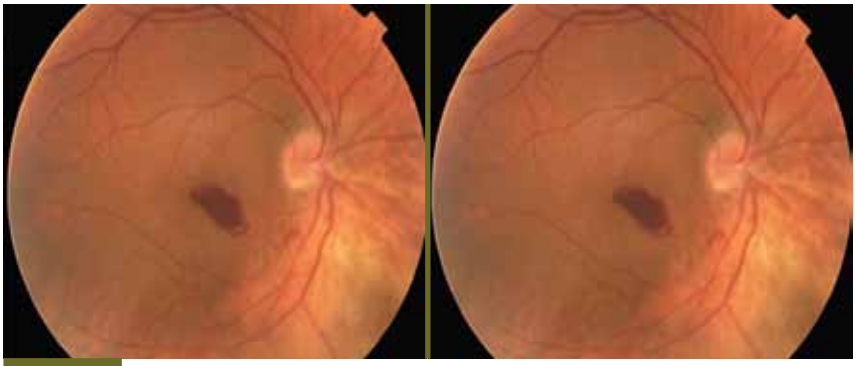

FIG. 1

and both Widal and Paul-Bunnell reactions were negative, as were all blood cultures performed. Given the persistence of the symptoms and the difficulties in diagnosis, the patient was transferred to the Hospital de Vila Real - Centro Hospitalar de Trás-os-Montes e Alto Douro on the 14th January 2008, and admitted to the Service of Medicine.

The patient's personal history highlighted bouts of childhood bronchiolitis and a case of bacterial meningitis as a complication of acute otitis media in 2003. During that hospitalization, he underwent surgery of the right ear, sustaining unilateral hypoacusis. The patient was a heavy smoker, but did not drink heavily or have engaged in risky behaviors, such as extramarital relations or illegal drug use. He had traveled to Spain and Luxembourg a few years earlier. The patient had always been a self-employed carpenter and worked with all types of wood and paint thinners. He had had no contact with animals and had not consumed any unpasteurized milk or milk derivatives. The family history revealed nothing of interest.

During initial observation, the patient was calm, alert, pale, and quite thin. His axillary temperature was 38ºC, blood pressure 100/60 mmHg, pulse 80 bpm, and respiratory rate 14 breaths/min. The oral cavity and the pharynx were normal, without ulcers or candidiasis. Cardiopulmonary auscultation was normal. The abdomen was soft and depressible, painless on palpitation, with enlarged spleen, with palpable border seven centimeters below the costal margin. There were no limb changes. There were no rashes, adenomegalies, cellulitis, or arthritis. The neurological exam was normal.

The laboratory exams performed on admission are shown in Table I. Other tests were ordered, including the Mantoux test, serology for cytomegaloviruses, Epstein-Barr, Human Immunodeficiency Virus (HIV), and Hepatitis B and C, the results of which were all negative, even for *Leishmania*. Additional complementary blood cultures were performed for aerobic and anaerobic bacteria, *Brucella*, and *Mycobacterium*. Thick and thin peripheral blood smears were negative for microorganisms and parasites. Aspiration of marrow from the sternum and a biopsy of the iliac crest were performed. In addition to a morphological study, stainings were performed in search of microorganisms, parasites, and myelocultures. There was no evidence of either neoplastic or granulomatous infiltration, or of hemophagocytosis. All cultures requested were persistently negative.

Analytically, the pancytopenia persisted, requiring red blood cell transfusions. Hypoalbuminemia and a very high ferritin level (> 2000 ng/mL) were also noted. The auto-immunity screening was normal. Electrophoresis of serum proteins showed IgG hypergamaglobulinemia, without monoclonal chains in subsequent study by immunoelectrophoresis. The analytic evolution is reflected in *Table I*.

A transthoracic echocardiogram showed no vegetations. Abdominal magnetic resonance revealed accentuated splenomegaly with sagittal diameter of 27 mm and structure suggestive of changes in vascularization, without appearance of splenic infarction or lymph nodes suggestive of lymphoma or tuberculosis, mild to moderate hepatomegaly, and a small amount of ascitic fluid. There were no retroperitoneal adenopathies. Given the clinical suspicion of leishmaniasis, we performed another bone marrow aspiration, once again negative for the parasite.

The patient continued to have spikes of fever of between 38ºC and 39ºC with no circadian rhythm, asthenia, anorexia, and weight loss. On the fifteenth day of hospitalization, when we were faced with the dilemma of deciding between the need for a diagnostic splenectomy or beginning treatment for VL, we obtained serological results that were for *Leishmania* and strongly suggestive of ongoing infection. Indirect immunoflourescence (IFA) and contraimmunoelectrophoresis (CIE), as well as immunoenzymatic reaction tests (ELISA and immunoblotting) were performed. With a diagnosis of visceral leishmaniasis/

TABLE II

	23/01/08	31/01/08	↓Treatment onset 05/03/08	07/02/08	11/02/08
Hemoglobin (g/dL)	8,8	9,6	9,9	10,2	11,5
Leukocytes/µL	2.900	4.900	8.700	11.000	10.200
Neutrophils (%)	51.8	65.3	64.4	66.7	67.7
Erythrocyte sedimentation rate (mm)	—	—	97	—	—
Platelets/ µL	155.000	150.000	260.000	265.000	307.000
INR	—	—	—	—	—
Glucose (mg/dL)	108	113	148	113	93
Creatinine (mg/dL)	0,6	0,7	0,6	0,5	0,6
Urea (mg/dL)	34	43	40	41	39
Sodium (mmol/L)	133	136	135	135	131
Potassium (mmol/L)	4,5	4,0	3,4	4,0	4,2
Total proteins (g/dL)	8,6	9,8	9,1	8,3	8,4
Albumin (g/dL)	2,5	2,6	2,9	2,7	2,9
ALT (U/L)	35	29	146	142	152
AST (U/L)	35	26	92	52	62
Alkaline phosphatase (IU/L)	72	83	89	79	104
GGT (IU/L)	46	37	60	47	54
Total bilirubin (mg/dL)	0,5	0,4	0,4	0,5	0,4
Direct bilirubin (mg/dL)	0,1	0,0	0,1	0,0	0,1
LDH (IU/L)	503	—	388	351	415
Ferritin (ng/ml)	—	—	—	—	—
CRP (mg/dL)	6,0	—	1,4	—	—

Kala-azar, confirmed by the clinical and laboratorial profile, and a lack of evidence of other disease, specifically in the hematologic arena, intravenous treatment with meglumine antimoniate ("Glucantime") was immediately started.

That same morning, the patient began to report blurred vision and decreased visual acuity, especially in the right eye. He had no pain or redness in his eyes. Emergency observation by Ophthalmology showed bilateral, retinal hemorrhages, prefovial on the right side *(Fig. 1)*. They prescribed oral corticotherapy because, in their experience, bilateral hemorrhages suggest a systemic process, like vasculitis, and over the next few days his vision improved.

Within forty-eight hours the fever broke, with simultaneous improvement of accompanying symptoms. Apyrexia and hemodynamic stability were maintained for the rest of the hospitalization, with total recovery from pancytopenia (*Table II*). There was a slight elevation of liver enzymes, possibly a side effect of the antimoniate. There were no electrocardiographic changes. The patient was discharged on the thirty-first day of hospitalization, completing the proposed treatment, administered intramuscularly, for twenty-eight days.

In a follow-up consultation with Internal Medicine three months after discharge, he was asymptomatic, with no signs of hepatosplenomegaly in a physical exam, and had regained the weight lost. In a reevaluation months later at the Ophthalmology Outpatient Clinic, he had no further complaints and the ocular fundi displayed no hemorrhaging.

DISCUSSION

Our country has geographically distributed areas of leishmaniasis that can be considered endemic: the metropolitan area of Lisbon, the Setúbal peninsula, the county of Alijó (Alto Douro), and the Algarve.[16] Our patient lived in the area of the Tua River basin, adjacent to the county of Alijó, offering a plausible epidemiological context for contracting leishmaniasis.

The patient presented the classical manifestations of VL (prolonged fever, constitutional symptoms, weight loss, and hepatosplenomegaly) and typical laboratory alterations, such as pancytopenia, hypergamaglobulinemia, and hypoalbuminemia.

As mentioned above, retinal hemorrhages are rare

manifestations of the disease. Still, their discovery should alert to the possibility of VL in a patient with systemic complaints in areas where *Leishmania* is endemic.[12] In our case, they were the only hemorrhagic manifestations of the disease and the only ocular finding, with complete regression during the course of treatment. It would be difficult to correlate these hemorrhages with platelet count, which was never lower than 88,000/mm^3.

The direct demonstration of amastigotes is sometimes difficult, especially in the early stage of the disease or in late phases of treatment, when the presence of parasites is low.[14] In turn, cultures are time-consuming methods and not always available in most medical centers.[14] In our case, while clinically suggestive, repeated attempts failed to isolate the parasite in the bone marrow and the diagnosis was confirmed by serological tests. The sensitivity of serological testing in immunocompetent patients is high, and higher than in patients infected with HIV (>90% vs. around 50%),[5] and varies depending upon the method used. However, in one published study, sensitivity and specificity of serological tests with HIV infection were between eighty and one hundred percent, respectively.[17] Seropositivity does not always imply the presence of parasites and active disease, since patients can continue to present positive titers for long periods following cure. Given the low sensitivity of serological tests, mainly in immunocompromised patients, associated with persistent seropositivity for extended periods, it is advantageous to include another method of complementary diagnosis.[14,17]

The detection of DNA specific to *Leishmania* by PCR, using DNA extracted from clinical samples, does not have the limitations mentioned in relation to blood testing. This procedure offers heightened sensitivity and specificity, allowing the rapid direct demonstration of active infection.[14] It was not performed in our case.

Antimonial therapy is normally safe. However, besides the usual minor side effects, there can be more severe ones, depending upon the dose, like cytopenia and electrocardiographical alterations, of which the prolongation of the QT interval and ventricular arrhythmia are the once that give most concern. There have also been reports of cases of renal failure, proteinuria, elevated pancreatic and liver enzymes, and occasional deaths from acute pancreatitis and cardiotoxicity.[3] In addition to clinical and laboratory monitoring, an ECG is recommended at the onset and then weekly during the course of treatment. [26] The only side effect experienced by our patient was a slight elevation of liver transaminases.

Generally, apyrexia occurs after a week of treatment, but the resolution of splenomegaly and hematological abnormalities may take weeks or months. [5] Follow-up parasitological tests are justified for any suspicion of recurrence. Secondary prophylaxis is recommended for patients immunocompromised by HIV infection, but there is no consensus regarding the optimum regimen.

CONCLUSION

We decided to report this case because of its rarity, due to the appearance of bilateral retinal hemorrhages. The difficulty of diagnosis due to the persistent failure to isolate parasites in the bone marrow also merits attention. During the course of treatment, the ocular manifestations of the disease were resolved, as is also described in the literature.

In conclusion, let us remember that HIV infection may increase the number of cases of the disease, extending the distribution of the disease outside of the restricted regions of vectors, partially modifying the traditional model of zoonotic/anthroponotic transmission.[16] For this reason, despite the rarity of the disease in our country, a diagnosis of VL should not be overlooked, particularly in patients infected by HIV. ■

References

1. Murray HW. Kala-Azar - Progress against a neglected disease. N Engl J Med 2002; 347: 1793-1794.

2. Jeronimo S, Sousa A, Pearson R. Leishmania Species: Visceral (Kala-azar), Cutaneous and Mucocutaneous Leishmaniasis. In Mandell, Douglas and Bennett´s Principles of Infectious Diseases, 6th Ed. Philadelphia. Churchill Livingstone 2005; 119: 3145-3156.

3. Leder K, Weller P. Epidemiology and clinical manifestations of leishmaniasis; Diagnosis of leishmaniasis; Treatment and prevention of leishmaniasis. Uptodate 2008.

4. Choi CM, Lerner EA. Leishmaniasis as an Emerging Infection. JID Symposium Proceedings 2001; 6: 175-182.

5. Herwaldt L. Barbara; Leismaniasis; Fauci, Braunwald, Kasper, Hauser, Longo, Jameson, Loscalzo (Eds.); In Harrison`s Principles of Internal Medicine 17th edition, p1296-1300.

6. Desjeux P, Alvar J. Leishmania/HIV co-infections: epidemiology in Europe. Ann Trop Med Parasitol 2003; 97 (suppl 1): S3-S15.

7. Paredes R, Munoz J, Diaz I et al. Leishmaniasis in HIV infection. J Postgraduate Med 2003; 49: 39-49.

8. Guerin PJ, Olliaro P, Sundar S et al. Visceral leishmaniasis: current status of control, diagnosis, and treatment and a proposed research and development agenda. Lancet Infect Dis 2002; 2: 494-501.

9. Pintado V, Martín-Rabadán P, Rivera ML et al. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected patients. Medicine 2001; 80: 54-73.

10. Klotz SA, Penn CC, Negvesky GJ et al. Fungal and Parasitic Infections of the Eye. Clinical Microbiology Reviews 2000;13: 662–685.

11. De Cock KM, Rees PH, Klauss V et al. Retinal hemorrhages in Kala Azar. Am J Trop Med Hyg 1982; 31(5): 927-930.

12. Montero JA, Ruiz-Moreno JM, Sanchis E. Intraretinal hemorrhage associated with leishmaniasis. Ophthalmic Surg Lasers Imaging 2003; 34(3); 212-214.

13. Abboud IA, Ragab HAA, Hanna LS. Experimental ocular leishmaniasis. Brit J Ophthal 1970);54: 256-262.

14. Pal S, Aggarwal G, Haldar A et al. Diagnosis of symptomatic kala-azar by polymerase chain reaction using patient`s blood. Med Sci Monit 2004; 10(1): MIT 1-5.

15. Medrano FJ, Canavate C, Leal M et al. The role of serology in the diagnosis and prognosis of visceral leishmaniasis in patients coinfected with human immunodeficiency virus type-1. Am J Trop Med Hyg 1998; 59(1): 155-162.

16. Marques N, Cabral S, Sá R, et al. Leishmaniose visceral e infecção por vírus da imunodeficiência humana, na era da terapêutica anti-retrovírica de alta eficácia. Acta Med Port 2007; 20: 291-298.

17. Piarroux R, Gambarelli F, Dumon H et al. Comparison of PCR with direct examination of bone marrow aspiration, myeloculture, and serology for diagnosis of visceral leishmaniasis. J Clin Microbiol 1994; 32(3): 746-749.

18. Braz RF, Nascimento et, Martins DR et al: The sensitivity and specificity of Leishmania chagasi recombinant K39 antigen in the diagnosis of American visceral leishmaniasis and in differentiating active from subclinical infection. Am J Trop Med Hyg 2002;67:344-348

19. Md Gulam Musawwir Khan, Mohammad Shafiul Alam, Milka P Podder, Makoto Itoh, Kazi M Jamil, Rashidul Haque and Yukiko Wagatsuma: Evaluation of rK-39 strip test using urine for diagnosis of visceral leishmaniasis in an endemic area in Bangladesh. Parasites & Vectors 2010, 3:114.

20. Murray HW, Berman J, Davies C et al. Advances in leishmaniasis. Lancet 2005; 366: 1561-1577.

21. Sundar S, More DK, Singh MK et al. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. Clin Infect Dis 2003; 31: 1104-1106.

22. Chappuis F, Sundar S, Hailu A et al. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol 2007; 5(11): 873-882.

23. Sundar S, Jha TK, Thakur CP et al. Oral miltefosine for Indian visceral leishmaniasis. N Engl J Med 2002; 347: 1739-1746.

24. Bhattacharya SK, Sinha PK, Sundar S et al. Phase 4 trial of miltefosine for the treatment of visceral leishmaniasis. J Infect Dis 2007; 196: 591.

25. Sindermann H, Engel KR, Fisher C et al. Oral miltefosine for leishmaniasis in immunocompromised patients: compassionate use in 39 patients with HIV infection. Clin Infect Dis 2004; 39:1520-1523.

26. Herwaldt BL, Berman JD. Recommendations for Treating Leishmaniasis with Sodium Stibogluconate (Pentostam) and Review of Pertinent Clinical Studies. Am J Trop Med Hyg 1992; 46(3): 296-306.