

Human immunodeficiency virus/*Leishmania infantum* in the first foci of urban American visceral leishmaniasis: clinical presentation from 1994 to 2010

Íuri Paz Lima^{[1],[2]}, Marina Costa Müller^{[1],[2]}, Thiago Ayres Holanda^[2], Michael Harhay^[2], Carlos Henrique Nery Costa^{[1],[2]} and Dorcas Lamounier Costa^{[1],[2],[3]}

[1]. Departamento de Medicina Comunitária, Universidade Federal do Piauí, Teresina, PI. [2]. Laboratório de Leishmaniose, Instituto de Doenças Tropicais Natan Portella, Teresina, PI. [3]. Departamento Materno Infantil, Universidade Federal do Piauí, Teresina, PI.

ABSTRACT

Introduction: Human immunodeficiency virus (HIV) coinfection with *Leishmania infantum* or *Leishmania donovani*, the agents of visceral leishmaniasis (or kala-azar), has become a fatal public health problem in the tropics where kala-azar is endemic. **Methods:** The clinical presentation of patients with HIV and *L. infantum* coinfection is described using two unique databases that together produce the largest case series of patients with kala-azar infected with HIV in South America. First, a retrospective study paired the list of all patients with kala-azar from 1994 to 2004 with another of all patients with HIV/AIDS from the reference hospital for both diseases in the City of Teresina, State of Piauí, Brazil. Beginning in 2005 through to 2010 this information was prospectively collected at the moment of hospitalization. **Results:** During the study, 256 admissions related to 224 patients with HIV/*L. infantum* coinfection were registered and most of them were males between 20-40 years of age. Most of the 224 patients were males between 20-40 years of age. HIV contraction was principally sexual. The most common symptoms and signs were pallor, fever, asthenia and hepatosplenomegaly. 16.8% of the cohort died. The primary risk factors associated to death were kidney or respiratory failure, somnolence, hemorrhagic manifestations and a syndrome of systemic inflammation. The diagnosis of HIV and kala-azar was made simultaneously in 124 patients. **Conclusions:** The urban association between HIV and kala-azar coinfection in South America is worrisome due to difficulty in establishing the diagnosis and higher mortality among the coinfecting than those with either disease independently. HIV/*L. infantum* coinfection exhibits some singular characteristics and due to its higher mortality it requires immediate assistance to patients and greater research on appropriate combination therapy.

Keywords: HIV. AIDS. Kala-azar. *Leishmania infantum*. Visceral leishmaniasis.

INTRODUCTION

About 350 million people are at risk of contracting leishmaniasis, one of the most neglected tropical diseases. Presently 21 states in Brazil annually report approximately 3,500 new cases of the disease¹. The human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) represents one of the biggest health problems globally. Since 1980, when the first patient was identified in Brazil more than 590 thousand patients have been diagnosed by the most recent count in December 2010³. Presently, Brazil accounts for a third of the diagnosed HIV patients in South America⁴. In Piauí, one of the country's poorest states, 3,500 patients have been identified. Men who have sex with men and bisexual patients historically represented most of the patients, aside intravenous drug users, both of which tended to cluster

in larger urban settings and major cities⁵. However, presently, most of the transmission of HIV is heterosexual, which followed interiorization and pauperization of the epidemic, progressing towards the poorer Northeast and North Regions⁶.

The pandemic of HIV/AIDS began in 1980, coinciding with the start of the urbanization of kala-azar in Brazil⁷. Since then, a similar association of both diseases has been described in several regions of the world^{8,9}. The urbanization of kala-azar in Brazil started in Teresina, northeast Brazil in 1981 and since 1994 the occurrence in HIV-infected patients has been registered. Teresina has the most documented cases of the association of kala-azar and HIV in Brazil¹⁰. This manuscript aims to describe the coinfection HIV/*L. infantum* in order to highlight the importance of the problem and to discuss the clinical characteristics and the identification of new patients with the combination of the infections.

METHODS

The study was based on the information collected in the Institute of Tropical Diseases Natan Portella, which is the only reference hospital for kala-azar and HIV/AIDS in Teresina,

Address to: Dr. Carlos Henrique Nery Costa. Laboratório de Leishmanioses/IDTNP. Rua Artur de Vasconcelos 151-Sul, 64001-450 Teresina, PI, Brasil.
Phone: 55 86 3222-4377; **Fax:** 55 86 3221-3062
e-mail: chncosta@gmail.com
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the capital of the State of Piauí. Due to the location of Teresina, many patients also live in the neighbor State of Maranhão, and others. The first coinfection was diagnosed in 1994. Therefore, 1994 was the first year of a retrospective study up to 2004, when patients were thereafter identified prospectively. The list of all patients with kala-azar was both electronically and manually paired with the list of all patients with HIV, both from the hospital list as well as the state health department. From 2005 to 2010 data was prospectively collected at hospital admission; patients admitted with kala-azar were also tested for HIV. Beginning in 2009, patients that tested positive for HIV in the public surveillance were also screened for *Leishmania* with serology. Only patients with a positive parasitological or reactive serology were included.

The diagnosis of kala-azar was based on the presence of the typical symptoms of fever, anemia, and hepatosplenomegaly (spleen and liver palpable) in addition to a positive serological or parasitological test. A standard data collection form was populated for all patients that included demographic, epidemiologic, clinical, laboratorial and therapeutic data that was analyzed with the statistical software STATA (College Station, TX, USA). A forward stepwise logistic regression analysis for the identification of independent predictors of death was performed. Only variables with a *p*-value less than 0.2 were included in this analysis.

Ethical considerations

The ethics committee of the Federal University of Piauí approved the study.

RESULTS

Between January of 1994 and December of 2010, 224 patients (admitted 256 times) were registered with HIV/*L. infantum* coinfection at the reference hospital. One hundred eighty five (83.2%) patients were male, principally between 20-40 years of age (143 (63.9%) patients), with age ranging from three months to 72 years. Most (84.4%) lived in cities. Nine children were identified, from three months to 11 years, with a mean age of 3.6 years. The main form of HIV contraction was sexual (86%). Intravenous drug use and vertical transmission corresponded to 2.9% each.

The average duration of symptoms was 67.5 days [95% confidence interval - (95%CI) 56.7 to 78.3 days]. The most frequent symptoms were pallor, fever and asthenia (**Table 1**). Any form of hemorrhagic phenomena, such as epistaxis, ecchymosis, and hematuria was observed in almost a third of the patients. A palpable spleen was observed in two-thirds of patients. The classic triad of fever, anemia and splenomegaly was observed in a little more than half of the patients. Of the 224 patients, 10.2% relapsed, at an average of 333 days after their initial treatment (95%CI: 184.1 to 482.6 days). Mortality occurred in 16.8% of the patients; of the 43 deceased patients, 42 died during the first admission. The mean time to death after the diagnosis was 24.2 days (95%CI: 19.4-29.1), the median, 23 days, and the maximum time was 64 days.

TABLE 1 - Clinical data of patients with human immunodeficiency virus and kala-azar diagnosed, between 1994 and 2010 in Teresina, State of Piauí, Brazil

Signs and symptoms	Frequency		95%CI of the proportion
	n	%	
All	256	100.0	-
Paleness	234	91.4	0.87-0.94
Fever	220	85.9	0.81-0.89
Asthenia	203	79.3	0.74-0.84
Weight loss	202	78.9	0.73-0.84
Apathy	192	75.0	0.69-0.80
Inappetence	176	68.7	0.63-0.74
Splenomegaly	165	64.4	0.58-0.70
Cough	135	52.7	0.46-0.59
Abdominal pain	134	52.3	0.46-0.58
Hepatomegaly	109	42.6	0.36-0.49
Chills	109	42.6	0.36-0.49
Diarrhea	107	41.8	0.36-0.48
Vomiting	88	34.3	0.29-0.40
Hemorrhagic phenomena	76	29.7	0.24-0.36
Dyspnea	73	28.5	0.23-0.34
Insomnia	54	21.1	0.16-0.27
Enlarged lymph nodes	52	20.3	0.16-0.26
Somnolence	51	19.9	0.15-0.25
Jaundice	46	17.9	0.13-0.23
Irritability	35	13.7	0.10-0.18
Edema	34	13.2	0.09-0.18
Alopecia	33	12.9	0.09-0.18
Constipation	31	12.1	0.08-0.17
Oliguria	23	9.0	0.06-0.13
Convulsions	18	7.0	0.04-0.11
Intercostal retraction	13	5.1	0.03-0.08
Impaired consciousness	9	3.5	0.02-0.07

95%CI: 95% confidence interval.

Using a multivariate regression analyses, the factors associated to death were acute kidney failure, respiratory distress, hemorrhagic phenomena and a syndrome of systemic inflammation. This syndrome was defined by the presence of at least one of the following signs: hyperventilation, tissue hypoperfusion, low oxygen venous saturation, cyanosis or organic dysfunction characterized by lactic acidosis, oliguria, or impaired consciousness (**Table 2**)¹¹.

Regarding laboratorial tests, most had pancytopenia, with hemoglobin less than 12g/dL in 97.2%, leucocytes bellow 5,000/ μ L in 89.2% and platelets less than 150,000/ μ L in 68.1%. Moreover, most (58.6%) patients had elevated aminotransferases and a minority (26.5%) had high serum creatinine (**Table 3**). Only 47 patients had the cluster of differentiation 4 (CD4) cell

count available in their medical records. Most had moderate to severe immunodeficiency. A CD4 count below 50 cells/ μ L was found in 13 (27.6%) patients, between 50 and 199 in 25 (53.2%) patients, between 200 and 499 cells/ μ L in seven (14.9%) patients and above 499 cells/ μ L in only two (4.3%) patients.

TABLE 2 - Factors associated with death in patients with human immunodeficiency virus and kala-azar, between 1994 and 2010 in Teresina, State of Piauí, Brazil

Factors associated with death	Odds ratio	p-value	95% CI
Systemic inflammatory response	19.4	0.002	3.0-125.4
Intercostal retraction	16.6	0.013	1.8-150.4
Renal failure	9.2	0.017	1.5-57.5
Hemorrhagic phenomena	6.5	0.003	1.9-22.6
Somnolence	4.4	0.018	1.3-15.1

95%CI: 95% confidence interval.

TABLE 3 - Laboratorial data of patients with human immunodeficiency virus and kala-azar, between 1994 and 2010 in Teresina, State of Piauí, Brazil

Laboratorial data	Frequency/total	Percentage
Hemoglobin (g/dL)		
<12	243/250	97.2
Leukocytes (mm ³)		
<5.000	224/251	89.2
Platelets (mm ³)		
<150.000	139/204	68.1
Pancytopenia	126/212	59.4
Aspartate aminotransferase (U/L)		
>38	110/197	55.8
Alanine aminotransferase (U/L)		
>41	65/195	33.3
Creatinin (mg/dL)		
>1.2	56/211	26.5
Urea (mg/dL)		
>50	49/209	23.4

TABLE 4 - Diagnostic tests of kala-azar in patients infected with human immunodeficiency virus, between 1994 and 2010 in Teresina, State of Piauí, Brazil

Diagnostic method	Frequency/total	Percentage
Immunofluorescent indirect test		
reactive	113/150	75.3
Bone marrow aspirate direct observation		
positive	161/242	66.5
Bone marrow culture		
positive	152/187	81.3

Parasitological examination by bone marrow culture was the most sensitive diagnostic tool (Table 4). The diagnosis of HIV infection and kala-azar was performed during the same hospitalization in 124 (55.4%) patients. Regarding to therapy, 117 (45.7%) of coinfecting patients received pentavalent antimonials, 142 (55.5%) received deoxicholate of amphotericin B and 14 (5.5%) received liposomal amphotericin B. Antibiotics were given to 183 (71.5%) patients and 136 (53.1%) received hemoderivatives (Table 5).

TABLE 5 - Therapy employed in patients with kala-azar infected with human immunodeficiency virus, between 1994 and 2010 in Teresina, State of Piauí, Brazil

Therapy	Frequency		95%CI
	n	%	
All patients	256	100.0	-
Inspecific therapy			
antibiotics	183	71.5	0.65-0.77
hemoderivative	136	53.1	0.47-0.59
Specific therapy			
pentavalent antimony	117	45.7	0.39-0.52
amphotericin B	142	55.5	0.49-0.62
liposomal amphotericin B	14	5.5	0.03-0.09

95%CI: 95% confidence interval.

DISCUSSION

The coinfection of HIV with viscerotropic *Leishmania* has been described in several areas of the world^{5,8,9,12,13}. This study describes the clinical and laboratorial profiles of this coinfection in the urban setting of the place where the present South American urban epidemic of kala-azar started. This case series is largest ever published in the region¹⁰.

The male predominance among these patients is expected since most patients with kala-azar are male and HIV is still more common in men in Northeast Brazil^{12,14}. New World kala-azar in immunocompetent patients afflicts mostly younger children¹⁴. However, coinfecting patients are usually older, as are the patients in this series. The median age-distribution of European coinfecting patients is 30-50 years, which follows the age pattern of intravenous drug use, since both diseases can be acquired by this mode of transmission^{5,9}. However, there are few documented users in this part of Brazil, possibly related to the low *per-capita* income of the region. Consequently, coinfection in Brazil is likely attributable to reactivation to past infection by *L. infantum* rather than to recently acquired disease.

These Brazilian coinfecting patients showed clinical similarity to those immunocompetent persons with kala-azar¹⁵ since most had pancytopenia, fever and hepatosplenomegaly^{14,16}. This similarity may have retarded the diagnosis of HIV before 2005 since doctors might not be aware of the high prevalence of the virus among adult kala-azar patients. However, the classical clinical signs of kala-azar were less frequent in HIV patients,

which may have led delay in recognizing kala-azar in patients with HIV. A key symptom is the enlargement of lymph nodes. Nevertheless common in East Africa, this sign is unusual in immunocompetent New World patients with kala-azar and, when present, may alert to the presence of HIV infection¹⁴. However, the most worrying aspect of the coinfection is the higher mortality¹⁷⁻²⁰. Yet, it is not clear if patients are dying from other comorbidities or due to complications of kala-azar itself. On the other hand, it seems reasonable to infer that diagnosis delay of kala-azar due to lack of the classical symptoms in some patients may have contributed to the higher mortality, although, obviously, other comorbid conditions certainly also contributed to it. There are many other forms of selection, observation or recording bias that may have influenced the final cohort and the existence of these biases must be considered when evaluating our results.

Most of the deceased patients died during the first hospitalization, which indicates that an early investigation of kala-azar in HIV febrile patients should be performed, coupled with the treatment of the complications of the disease and of other opportunistic infections. The five independent identified risk factors for death were all related to the sepsis syndrome. The concept of sepsis, or systemic inflammatory response syndrome, nevertheless elusive, represents the conceptual unit of systemic inflammation caused by infection. The present model, although not identical to that of risk factors for visceral leishmaniasis (VL) in previously immunocompetent patients^{21,22} represents the feature of systemic inflammation of lethal VL. Therefore, the evidence of the syndrome of systemic inflammation or dyspnea seems to be a clear indication for referral to an intensive care unit. Symptoms and signs such as somnolence, bleeding, sepsis, or kidney failure should also signal the likelihood of severe kala-azar and thus rapid medical intervention, having in mind that they may not be directly caused by *Leishmania*, but also by other opportunistic agents, like bacterial coinfection and sepsis. Although dyspnea is a complication of severe kala-azar, the high frequency of this complication suggests that pneumonia caused by *Pneumocystis jiroveci* may be another related opportunistic infection that should be evaluated in presenting patients.

The low proportion of relapse of kala-azar seen in these patients differs from the literature^{8,13,17,23}. It is unlikely that this could be attributed to the availability of highly active antiretroviral therapy (HAART), since the drug was also available to the patients included in other published case series. The long hospital stays of patients with this coinfection, and thus the routine weekly secondary prophylaxis with amphotericin B may have contributed to this finding. However, due to the broad reference area of the hospital, many patients may had not returned, being treated elsewhere.

Blood count with pancytopenia in patients with HIV should alert physicians to the diagnosis of kala-azar, since most have it; such a common alteration leads to higher pre-test probability of other subsequent diagnostic tests, and increases the likelihood of the diagnosis of kala-azar. Although medications and other diseases such as zidovudine and disseminated infection by *Mycobacteria avium* and *Mycobacterium intracellulare* in HIV patients can also lead to

pancytopenia. Therefore, evidence of fever and splenomegaly indicates that kala-azar should also be investigated. Liver enzymes and kidney function should be collected since liver involvement is associated to disease severity. Particularly risky is renal failure, probably due to antimony accumulation followed by cardiac or pancreatic toxicity²⁴.

The diagnosis of kala-azar in patients with HIV is troublesome due to the low sensitivity of the tests. Available parasitological tests do have a high specificity. Spleen aspiration is faster, more sensible, but with a higher risk of death, particularly in patients with more severe disease, there are limits on its use. Bone marrow aspirates show a sensitivity varying from 67% to 95%^{25,26}; lower sensitivity could be attributed to sample dilution with capillary blood at the moment of aspiration or to insufficient amount of time spent in examining the smear from the aspirate²⁷. Nevertheless, compared to other published series²⁸, the sensitivity was too low in this series. The dependence of the parasitological diagnosis on culture, which is more sensitive, may delay the beginning of treatment from one to four weeks and consequently impact the probability of survival. New culture technologies, such as the micro culture, should be studied in HIV infected patients with kala-azar.

The simultaneous diagnosis of HIV and kala-azar at the same hospitalization suggests that kala-azar may have been the first opportunistic disease and the indication for the diagnosis of HIV^{3,18,20,29}. Therefore, in endemic areas of kala-azar it is important to screen all patients with kala-azar for HIV, and kala-azar should be investigated in all symptomatic patients of HIV. Although most patients already had immunodeficiency^{16,29,30}, in some HIV patients the CD4 count was within the normal range, a typical situation of kala-azar in immunocompetent host. The use of both pentavalent antimonium and amphotericin B highlights the lack of adequate recommendations or evidence for the use of either drug to treat coinfecting patients. This might be partly attributable to a lack of diagnosis of HIV infection at the time of therapeutic choice, further highlighting the need for greater awareness of this coinfection.

In summary, the coinfection of HIV and *L. infantum* coinfection is a very serious and growing public health problem in Brazil due to the incidence of both infections, difficulties in the diagnosis and to the higher mortality associated with coinfection. Moreover, the urbanization of kala-azar across South America, the lack of effective measures for its control, and the expansion of HIV to endemic areas of kala-azar such as small cities of Brazil⁷ indicate that the problem is going to remain for a long time unless national priorities and resources are mobilized to better study, document, identify and treat those patients³¹.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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