

## CASE STUDY OF A PATIENT WITH HIV-AIDS AND VISCERAL LEISHMANIASIS CO-INFECTION IN MULTIPLE EPISODES

Elis Dionísio da SILVA(1,2,3), Luiz Dias de ANDRADE(3), Paulo Sérgio Ramos de ARAÚJO(3,4), Vera Magalhães SILVEIRA(4),  
Carlos Eduardo PADILHA(5), Maria Almerice Lopes da SILVA(3) & Zulma Maria de MEDEIROS(3,6)

### SUMMARY

Report of a 45-year-old male farmer, a resident in the forest zone of Pernambuco, who was diagnosed with human immunodeficiency virus (HIV) in 1999 and treated using antiretroviral (ARV) drugs. In 2005, the first episode of visceral leishmaniasis (VL), as assessed by parasitological diagnosis of bone marrow aspirate, was recorded. When admitted to the hospital, the patient presented fever, hepatosplenomegaly, weight loss, and diarrhea. Since then, six additional episodes of VL occurred, with a frequency rate of one per year (2005-2012, except in 2008). In 2011, the patient presented a disseminated skin lesion caused by the amastigotes of *Leishmania*, as identified by histopathological assessment of skin biopsy samples. In 2005, he was treated with N-methyl-glucamine-antimony and amphotericin B deoxycholate. However, since 2006 because of a reported toxicity, the drug of choice was liposomal amphotericin B. As recommended by the Ministry of Health, this report emphasizes the need for HIV patients living in VL endemic areas to include this parasitosis in their follow-up protocol, particularly after the first infection of VL.

**KEYWORDS:** Co-infection; Visceral leishmaniasis; HIV infection; AIDS.

### INTRODUCTION

Cases of visceral leishmaniasis (VL) co-infection with human immunodeficiency virus (HIV) and acquired immune deficiency syndrome AIDS (VL/HIV-AIDS) have been registered in 35 countries, mainly in southwestern Europe. VL/HIV-AIDS co-infections increase in areas where these two diseases coexist, as observed in Asian, African, and Latin American countries. In the latter group, Brazil has the highest number of cases<sup>1</sup>.

In 2011, in Brazil, VL appeared in 22 of the 27 Brazilian states, covering urban and suburban areas. Between 1998 and 2009, the annual average was 3,349 cases<sup>7</sup>. From 1980 to 2011 in Brazil, 608,230 cases of AIDS were reported. This epidemic tends to spread to poorly inhabited macro-regions as well as to medium and small cities<sup>8</sup>. When AIDS and VL databases were correlated, 176 cases of VL/HIV-AIDS co-infection were detected among the Federal States<sup>7</sup>.

Several episodes of VL are frequent in cases of VL/HIV-AIDS co-infection. According to BOURGEOIS *et al.*, 2010, these patients present a novel nosological entity called 'active chronic visceral leishmaniasis'. This condition may be termed 'chronic' because of the presence of relapses over a period of several years and 'active' because of the continuous blood circulation of the parasite. On the other hand,

it's impossible to know if repeated episodes are relapses or reinfections by using conventional parasitological and immunological methods<sup>15</sup>. Some studies show that individuals with HIV/AIDS and infected with VL often present atypical clinical manifestations and high incidence of relapse<sup>5,22,24,25</sup>. Molecular methods confirm that more than 90% of these cases are relapses, rather than reinfections<sup>27</sup>. The discrimination between relapses and reinfection can be made by molecular techniques based on restriction fragment length polymorphism (RFLP) analysis. The use of this technology may provide the physician with more information to determine *Leishmania* infections in patients who do not respond to treatment<sup>20</sup>.

Professionals, who treat patients with HIV/AIDS, report that this co-infection was not prioritized because of the variety of diseases related to immunosuppression, in addition to not being included among the AIDS-defining conditions<sup>11</sup>. To alert healthcare professionals about this association, we describe the case of a patient presenting multiple VL/HIV-AIDS co-infections during the seven years of evolution of this disease.

### CASE REPORT

In 1999, a 31-year-old male farmer, who was a resident in the forest zone of Pernambuco, was admitted to the Clinics Hospital of the Federal University of Pernambuco. At the time of admission, he presented with

(1) Postgraduate Course of Biology applied to Health, Federal University of Pernambuco, PE, Brazil. E-mail: dionisio.elis@gmail.com

(2) Graduate Course on Biological Sciences, Institute of Biological Sciences, University of Pernambuco, PE, Brazil.

(3) Parasitology Department, Aggeu Magalhães Research Center, Oswaldo Cruz Foundation, PE, Brazil. E-mails: dias@cpqam.fiocruz.br, almerice@cpqam.fiocruz.br

(4) Tropical Medicine Department, Clinical Hospital, Federal University of Pernambuco, PE, Brazil. E-mails: psergio@cpqam.fiocruz.br, vemagalhaes@uol.com.br

(5) Service of Infectious and Parasitic Diseases, Clinical Hospital, Federal University of Pernambuco, Brazil. E-mail: cegpadilha@gmail.com

(6) Pathology Department, Institute of Biological Sciences, University of Pernambuco, PE, Brazil. E-mail: medeiros@cpqam.fiocruz.br

**Correspondence to:** Zulma M. Medeiros, Centro de Pesquisas Aggeu Magalhães CPqAM/Fiocruz, Av. Professor Moraes Rego s/n, 50670-420 Cidade Universitária, Recife, PE, Brazil. E-mail: medeiros@cpqam.fiocruz.br

asthenia and headache, and had diarrhea for at least 30 days. He was diagnosed with HIV, and began receiving antiretroviral therapy (ART) with stavudine, lamivudine, and efavirenz. Meanwhile, his 25-year-old partner and 9-month-old daughter were diagnosed with HIV infection.

Five years after initiating ART, the patient presented virological failure; after genotyping, his treatment was changed to tenofovir, lamivudine, and lopinavir/ritonavir. In 2005, he was diagnosed with VL as assessed by directly testing *Leishmania* in the bone marrow aspirate and initially treated with N-methyl-glucamine-antimony. However, because of pancreatitis, the patient began receiving amphotericin B, which was then replaced by a liposomal formulation because of the onset of renal failure.

In 2011, the patient presented disseminated cutaneous lesions caused by *Leishmania*, as assessed by histopathological analysis of skin biopsy samples. In July 2012, the patient was readmitted for presenting febrile disease with splenomegaly and pancytopenia, in addition to showing

positive results for laboratory tests for leishmaniasis (Table 1). After administration of liposomal amphotericin B deoxycholate, the patient's condition improved, and he was discharged upon recommendation of a secondary prophylaxis by administering liposomal amphotericin B twice a week. Between 2005 and 2012, seven VL infections occurred, as shown in the Table 1.

## DISCUSSION

This case describes some of the many clinical, diagnostic, and epidemiologic aspects of VL/HIV-AIDS co-infection. Immunosuppression caused by HIV might lead to the development of symptomatic VL<sup>14</sup>. In turn, VL might promote the clinical progression of HIV and of AIDS-defining conditions, thus, reducing the possibility of recovery after treatment and increasing the incidence of relapse<sup>11</sup>. This report showed that individuals with HIV/AIDS and living in endemic areas of VL should include VL assessment in their follow-up protocol. After the first co-infection, by means of clinical and laboratory support, a follow-up

**Table 1**  
Description of clinical events, additional diagnostics, and treatment for the case of co-infection from Pernambuco, between 1999 and 2012

Period (month/year)	Clinical events	CD4+ T cells (cells/mm <sup>3</sup> )	Viral load (copies/mL)	Laboratory diagnosis	Treatment	Prophylactic treatment
09/99	Positive for HIV	N.A.	N.A.	N.A.	d4T + 3TC + EFV	N.A.
05/04		24	208,000	N.A.	TDF + 3TC + LPVr	N.A.
03/05		154	29,000	N.A.	TDF + 3TC + LPVr	N.A.
06/05	Visceral leishmaniasis (Hepatosplenomegaly/ diarrhea/fever/ cachexia/ pancytopenia)	N.A.	N.A.	B.M. aspirate	TDF + 3TC + LPVr N-methyl-glucamine-antimony <sup>a</sup> , amphotericin B <sup>b</sup> , liposomal amphotericin	N.A.
11/05		58	87,800	N.A.	TDF + 3TC + LPVr	N.A.
02/06	Visceral leishmaniasis (Second infection)	170	<50	B.M. aspirate	TDF + 3TC + LPVr liposomal amphotericin	N-methyl-glucamine-antimony
02/07	Visceral leishmaniasis (Third infection)	72	N.A.	B.M. aspirate	TDF + 3TC + LPVr liposomal amphotericin	N.D
06/08		113	<50	N.A.	TDF + 3TC + LPVr	Amphotericin B
07/09	Visceral leishmaniasis (Fourth infection)	141	<50	B.M. aspirate	TDF + 3TC + LPVr liposomal amphotericin	Amphotericin B
05/10	Visceral leishmaniasis (Fifth infection)	83	<50	B.M. aspirate	TDF + 3TC + LPVr liposomal amphotericin	Amphotericin B
05/11	Skin lesions on the forehead/ right forearm; Visceral leishmaniasis (Sixth infection)	120	<50	Skin biopsy, rK39 rapid test, DAT, latex agglutination test, and PCR	TDF + 3TC + LPVr liposomal amphotericin	Liposomal amphotericin
07/12	Visceral leishmaniasis (Seventh infection) (Splenomegaly/diarrhea/ fever/cachexia/pancytopenia)	114	<50	rK39 rapid test, DAT, latex agglutination test, and PCR	TDF + 3TC + LPVr amphotericin B <sup>b</sup> , liposomal amphotericin	Liposomal amphotericin

HIV, human immunodeficiency virus; N.A., not available; N.D., not done; B.M. aspirate, bone marrow aspirate; d4T, stavudine; 3TC, lamivudine; EFV, efavirenz; TDF, tenofovir; LPVr, lopinavir/ritonavir; DAT, direct agglutination test; PCR, polymerase chain reaction. <sup>a</sup>Developed acute pancreatitis. <sup>b</sup>Developed renal failure.

protocol of the patient should be created for early detection of relapse and re-infection.

One of the common features of co-infection is the increased tendency of relapse, observed in 37-80% of the patients<sup>22</sup>. Additionally, in some cases a chronic course with multiple occurrences might take place. This can be attributed not only to immunodeficiency but also to re-infection, host deficiencies correlating with ART, secondary prophylaxis, and CD4+ lymphocyte count<sup>16,18</sup>. CD4+ lymphocyte count is one of the most significant prognostic factors for survival<sup>11,22</sup>. VL usually appears as an opportunistic disease in HIV patients when CD4+ cell count is less than 200 cells/mm<sup>3</sup> (1,6,12,13,17,25). During the seven years of follow-up, the patient presented a CD4+ cell count  $\leq$  170 cells/mm<sup>3</sup>. This represents an important predictor of relapse. Relapses of VL are suggested to occur mainly in individuals with poor responses to antiretroviral treatment who have no improvement in CD4+ counts with a few exceptions<sup>3,9</sup>.

Based on clinical and biological [polymerase chain reaction (PCR)-based] follow-up, an 'active chronic visceral leishmaniasis'<sup>3,5</sup> has been proposed by BOURGEOIS *et al.* (2010). In our case, only the 6<sup>th</sup> and 7<sup>th</sup> episodes were able to have the peripheral blood (PB) analyzed by PCR, which showed positive results for *Leishmania* spp. As PCR-RFLP was only found in the 7<sup>th</sup> sample episode, the etiological agent is *Leishmania chagasi*, according to the pattern of bands defined by SCHONIAN *et al.* (2003)<sup>26</sup>. Due to the absence of PB samples in previous episodes, the analysis by PCR-RFLP was not made. Therefore, it wasn't possible to distinguish between relapse and reinfection or characterize the case as 'chronic visceral leishmaniasis'. Despite the medical importance of a clinical and laboratory monitoring of coinfecting patients, this practice is still little used<sup>12,19,20</sup>.

ART plays an important role in reducing the effect of opportunistic diseases and in recent studies has shown a reduction in the incidence of VL. Studies in individuals with HIV/AIDS treated using ARV drugs showed a similar incidence of VL relapse when compared to studies of the pre-highly active antiretroviral therapy (HAART) era<sup>11,14</sup>. The increased survival resulting from ART might partially explain the high incidence of relapse observed in this population<sup>18</sup>. In the present study, during the eight years of follow-up, we observed seven VL infections, despite the patient receiving ART before the first infection.

VL manifestations associated with HIV infection might appear in a classical form, particularly in patients from VL-endemic areas, as well as with relatively aggressive symptoms that are sometimes non-specific and difficult to clinically diagnose<sup>11</sup>. In individuals with HIV/AIDS and presenting symptoms such as asthenia, anorexia, and weight loss, VL might be responsible for 7-23% of instances of fever of unknown origin<sup>11</sup>. This patient presented classic clinical manifestations during the study period, although in 2011, we observed the formation of skin lesions because of the parasite, as assessed by histopathological analysis.

Among the previously treated VL cases, several patients present a skin condition characterized by macular, papular, or nodular lesions, called Post-kala-azar dermal Leishmaniasis (PKDL) caused by the amastigotes of *Leishmania donovani* on the Indian subcontinent (India, Nepal, Bangladesh) and east Africa (Sudan, Ethiopia, Kenya) and caused by *Leishmania chagasi* in South America where it is rarely reported, as well as its presence in HIV positives<sup>2,4,23,28</sup>. It is worth noticing that

exclusive involvement of the skin is an unusual condition, because the simultaneous appearance of skin lesions along with other VL manifestations was more frequently observed<sup>21</sup>. In this case, the skin lesion suggests a clinical PKDL, which developed five years after the first VL episodes, administration of multiple therapeutic regimens, and treatments of discontinuous secondary prophylaxis. Although it has been viewed amastigotes in biopsy specimens obtained from skin lesions, the hypothesis of PKDL can be suggested but not stated categorically because there was no characterization of *Leishmania* species involved in the cutaneous lesions, and may have been an infection of some sort cutaneous *Leishmania* endemic to the region as *L. braziliensis*.

Several studies on co-infected individuals show that they present a decrease in anti-*Leishmania* antibody levels in the peripheral blood<sup>11</sup>; that is, in only 40-50% of VL/HIV-AIDS cases, specific antibodies are detected<sup>1</sup>. Conversely, assessment of *Leishmania* antigen in urine by latex agglutination test showed a sensitivity of 85-100%<sup>1</sup>. Polymerase chain reaction (PCR) in peripheral blood and bone marrow is a useful tool to diagnose, for follow-up, and detect relapses<sup>22</sup>. Although the literature shows that serological analyses are not the most convenient in patients presenting co-infection<sup>1,6</sup>, two serological tests (direct agglutination test and rK39-based rapid immunochromatographic test) performed enabled the diagnosis of such cases in 2011 and 2012. In the same years, latex agglutination test and PCR test showed positive results, thus, confirming the data in the literature. Similar to the finding in our study, CAVALCANTI *et al.* (2012), described a series of case studies of co-infection in the main hospitals of Recife, Brazil<sup>10</sup>.

There is currently sufficient evidence suggesting that secondary prophylaxis provides some protective effect but does not completely prevent the occurrence of relapse<sup>11</sup>. A meta-analysis study described that the average incidence of relapse in patients who did not receive secondary prophylaxis was 67%, whereas in those who received it was 31%<sup>16</sup>. Current recommendations from the Ministry of Health of Brazil<sup>2</sup> for the diagnosis, treatment, and follow-up of patients presenting co-infection state that the "efficacy of the secondary prophylaxis after the first successfully treated VL infection, was not completely established." The suggested secondary prophylaxis (Table 1) was poorly adopted, thus, compromising the clinical follow-up. Based on this case study and literature review, it is evident that co-infection presents typical clinical, diagnostic, and therapeutic features, and can be observed in the prognosis of the disease. Therefore, prospective studies are required to clarify gaps such as the efficacy of secondary prophylaxis and need for clinical and laboratory monitoring tools for the early assessment of relapse or re-infection.

## RESUMO

### Estudo de caso de paciente com múltiplos episódios da coinfeção HIV-AIDS e leishmaniose visceral

Relato de caso de paciente masculino de 45 anos, agricultor, residente na zona da mata do Estado de Pernambuco, diagnosticado com HIV em 1999 e em uso de ARV. Em 2005 foi registrada a primeira ocorrência de LV através do diagnóstico parasitológico a partir do aspirado da medula óssea. À admissão no hospital apresentava-se com febre, hepatoesplenomegalia, perda de peso e diarreia. Desde então houve a ocorrência de mais sete episódios de LV, tendo ocorrido em média, um

evento a cada ano (2005-2012 exceto em 2008). O paciente apresentou, em 2011, um quadro cutâneo disseminado, sendo realizada biopsia de pele que evidenciou formas amastigotas de *Leishmania* no exame histopatológico. Em 2005, o tratamento foi realizado com antimoniato de N-metil-glucamina e anfotericina B desoxicolato, mas desde 2006, devido à toxicidade, o medicamento de escolha foi a anfotericina B lipossomal. Como recomendado pelo Ministério da Saúde, esse relato reforça a necessidade de que os casos de HIV residentes em área endêmica de LV deverão ter inserido em seu protocolo de acompanhamento essa parasitose, principalmente após o primeiro episódio.

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### REFERENCES

1. Alvar J, Cañavate C, Gutiérrez-Solar B, Jiménez M, Laguna F, López-Vélez R, *et al.* *Leishmania* and human immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev.* 1997;10:298-319.
2. Badaró R, Carvalho EM, Rocha H, Queiroz AC, Jones TC. *Leishmania donovani*: an opportunistic microbe associated with progressive disease in three immunocompromised patients. *Lancet.* 1986;1(8482):647-8.
3. Berenguer J, Cosin J, Miralles P, López JC, Padilla B. Discontinuation of secondary anti-*Leishmania* prophylaxis in HIV-infected patients who have responded to highly active antiretroviral therapy. *AIDS.* 2000;14:2946-8.
4. Bittencourt A, Silva N, Straatmann A, Nunes VL, Follador I, Badaró R. Post kala-azar dermal leishmaniasis associated with AIDS. *Braz J Infect Dis.* 2002;6:313-6.
5. Bourgeois N, Bastien P, Reynes J, Makinson A, Rouanet I, Lachaud L. 'Active chronic visceral leishmaniasis' in HIV-1-infected patients demonstrated by biological and clinical long-term follow-up of 10 patients. *HIV Med.* 2010;11:670-3.
6. Bourgeois N, Lachaud L, Reynes J, Rouanet I, Mahamat A, Bastien P. Long-term monitoring of visceral leishmaniasis in patients with AIDS: relapse risk factors, value of polymerase chain reaction, and potential impact on secondary prophylaxis. *J Acquir Immune Defic Syndr.* 2008;48:13-9.
7. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Manual de recomendações para diagnóstico, tratamento e acompanhamento de pacientes com a coinfeção *Leishmania*-HIV. Brasília: Ministério da Saúde; 2011.
8. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Aids no Brasil. Epidemia concentrada e estabilizada em populações de maior vulnerabilidade. *Bol Epidemiol AIDS/DST.* 2011;8(1):10-5.
9. Casado JL, Lopez-Velez R, Pintado V, Quereda C, Antela A, Moreno S. Relapsing visceral leishmaniasis in HIV-infected patients undergoing successful protease inhibitor therapy. *Eur J Clin Microbiol Infect Dis.* 2001;20:202-5.
10. Cavalcanti ATA, Medeiros Z, Lopes F, Andrade L, Ferreira VM, Magalhães V, *et al.* Diagnosing visceral leishmaniasis and HIV/AIDS co-infection: a case series study in Pernambuco, Brazil. *Rev Inst Med Trop Sao Paulo.* 2012;54:43-7.
11. Cota GF, Sousa MR, Rabello A. Predictors of visceral leishmaniasis relapse in HIV patients: a systematic review. *PLoS Negl Trop Dis.* 2011;5(6):e1153.
12. Cruz I, Nieto J, Moreno J, Cañavate C, Desjeux P, Alvar J. *Leishmania*/HIV co-infections in the second decade. *Indian J Med Res.* 2006;123:357-88.
13. Daher EF, Fonseca PP, Gerhard ES, Leitão TM, Silva Júnior GB. Clinical and epidemiological features of visceral leishmaniasis and HIV co-infection in fifteen patients from Brazil. *J Parasitol.* 2009;95:652-5.
14. Fernández Cotarelo MJ, Abellán Martínez J, Guerra Vales JM, Martínez Sánchez P, Rodrigo Gómez De La Bárcena M, Salto Fernández E. Effect of highly active antiretroviral therapy on the incidence and clinical manifestations of visceral leishmaniasis in human immunodeficiency virus-infected patients. *Clin Infect Dis.* 2003;37:973-7.
15. Hailu A. Pre- and post-treatment antibody levels in visceral leishmaniasis. *Trans R Soc Trop Med Hyg.* 1990;84:673-5.
16. Kubar J, Marty P, Lelièvre A, Quaranta JF, Staccini P, Caroli-Bosc C, *et al.* Visceral leishmaniasis in HIV-positive patients: primary infection, reactivation and latent infection. Impact of the CD4+ T-lymphocyte counts. *AIDS.* 1998;12:2147-53.
17. Lopez-Velez R, Perez-Molina JA, Guerrero A, Baquero F, Villarrubia J, Escribano L, *et al.* Clinicoepidemiologic characteristics, prognostic factors, and survival analysis of patients coinfecting with human immunodeficiency virus and *Leishmania* in an area of Madrid, Spain. *Am J Trop Med Hyg.* 1998;58:436-43.
18. Maia-Elkhoury AN, Alves WA, Sousa-Gomes ML, Sena JM, Luna EA. Visceral leishmaniasis in Brazil: trends and challenges. *Cad Saúde Pública.* 2008;24:2941-7.
19. Marfurt J, Niederwieser I, Makia ND, Beck HP, Felger I. Diagnostic genotyping of Old and New World *Leishmania* species by PCR- RFLP. *Diagn Microbiol Infect Dis.* 2003;46:115-24.
20. Morales MA, Cruz I, Rubio JM, Chicharro C, Cañavate C, Laguna F, *et al.* Relapses versus reinfections in patients coinfecting with *Leishmania infantum* and human immunodeficiency virus type 1. *J Infect Dis.* 2002;185:1533-7.
21. Orsini M, Silva M, Luz ZM, Disch J, Fernandes O, Moreira D, *et al.* Identification of *Leishmania chagasi* from skin in *Leishmania*/HIV co-infection: a case report. *Rev Soc Bras Med Trop.* 2002;35:259-62.
22. Pintado V, Martín-Rabadán P, Rivera ML, Moreno S, Bouza E. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected and non-HIV infected patients. *Medicine (Baltimore).* 2001;80:54-73.
23. Prata A, Domingues A. Leishmaniíode dérmico. *O Hospital.* 1955;50:93-113.
24. Rosenthal E, Marty P, del Giudice P, Pradier C, Ceppi C, Gastaut JA, *et al.* HIV and *Leishmania* coinfection: a review of 91 cases with focus on atypical locations of *Leishmania*. *Clin Infect Dis.* 2000;31:1093-5.
25. Russo R, Laguna F, López-Vélez R, Medrano FJ, Rosenthal E, Cacopardo B, *et al.* Visceral leishmaniasis in those infected with HIV: clinical aspects and other opportunistic infections. *Ann Trop Med Parasitol.* 2003;97(Suppl 1):S99-S105.
26. Schonian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HD, Presber W, *et al.* PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. *Diagn Microbiol Infect Dis.* 2003;47:349-58.
27. World Health Organization. 5<sup>th</sup> Consultative Meeting on *Leishmania*/HIV Coinfection; 2007 Mar 20-22; Addis Ababa, ET. Geneva: World Health Organization; 2007.
28. Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM. Post kala-azar dermal leishmaniasis. *Lancet Infect Dis.* 2003;3:87-98.

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