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ORIGINAL ARTICLE

Oral leishmaniasis: a clinicopathological study of 11 cases

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Leishmaniasis is a parasitic disease with diverse clinical manifestations, and considered a public health problem in endemic countries such as Brazil. Mucosal lesions usually involve the upper respiratory tract, with a predilection for nose and larynx. Oral involvement is unusual and in most cases it becomes evident after several years of resolution of the original cutaneous lesions. Oral lesions classically appear as mucosal ulcerations, mainly in the hard or soft palate. This report describes the clinicopathological data of 11 cases of mucocutaneous leishmaniasis with oral manifestations. Two cases of *Leishmania (Viannia) braziliensis* and one case of *Leishmania (Leishmania) amazonensis* were confirmed by polymerase chain reaction-restriction fragment length polymorphism or DNA sequencing in mucosal samples.
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Keywords: mucocutaneous leishmaniasis; mouth; PCR-RFLP; *Leishmania (V.) braziliensis*; *Leishmania (L.) amazonensis*

Introduction

Leishmaniasis is a parasitic disease caused by a protozoon of the genus *Leishmania* transmitted by sandfly vectors of the genus *Phlebotomus* or *Lutzomyia* (Chaudhry *et al*, 1999; Costa *et al*, 2003). Leishmaniasis is endemic in 88 countries, particularly localized in areas of the tropics, subtropics, and southern Europe, in settings ranging from rain forests in the Americas to deserts in western Asia, and from rural to peri-urban areas (Herwaldt, 1999). Leishmaniasis is classified as cutaneous, mucocutaneous and visceral or kala-azar, with a wide spectrum of clinical manifestations (Silveira *et al*, 2004). Most cases of cutaneous leishmaniasis occur in Iran, Afghanistan, Syria, Saudi Arabia, Brazil and Peru, and more than 90% of visceral leishmaniasis are endemic in Bangladesh, Brazil, India and Sudan (Desjeux, 2004). Annual world incidence is estimated at 1–1.5 million cases of cutaneous and 0.5 million of visceral leishmaniasis. Overall prevalence is 12 million people and the population at risk is 350 million (Desjeux, 1996).

Mucosal involvement in leishmaniasis is uncommon and results from haematogenous or lymphatic dissemination of amastigotes from the skin to the nasooropharyngeal mucosa. In most cases, mucosal leishmaniasis becomes evident after some years of resolution of the preceding cutaneous lesions, but it can develop while the skin lesions are still present (Herwaldt, 1999). Mucocutaneous leishmaniasis is endemic in some regions of Brazil affecting usually the upper respiratory tract with predilection for the nose and larynx. On the other hand, oral manifestations, as in other parts of the world, are exceptional (Chaudhry *et al*, 1999). This study describes the clinicopathological characteristics of 11 cases of leishmaniasis with oral manifestations.

Patients and methods

Demographic data, clinical informations, laboratory features, treatment and outcome of 11 patients with leishmaniasis with oral manifestations were reviewed and described. Eight cases were retrieved from the files of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil, one from the Dental School of Piracicaba, University of Campinas, Brazil, and two from Facultad de Odontología, Universidad Mariano Gálvez, Guatemala, Guatemala.

Results

Clinical findings

At the time of diagnosis, the age of the patients ranged from 19 to 76 years with a mean of 49.2 years. Nine patients were male and two female, seven were White, two African-American and two Mestizos from

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Guatemala. Most of the patients (72.7%) were rural workers. Eight patients presented mucocutaneous and three mucosal leishmaniasis. Six of eight patients with mucocutaneous leishmaniasis had skin lesions on the face. However, lesions were also found in the arms, legs, mammilla, scrotum and penis. Mucosal disease was most commonly found in the palate followed by the oropharynx. Hard and or soft palate were affected in eight cases, oropharynx in five cases, nasal mucosa in five, upper lip in three and tongue, labial commisure and larynx in one case each (Figures 1, 2, 3, 4) (Table 1)

Laboratory features

Leishmanian skin test (LST) was performed in six patients, three were positive and three negative. Interestingly, all cases positive for the LST were parasite poor in the biopsy tissues. On the other hand, the LST-negative cases were strongly positive for amastigotes in the histopathological material. Microscopically there was predominance of a dense mononuclear inflammation, with variable amounts of round to oval bodies compatible with *Leishmania* amastigotes in the cytoplasm of macrophages (Figure 5). Only one patient was positive for anti-HIV serology. Species identification was performed in five cases, four cases by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and one by immunohistochemistry for *Leishmania (Viannia) braziliensis* (mouse polyclonal antibody, 1/5000, Medical School of the University of São Paulo) (Figure 6). Using two restriction enzymes,

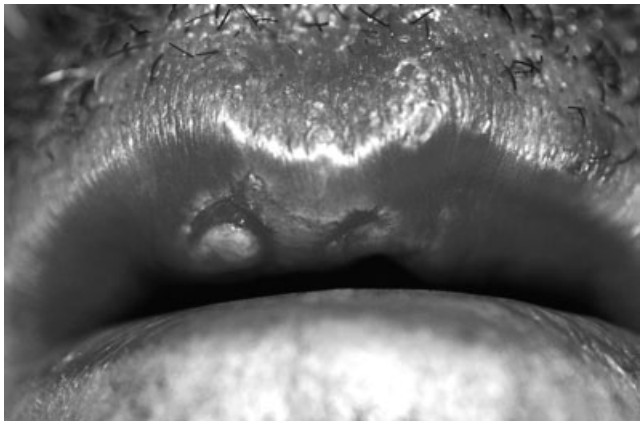

Figure 1 Lip leishmaniasis in a 39-year-old patient presenting as erythematous areas and small ulcerations (patient 6)

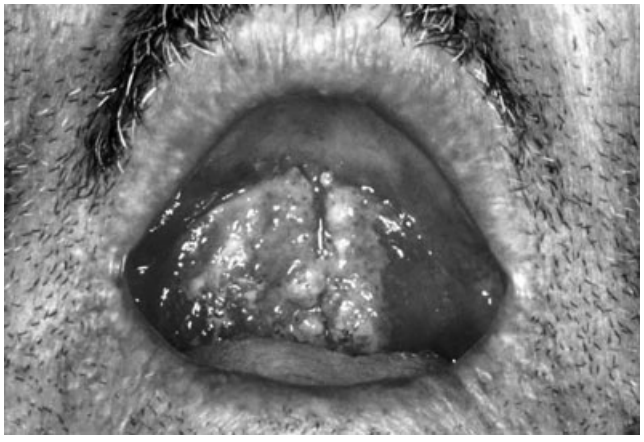

Figure 2 Extensive ulcerative lesion on the hard and soft palate (patient 4)

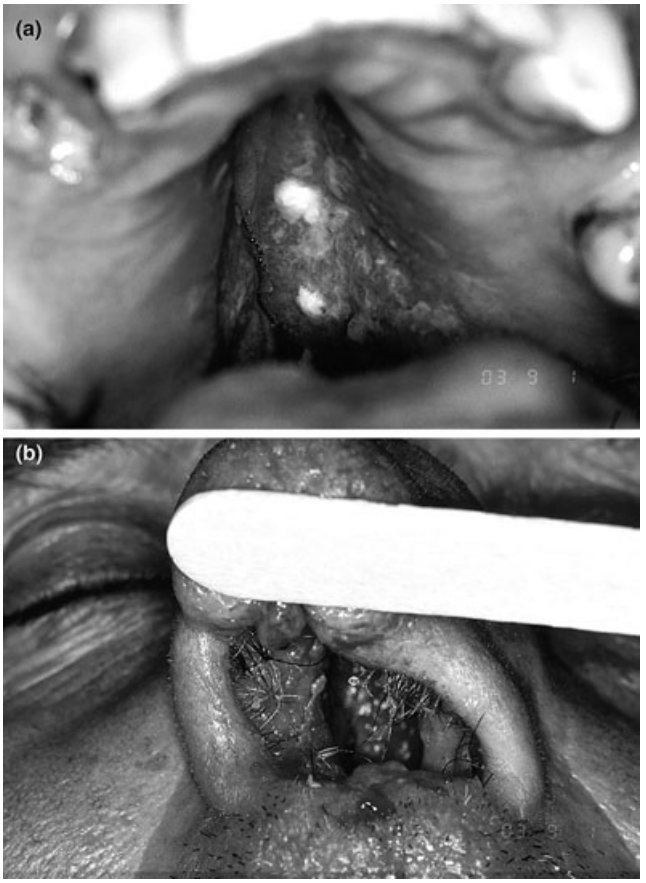

Figure 3 (**a**) Ulceration with granulomatous surface on the midline of the hard palate, (**b**) involvement of nasal mucosa (patient 8)

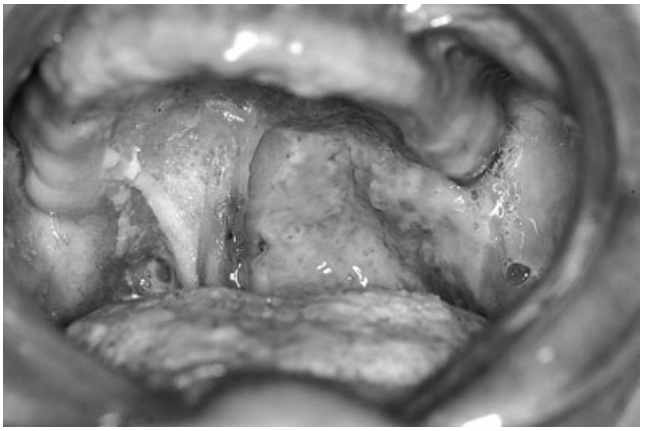

Figure 4 Ulcerative lesion with necrotic tissue on the hard and soft palate (patient 9)

Table 1 Demographic and clinical data of 11 patients with oral manifestations of leishmaniasis

	Demographic data								
Patient no.	*Age (years)*	*Sex*	*Race*	*Occupation*	*Year*[a]	*Time of disease (years)*	*Clinical form*	*Skin lesions*	*Mucosal lesions*
1	37	M	A	Rural worker	1985	2	MCL	Face, legs, arms, mammilla	Upper lip, tongue
2	62	M	W	Shop man	1986	1	MCL	Face	Soft palate
3	76	F	W	Rural worker	1996	3	MCL	Left arm	Hard and soft palate, oropharynx, nasal mucosa, larynx
4	65	M	W	Rural worker	1996	1	MCL	Face, left leg, scrotum	Hard and soft palate, oropharynx
5	56	M	W	Rural worker	2000	10	MCL	Penis	Soft palate, oropharynx
6	39	M	W	Rural worker	2001	3	MCL	Face	Upper lip, hard palate, nasal mucosa
7	39	M	A	Rural worker	2001	1	ML	Absent	Hard and soft palate, oropharynx, nasal mucosa
8	65	M	W	Rural worker	2003	3	MCL	Face	Hard and soft palate, oropharynx, nasal mucosa
9	47	F	W	n.a.	2005	n.a.	ML	Absent	Hard and soft palate
10	36	M	MG	Rural worker	1998	3	ML	Absent	Upper lip, nasal mucosa
11	19	M	MG	n.a.	2001	n.a.	MCL	Ear helix	Labial commissure

F, female; M, male; W, white; A, African-American; MG, Mestizos from Guatemala; MCL, mucocutaneous leishmaniasis; ML, mucosal leishmaniasis; n.a., not available.
[a]Year at first consultation.

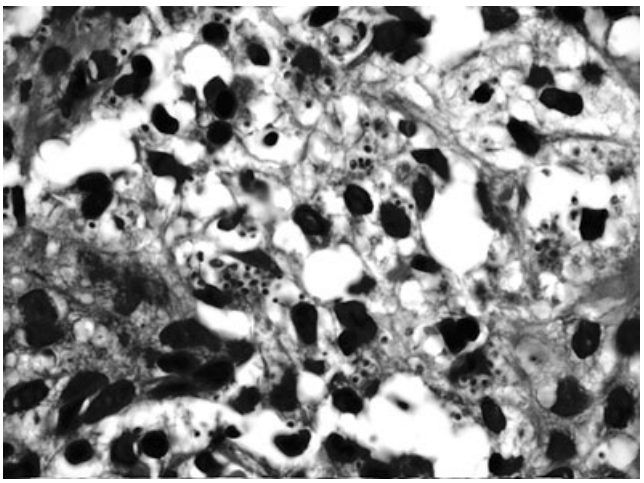

Figure 5 Histological view showing a dense mononuclear inflammation, with variable amounts of round to oval bodies compatible with *Leishmania* amastigotes in the cytoplasm of macrophages (hematoxylin and eosin ×400)

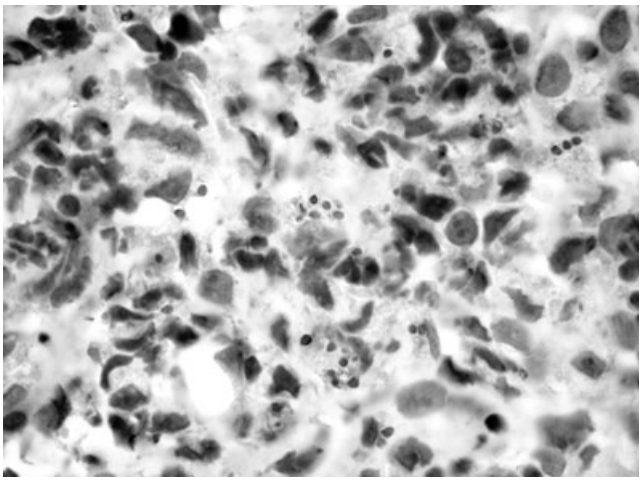

Figure 6 Species identification by immunohistochemistry for *Leishmania (V.) braziliensis* (×400)

*Bsr*I and *Hae*III, one case was identified as *Leishmania (Viannia) braziliensis* and another one as *L. (L.) amazonensis* (Figure 4). The PCR-RFLP technique did not permit the species identification in cases 3 and 4. In case 4, DNA sequencing of the PCR product confirmed *L. (V.) braziliensis* species. Serological test for Chagas' disease is performed usually before initiating antimonial therapy, as it can provoke heart toxicity. From eight cases, six showed positivity for *Trypanosoma cruzi* (Table 2).

Treatment and outcome

Patients 1–8 were treated with meglumin antimony, 15 mg kg^{-1} of body weight per day, for different periods of time ranging from 8 to 89 days. Patient 3 received meglumin antimony and amphotericin B 2 g (total dose). Patients 10 and 11 were treated with meglumin antimony; however, we did not have information about the dosage, period of treatment and outcome. The HIV-positive patient died 2 days after the diagnosis of leishmaniasis. On the other hand, cases 1–8, HIV-negative patients, were considered cured after different periods of follow-up (Table 2).

Discussion

Leishmaniasis is a vector-born disease caused by obligate intramacrophage protozoa of the genus *Leishmania* (Herwaldt, 1999). Leishmaniasis in humans has been

Table 2 Laboratory features, treatment and outcome data of 11 patients with oral manifestations of leishmaniasis

Patient no.	*HIV*	*LST*	*Presence of leishmania in biopsy*	*PCR-RFLP*	*Serological for Chagas' disease*[b]	*Treatment*	*Outcome*
1	(−)	(+)	(−)	n.p.	(+)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 20 days	Healed without recurrences at 17 years follow-up
2	(−)	n.p.	(−)	n.p.	(−)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 89 days	Decrease of nasal infiltration and heal of palate lesion at 1 year follow-up
3	(−)	(+)	(−)	*Leishmania* spp.	(+)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 8 days + amphotericin B 2 g[c]	Healed without recurrences at 3 years follow-up
4	(−)	(−)	(+)	*L. (V.) braziliensis*[a]	(+)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 23 days	Healed without recurrences at 1 year follow-up
5	(−)	(+)	(−)	n.p.	(+)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 60 days	Healed without recurrences at 2 years follow-up
6	(−)	(−)	(+)	n.p.	(−)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 60 days	Healed without recurrences at 2 years follow-up
7	(−)	(+)	(+)	*L. (L.) amazonensis*	(+)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 28 days	Healed without recurrences at 2 years follow-up
8	(−)	(−)	(+)	*L. (V.) braziliensis*	(+)	Meglumin antimony 15 mg Sb^v kg^{-1} day^{-1} for 60 days	Healed without recurrences at 2 years follow-up
9	(+)	n.p.	(+)	n.p.	n.p.	n.p.	Died 2 days after the diagnosis
10	n.p.	n.p.	(+)	n.p.	n.p.	Meglumin antimony	n.a.
11	n.p.	n.p.	(−)	n.p.	n.p.	Meglumin antimony	n.a.

LST, leishmanian skin test; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; (+), positive; (−), negative; n.p., not performed; n.a., not available.
[a]Species identification confirmed by DNA sequencing; [b]probably by cross-reaction; [c]total dose.

classified in three major clinical types: visceral, cutaneous and mucocutaneous. The visceral form of leishmaniasis is caused mainly by *L. infantum* and *L. donovani*, which is endemic in the western Mediterranean. Cutaneous leishmaniasis is caused by *L. tropica* or *L. major* and the mucocutaneous variant is mainly due to *L. (V.) braziliensis*, but also *L. panamensis*, *L. guyanensis* and *L. (L.) amazonensis*, which is endemic in South America (Herwaldt, 1999; Milian *et al*, 2002). *Leishmania (L.) amazonensis* is responsible for the so-called New World (American) cutaneous leishmaniasis (Herwaldt, 1999); however it had also been implicated in non-cutaneous forms of the disease such as mucosal, visceral, and post-kala-azar dermal leishmaniasis (Abreu-Silva *et al*, 2003). There are about 24 infective *Leishmania* species, however single specie can produce more than one clinical form of the disease, and each form can be caused by multiple species (Aliaga *et al*, 2003). In endemic regions the protozoa may persist as an asymptomatic parasite for years, and situations of cellular immune suppression can contribute to induce the development of clinical manifestations (Milian *et al*, 2002).

Mucosal disease usually affects the upper respiratory tract with a predilection for the nose and larynx. Typically, mucosal disease follows an initial cutaneous lesion that has often healed by the time mucosal involvement is evident (Chaudhry *et al*, 1999). Parasites localized in the nasal, oral, and pharyngeal mucosa give rise to mucocutaneous ulcerative lesions in up to 5% of persons with a history of cutaneous lesions (Magill, 1995). In our cases, only two patients (cases 7 and 9) did not relate a preceding skin lesion. Observations in Brazil indicate that *L. (V.) braziliensis* may disseminate to distant sites during an early phase of infection, before the skin ulcer develops (Marsden *et al*, 1985). This may explain why in these two patients skin lesion was not observed. In addition, primary mucosal leishmaniasis may occur in both immunosuppressed and immunocompetent patients (Guddo *et al*, 2005).

Oral lesions usually appear as ulceration in the hard or soft palate. However, they can affect any site and also may present as exophytic, nodular and indurated lesions (Vazquez-Piñeiro *et al*, 1998; Milian *et al*, 2002). Isolated leishmaniasis in the tongue of immunocompetent individuals is extremely rare (Habibzadeh *et al*, 2005). Nevertheless, there are some reports of leishmaniasis in the tongue of immunodeficient patients (Alrajhi *et al*, 1998; Iborra *et al*, 1998; Vazquez-Piñeiro *et al*, 1998).

It is well established that leishmaniasis can be a presenting feature of HIV and/or AIDS (Chaudhry *et al*, 1999). In Brazil, almost 100 cases of *Leishmania*/HIV co-infection has been reported (Rosatelli *et al*, 1998; Rabello *et al*, 2003). However, few reports describe oral manifestations of this disease. Impaired immune system, as well as HIV infection, results in increased susceptibility to leishmaniasis, frequently with an atypical course and a reduced response to treatment (Chaudhry *et al*, 1999). HIV immunosuppression facilitates intracellular growth of *Leishmania* in macrophages. Furthermore, *Leishmania* can induce the activation of HIV in latently infected monocytic and T cells (Wolday *et al*, 1999; Choi and Lerner, 2002). In Brazil, most of the cases of mucosal leishmaniasis caused by *L. (V.) braziliensis* occur in immunocompetent patients.

Several methods have been used for the diagnosis of leishmaniasis. Serological diagnosis such as

Montenegro's reaction can be helpful as an indicator of the prevalence of cutaneous and mucocutaneous leishmaniasis (Sassi *et al*, 1999; Singh and Sivakumar, 2003). In the tissue, leishmaniasis is characterized by a subepithelial non-necrotizing granulomatous inflammatory reaction with lymphocytes, plasma cells, and histiocytes. Inclusion within the cytoplasm of histiocytes seen in hematoxylin-eosin staining may suggest the possibility of leishmania, however, other organisms must be considered such as toxoplasma, and histoplasma. The identification of leishmania is possible by Giemsa stain and/or specific antibodies (Figure 5) (Hofman *et al*, 2003). Nevertheless, mucosal leishmaniasis can be difficult to diagnose, even when clinically active, because amastigotes usually are scarce. Other conventional methods for parasitological diagnosis include *in vitro* culture of infected tissue or inoculation into animals (Herwaldt, 1999). Species identification can be made by various molecular methods including PCR and monoclonal antibodies (Rodriguez *et al*, 1994; Wilson, 1995; Medeiros *et al*, 2002). In terms of mucosal leishmaniasis, reports in the literature have shown that the sensitivity of detection by PCR varied from 47.4% to 83.3% (Uezato *et al*, 1998; Pinero *et al*, 1999; Onuma *et al*, 2001). The detection of parasite DNA is sufficient for diagnosis purposes, however identification of parasite species may contribute for a better understanding of leishmaniasis (Disch *et al*, 2005; Oliveira *et al*, 2005).

In the laboratory of one of the authors (A.M.R.), PCR-RFLP has been used to identify species of *Leishmania* in the skin or mucous samples from patients with leishmaniasis (Garcia *et al*, 2005). In the present casuistry, in two of four cases analyzed, species identification was possible by PCR-RFLP using two restriction enzymes. The identification of *L. (L.) amazonensis* in one case of mucosal leishmaniasis was interesting, as *L. (V.) braziliensis* is the species more commonly found in Brazilian mucocutaneous leishmaniasis (Figure 6).

Six of our patients were positive in the serological test for Chagas' disease. This fact could be explained by the cross-reaction of the serological test between *Leishmania* and *Trypanosoma cruzi*. Several conventional serological tests as well as enzyme-linked immunosorbent assays, commercially available in Brazil, showed cross-reactivity to sera of patients with other diseases. These false-positive results are frequent in patients with leishmaniasis (Carvalho *et al*, 1993).

The treatment of choice calls for pentavalent ammonium compounds such as sodium stibogluconate and meglumin antimony. The Centers for Disease Control (CDC) and the World Health Organization (WHO) recommend 20 mg kg^{-1} of body weight per day to a ceiling of 850 mg day^{-1} for a minimum of 20 days and extending for at least 2 weeks after apparent parasitological cure (WHO, 1990). Contrary to skin lesions, oral lesions usually heal without significant scars. The DNA of *L. (V.) braziliensis* has been successfully demonstrated in the peripheral blood (Guevara *et al*, 1993) and in scars (Schubach *et al*, 2001) of patients many years after clinical cure (Schubach *et al*, 1998). The findings of *L. (V.) braziliensis* in healed cutaneous lesions and in the blood of cured patients lead us to suppose that some lesions can recur after long periods.

In summary, it is important to emphasize that, although uncommon, leishmaniasis can affect the oral mucosa and it can be the first sign of the disease. Although oral leishmaniasis affects mainly immunocompetent patients, it must be included as one of the infectious diseases of HIV-positive patients in endemic and non-endemic countries.

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