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CASE REPORT

L. (L.) chagasi IN AIDS AND VISCERAL LEISHMANIASIS (KALA-AZAR) CO-INFECTION

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SUMMARY

Concomitant skin lesions in visceral leishmaniasis (VL) or kala-azar are rare, being more common the description of post-kala-azar dermal leishmaniasis occurring post treatment of kala-azar. Skin lesions caused by *Leishmania donovani* are frequently seen in the aids-VL co-infection. In Brazil cutaneous or mucosal forms of tegumentary leishmaniasis concomitant with aids are more commonly registered. Here we present a case of aids-VL co-infection, with unusual cutaneous and digestive compromising attributed to *L. (L.) chagasi*, with special attention to ecthymatous aspect of the lesion, allied to the absence of parasite on the histological skin biopsy.

KEYWORDS: AIDS-VL-co-infection; Digestive compromising leishmaniasis; Ecthymatous lesion.

INTRODUCTION

The leishmaniasis in their tegumentary and visceral forms may be caused by 15 different leishmania species, which zoonotic cycle is well established. The visceral leishmaniasis (VL) or kala-azar is caused in the Old World by *Leishmania donovani* and, in both, in the Old and New World by *L. infantum*. In the New World, *L. (L.) chagasi*, transmitted by the vector *Lutzomyia longipalpis*, can be considered as synonym of *L. infantum*, although some authors consider them as distinct species. Its incidence is increasing in the last years, being directly related to the global climatic heating and to the environmental modifications provoked by the man[11]. In Brazil, the dog becomes responsible as the main reservoir of leishmania parasite[20].

The susceptibility to VL is low, being associated with the age (children below two years are more affected), malnutrition and immune compromising[5,11]. In Brazil, where aids and leishmaniasis are endemic, the aids-leishmaniasis co-infection is more described in their cutaneous or mucosal forms of tegumentary leishmaniasis[6,19]. Fortunately, the incidence of that co-infection is decreasing in Brazil, attributed to the free governmental distribution of anti-retroviral drugs to the patients[18].

Specific skin lesions of leishmaniasis concomitantly to kala-azar or VL are rare, being more common the description of cutaneous or mucosal involvement occurring post treatment of kala-azar (post-kala-azar dermal leishmaniasis, PKDL)[22]. When cutaneous compromising exists in VL, in general it happens in association with aids coursing with low count of CD4 T cells[4,8,10,16]. Classic PKDL due to *L. (L.) chagasi* is not commonly described in Brazil[3]. In advance we are describing a case of aids-VL co-infection, whose patient presented digestive and ecthymatous lesions attributed to leishmaniasis, which leishmania species detection in the skin was only permitted by the PCR-RFLP technique employment.

CASE REPORT

A Brazilian male, 23 y-old, natural of Bahia and resident in São Paulo State, user of illicit drugs was admitted to the hospital in May of 1995 with fever, abdominal pain and diarrhea since 20 days ago. The continuous abdominal pain with moderate intensity was located in the right hypochondria region and worsened with feeding, associated to nauseas and gastric fullness, with duration of approximately 30 minutes. The diarrhea was characterized by being liquid, with no blood, mucus or pus, twice a day. On the physical exam, the patient was icteric, and polyadenomegaly besides hepatic and spleen enlargement were present. ELISA and WB confirmed the infection by HIV. The count of total lymphocytes showed 408 cells/mm^3 (CD4$^+$ 9.0/mm^3 and CD8$^+$ 63/mm^3). The bone marrow imprint showed leishmanias in histiocytes, while, in the hepatic biopsy, they were not seen. The patient was treated with meglumin antimoniate by 20 days, 20 mg/kg/day, leaving the hospital after two months, going back to Bahia. In November of 1998, he was hospitalized again, presenting diarrhea now with blood, three episodes a day. On this occasion, papular lesions were observed, some ulcerated, in the abdomen and in the left thigh. Endoscope digestive examination

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evidenced severe gastric and duodenal inflammation, when corresponding biopsies showed presence of amastigotes forms of leishmania (Fig. 1). Indirect immunofluorescence test for leishmania resulted 1/8, and the skin biopsy did not show any parasite (Fig. 2). One fragment of the cutaneous biopsy was used in PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) for leishmania identification. A specific pair of primers for a sequence of 120 bp of kDNA minicircle, common to all leishmania species, was used, as described elsewhere[9,14]. The analysis of the PCR product digested by *Hae*III enzyme suggested the species *L. (L.) chagasi*, since its pattern was similar to the *L. (L.) chagasi* maintained in culture (Fig. 3). In this occasion, antiretroviral drugs and amphotericin B were prescribed. In February of 1999, the $CD4^+$ count resulted 132 cells/mm^3 and $CD8^+$ 857/mm^3. The symptoms of VL regressed and, after an accumulated dose of 3,420g of amphotericin B, the control bone marrow exam was considered normal, with lack of parasites. However, in July of 1999, there was decreasing of $CD4^+$ (31/mm^3) and of $CD8^+$ count cells (477/mm^3), besides increasing of virus load. In August of 1999 the patient came to die due to bacterial pneumonia, not being submitted to necropsy.

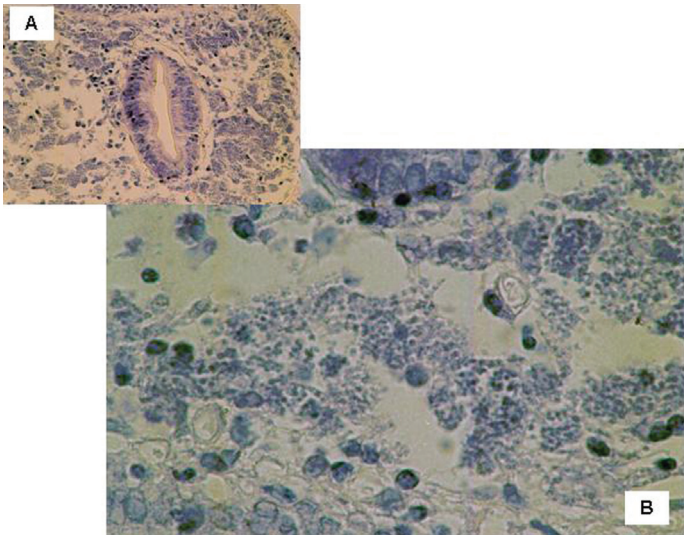

Fig. 1 - Histopathology of gastric biopsy from a patient with VL-aids co-infection: A: light and unspecific chronic gastritis, associated with great amount of round structures with eccentric nucleus (HE, 400X). B: Amastigotes forms of *Leishmania* spp. (GIEMSA, 1000X)

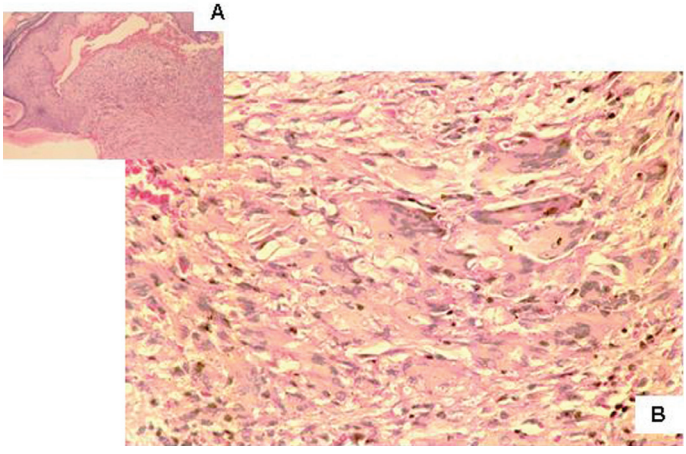

Fig. 2 - Histopathology of skin biopsy (ecthymatous lesion) from a patient with aids-VL co-infection: A: fibrosis of dermis, and light to moderate unspecific chronic inflammation (HE, 100X). B: small focal granuloma and lymphocytic septal pannyculitis, with no presence of amastigotes forms of *Leishmania* spp. (HE, 600X)

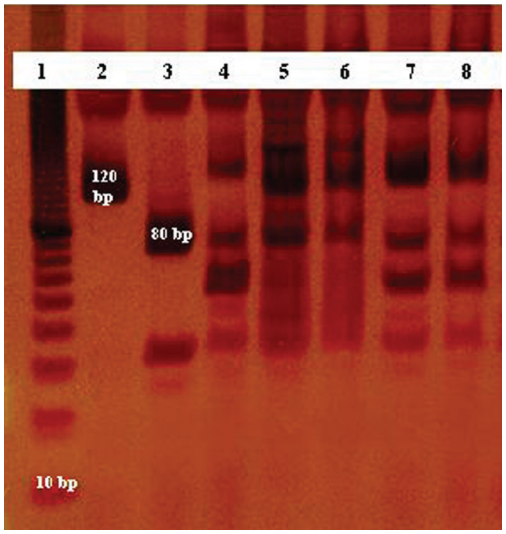

Fig. 3 - Gel of acrylamide 10%: enzymatic digestion with *Hae*III of a 120 bp DNA fragment amplified by PCR with specific primers for kDNA minicircle common to all species of *Leishmania* (to consult methodology seen GARCIA *et al.*[9]). The numbered columns correspond to samples of leishmanias maintained in culture medians, and samples from patients: 1. MW ladder 10 bp; 2. *L. (L.) amazonensis* (IFLA/BR/67/PH8); 3. *L. (V.) braziliensis* (LBB 2903); 4. *L. (L.) chagasi* (VL 9.3); 5,6. Blood samples from one patient with kala-azar; 7. Skin sample from the present case with aids and VL co-infection; 8. Blood sample from another patient with kala-azar. Observe the similarity obtained by the enzymatic restriction in samples represented in columns 7 and 8 compared to the culture of *L. (L.) chagasi* (column 4). The same could not be confirmed for blood samples represented in columns 5 and 6.

DISCUSSION

The present case shows the association of VL and aids, which both diagnoses were made in the first attendance. The symptoms of VL got better after a treatment cycle with antimonial drug but three and a half years after that, the patient presented recurrence of VL, with digestive symptoms, associated to the upcoming of ecthymatous skin lesions. In that occasion, two clinical aspects deserve importance: the digestive compromising with leishmania identification in the gastric and duodenal biopsies, and the skin lesions, with granuloma description at histopathology, even so with no parasite demonstration.

In relation to the digestive compromising in VL, there are wide descriptions in immune compromised patients, among them, in patients with aids[2,6] and in diabetes[1]. It is important pay attention for symptoms of chronic diarrhea, as well as for symptoms no usual, as asthenia, anorexia, dysphagia and odynophagia, associated to weight loss[2]. In this last citation, the authors identified the species *L. infantum* in gastro duodenal sample submitted to cultivation and enzymatic analysis.

Another atypical VL compromising was related in aids patient,

when *Leishmania chagasi* was detected by PCR in the pleural and ascitic fluid[7].

The skin lesions presented in aids-VL co-infection may be specific or not. There is description of the presence of leishmania in concomitant diagnosed dermatoses, as herpes simplex and zoster, Kaposi's sarcoma, dermatofibroma, even in apparently normal skin. This observation is attributed to the presence of leishmania in cutaneous histiocytes, seen especially in immunocompromised patients. Specific skin lesions of leishmaniasis may comprise many appearances; being papules and nodules more commonly described[4].

The histopathology exam of skin lesion seen in this case showed presence of granulomatous inflammatory infiltration, allied to a lymphocytic septal panniculytis. In compensation, with a paradoxical absence of leishmania identification in hepatic and skin biopsies, its presence was confirmed in the bone marrow and in gastric ones. The demonstration of granulomatous inflammatory infiltration with presence of lymphocyte cells can justify the absence of parasites on histopathological exam. Our experience has been showing the presence of amastigotes forms in nearly 50% of skin biopsies[14]. The literature shows numerous amastigotes forms identified in skin samples from nodules in aids-VL co-infection[6,8,10].

In the present description it is focused the identification of *L. (L.) chagasi* only allowed by the employment of PCR-RFLP in extracted DNA from ecthymatous skin sample of a patient with aids and VL. It could be questioned the presence of leishmanias' DNA in blood instead of skin itself on skin sample utilized for PCR. The description of granulomatous inflammatory infiltration with giant cells favors the presence of leishmanias' DNA inside macrophages, despite the fact that amastigotes forms had not been visualized on histopathological exam. In that approach, ORSINI *et al.* (2002)[16] also demonstrated the identification of *L. (L.) chagasi* in extracted DNA from parasites cultivated from skin lesion sample. One more reason to justify the positive PCR for leishmania on skin sample could be endorsed by the response to antimonial treatment, with cicatrization of ecthymatous skin lesions.

In Sudan, the species *L. donovani* can damage the oral mucosa, besides the skin. In some patients, compromising of the mucosal tract occurs after the treatment of VL or, occasionally, concomitantly to the kala-azar. Studies of molecular DNA markers of parasites from mucosal lesions of leishmaniasis and from blood samples of patients with kala-azar have shown they represent different strains of *L. donovani*[13]. A similar study of genetic polymorphisms distinguishes *L. donovani* strain responsible for kala-azar from that in PKDL[21].

In Brazil, reports of cutaneous lesions attributed to leishmaniasis in aids-VL co-infection are scarce[16]. Probably it happens because the lesions may shape common dermatoses, such as dermatofibroma, lichen, drug eruption[4].

Recent reports have been showing an immune reconstitution inflammatory syndrome (IRIS) associated with parasitic infections following antiretroviral treatment. IRIS consists in clinical presentation or deterioration of opportunistic infections that result from enhancement of pathogen-specific immune responses among patients responding to antiretroviral treatment[12]. For tegumentary leishmaniasis IRIS was described in two patients when CD4 T cell counts were recovering and virus load were decreasing[17]. The concept of IRIS can not be credited to the present case, since both VL and aids, such as cutaneous and abdominal compromising, had began before starting antiretroviral therapy for aids.

Finally, keeping similarity to reports of different strains of *L. donovani* responsible for cutaneous and mucosal lesions in patients from Sudan and India[13,21], genetic studies of strains of *L. (L.) chagasi* that affect the skin could be interesting to distinguish them of those that only affect internal organs in VL, mainly for the possibility of prediction of mucosal compromising. In this report it was possible to show similarity, using enzymatic digestion with *Hae*III, of a 120 bp DNA fragment amplified from skin lesion from this patient with aids-VL co-infection and from blood sample collected from another patient with VL. The same could not be observed when they were compared with blood sample from a third patient with VL. For the proposal of genetic studies that could support differences or similarities of strains of *Leishmania* it will be necessary to obtain skin and blood samples from the same patient such as from distinct patients.

RESUMO

***L. (L.) chagasi* em lesões cutâneas na co-infecção aids-calazar.**

Lesões cutâneas, na vigência da leishmaniose visceral (LV) ou calazar, são raramente observadas, sendo mais comum a ocorrência após o tratamento do calazar, conhecidas como lesões dérmicas pós calazar. Lesões cutâneas causadas por *Leishmania donovani* são freqüentemente observadas na co-infecção AIDS-LV. No Brasil, a concomitância das formas cutânea ou mucosa da leishmaniose tegumentar com a AIDS é mais comumente relatada. A seguir, relata-se um caso de co-infecção AIDS-LV com inusitado comprometimento digestivo e cutâneo, atribuído a *L. (L.) chagasi*, chamando a atenção para o aspecto ectimatóide da lesão cutânea, aliado à ausência do parasito ao exame histopatológico da pele.

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