

## Identification of *Leishmania chagasi* from skin in *Leishmania*/HIV co-infection: a case report

Identificação de *Leishmania chagasi* na pele em co-infecção  
*Leishmania*/HIV: relato de caso

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**Abstract** A case of HIV/*Leishmania* co-infection presenting both visceral and cutaneous manifestations is reported. *Leishmania* infection was confirmed by conventional methods (parasitological approach and serology) and by PCR. *Leishmania chagasi* isolated from the skin lesion was characterized by enzyme electrophoresis and by restriction fragment length polymorphism of the internal transcribed spacer of the ribosomal gene.

**Key-words:** *Leishmania*. HIV.

**Resumo** É descrito um caso de co-infecção *Leishmania*/HIV com manifestações cutâneas e visceral. Infecção pela *leishmania* foi confirmada através de métodos convencionais (parasitológicos e sorológicos) e através da PCR. A espécie *Leishmania chagasi* isolada da pele foi caracterizada por eletroforese enzimática e por polimorfismo de fragmento obtido por enzima de restrição.

**Palavras-chaves:** *Leishmania*. HIV.

Co-infection by *Leishmania* spp and HIV has been considered an emerging disease in several parts of the world<sup>11</sup>. In Brazil, more than 200,000 cases of leishmaniasis have been notified over the last decade<sup>1</sup>. Between 1980 and 1991, the Brazilian Ministry of Health recorded an annual mean of 27,000 new cases of cutaneous (CL) and muco-cutaneous leishmaniasis (ML) and 2,800 new cases of visceral infection. These figures in the annual reports have increased during the past three years reaching 35000 cases of CL and 3,500 patients with visceral leishmaniasis (VL)<sup>3</sup>. In Minas Gerais state, records have shown 1569 cases of VL (population of 15 million people), 30% (445) of which were from the metropolitan region.

Growth of the Brazilian population, acceleration of urbanization processes, man-made environmental changes and rural-urban migration contributed to the increased and modified geographic spread of

*Leishmania* spp parasite. Such that, nowadays many people living in dense urban zones live in contact with the causative agent. During the past decade, many urban outbreaks of VL caused by *Leishmania* (*Leishmania*) *chagasi* have been reported in Brazil<sup>7 10</sup>.

Despite of the reduction in mortality after the introduction of anti-HIV drugs, recent data suggest that the AIDS epidemic is still spreading among the Brazilian population, reaching small cities, affecting women in the same frequency as men and extending to less educated populations. According to the National AIDS Program of the Brazilian Ministry of Health, 215,810 cases of AIDS had been notified up until November 1999, with an incidence rate of 90/100,000 inhabitants. Approximately 114,500 (50% of the reported cases) cumulative deaths secondary to AIDS were recorded by 2001 (Boletim Epidemiológico AIDS, 2001).

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According to estimates of the World Health Organization (WHO), 2-9% of the South European AIDS patients develop VL. Gradoni et al<sup>5</sup> observed a 500 times higher prevalence of *Leishmania* spp infection among HIV infected subjects in Sicily.

In Brazil, out of 61 actively searched *Leishmania*/HIV co-infected cases, 64% were CL or ML and 36% presented the visceral form of the disease. In only

nine patients the *Leishmania* species was characterized and in all cases belonged to the *Viannia* subgenus (A. Rabello: *personal communication, 1999*).

This paper reports a fatal case of *Leishmania*/HIV co-infection presenting both cutaneous and visceral manifestations that were caused by *Leishmania (Leishmania) chagasi*.

#### CASE REPORT

In December 1995, after successive episodes of sinusitis and pneumonia, HIV infection was diagnosed in a 43-year-old patient from Belo Horizonte, Minas Gerais State, Brazil. HIV serology was performed by ELISA and confirmed by Western Blot. At that time, the patient presented a good healthy condition and clinical manifestation of seborrheic dermatitis.

In January 1996, with 87 CD4 positive lymphocyte/mm<sup>3</sup> and viral load of 73,000 RNA copies/ml, the patient received zidovudine, didanosine and trimethoprim-sulfamethoxazole. Clinical conditions remained unchanged until March 1997, when the patient presented fever (38°C), weight loss (7.5kg) and splenomegaly. Investigation of the putative etiology of the fever of unknown origin was not successful. X-ray and computerized tomography of the thorax and abdominal ultrasonography were normal, as well as the otorhinolaryngology exam. Urine and blood laboratory analysis also did not reveal any abnormalities. Serology for CMV infection, Epstein-Barr virus infection, syphilis, brucellosis and toxoplasmosis were negative. Microbiologic culture of urine and faeces and investigation of *Mycobacterium* sp were negative. Direct examination of the bone marrow showed no pathologic abnormalities. Anti-retroviral therapy was changed to stavudine and lamivudine which induced clinical improvement with reduction of the spleen size and regression of the fever leading to the patient's return to normal daily activities.

In January 1998, the patient presented diarrhea and *Shigella* sp and *Cryptosporidium* sp were identified in the feces. Treatment (floxacin for 15 days) was considered successful. One month later, there was a progressive worsening of symptoms, with alopecia, caquexia and peripheral neuritis.

In August 1998, the patient presented pancytopenia (platelets: 34,000/ml, leukocytes: 1,200/ml, granulocytes:

700/ml, hemoglobin: 8.1%) and alterations in several biochemical parameters (aspartate aminotransferase: 71 mg%, alanine aminotransferase: 40mg%, alkaline phosphatase: 150mg%, prothrombin activity: 85%) were observed. In December, 1998, cutaneous and oral-pharyngeal lesions suggestive of herpes zoster, appeared. Fluconazole and acyclovir achieved total recovery of the oral-pharyngeal lesions and partial recovery of the cutaneous lesions was observed after 14 days of treatment. However, the cutaneous hyperkeratosis lesions did not heal completely and a biopsy was taken in June 1999. Although initial histopathological diagnosis of keratoacanthoma was not confirmed, during a microscopic revision of the slides *Leishmania* spp amastigotes were found in the interior of macrophages in a mild lymphohistiocytary inflammatory dermal infiltrate (Figure 1). In August, the patient presented new cutaneous lesions located in the upper and lower limbs and hepatosplenomegaly. A skin biopsy from the new lesions was performed and *Leishmania* spp was detected by imprint and culture. Hemoculture and PCR using DNA extracted from peripheral mononuclear cells or skin with a set of primers directed towards the minicircle conserved region, were positive. Parasites isolated from the biopsy specimen were submitted to *in vitro* culture and DNA was extracted. Electrophoresis of parasite enzymes identified *L. chagasi* as the causative agent. Restriction fragment length polymorphism of amplicons corresponding to the internal transcribed spacers of the ribosomal gene confirmed this finding (Figure 2).

Treatment with meglumine antimoniate (10mg/kg for 40 days) was initiated and led to partial improvement of the skin lesions, regression of the hepatosplenomegaly and negative hemoculture. In November the patient was hospitalized, presenting fever, progressive dyspnea, caquexia and disseminated cutaneous lesions. Supportive measures were implemented, but nevertheless the patient died on December 7<sup>th</sup>.

#### DISCUSSION

Both AIDS and leishmaniasis are highly prevalent in Brazil and the number of cases of co-infections is growing<sup>9</sup> In the immune competent patient CL is commonly characterized by a single ulcer that responds satisfactorily to treatment. Cure is assured for 95% of the patients for whom treatment has been applied in time<sup>6</sup>. Cure and resistance to infection demands an

adequate cellular immune response of the Th1 type, with production of gamma-interferon and IL-12. The infection leads to disease and its complications when a cellular immune response of the type Th2 is elicited<sup>9</sup>. Similarly the development of AIDS in an HIV infected patient is associated with a Th2 profile. HIV-*Leishmania* co-infection favors the Th2 response with consequent aggravation of both infections.

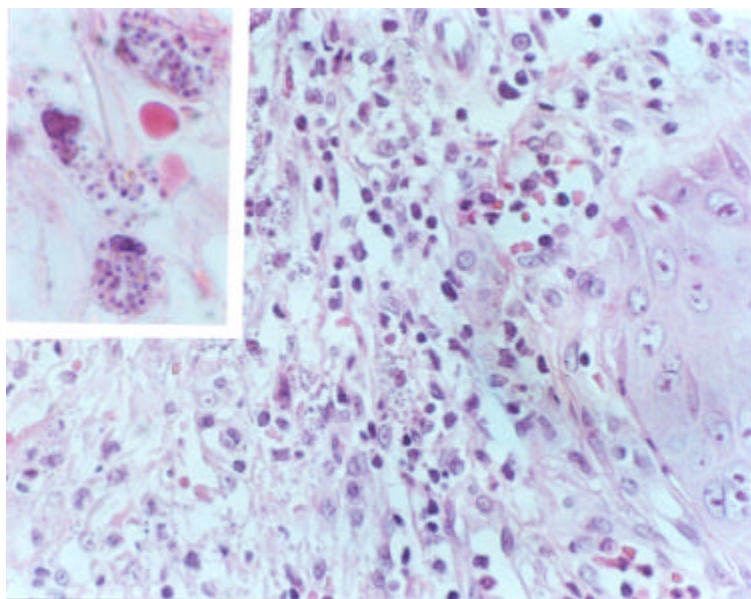


Figure 1 - Lymphohistiocytic inflammatory infiltrate in the dermal surface with amastigotes in the interior of macrophages (HE, 400X). On the left corner, macrophages with amastigotes (HE, 1000X).

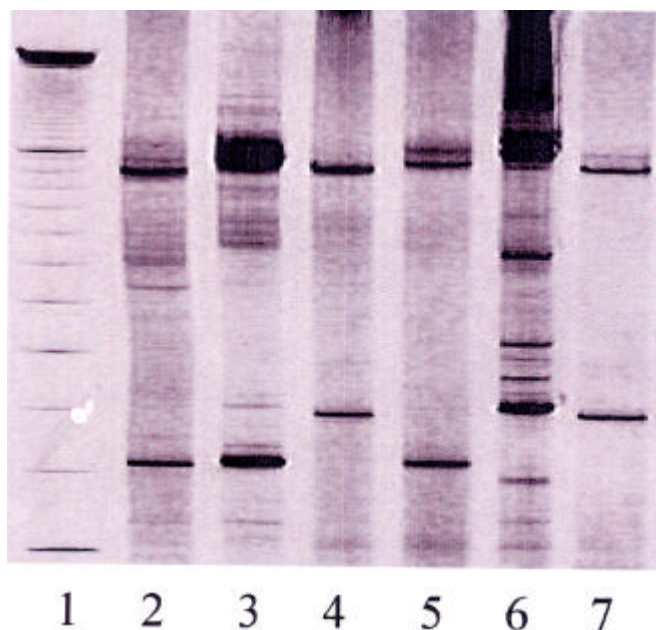


Figure 2 - Profiles of *Ecor-I* amplified KDNA of *Leishmania* spp: Lane 1: 100 bp ladder. Lane 2: *L. lainsoni*. Lane 3: *L. guyanensis*. Lane 4: *L. chagasi* (patient). Lane 5: *L. guyanensis*. Lane 6: *L. mexicana*. Lane 7: *L. chagasi*.

The patient here described presented cutaneous and visceral manifestation. Initial confirmation of infection by *Leishmania* spp was performed by microscopic examination of the skin biopsy. Cutaneous manifestations of *L. chagasi* in HIV infected patients has been observed by several other authors<sup>2,4,8</sup>.

In this patient, the diagnosis of leishmaniasis was not anticipated. Spleen enlargement was attributed to specific reaction to HIV and/or drugs and *Leishmania* spp amastigotes were not identified at pathologic examination. Treatment of the *Leishmania* infection was

therefore delayed leading to aggravation of the patient's situation. The described case illustrates the severity of an association of *Leishmania* spp infection and HIV and

underlines the need for inclusion of *Leishmania* spp evaluation in AIDS patients in regions where leishmaniasis occurs.

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