Exclusive Primary Lesion of Oral Leishmaniasis with Immunohistochemical Diagnosis

Tatiana Fernandes Araujo Almeida¹ · Esmeralda Maria da Silveira¹ ·
Cássio Roberto Rocha dos Santos¹ · Jorge Esquiche León² ·
Ana Terezinha Marques Mesquita¹

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Abstract A case of oral leishmaniasis without cutaneous involvement affecting the upper alveolar ridge mucosa/gingiva and the hard palate is reported in a 41-year-old Brazilian man. Microscopic examination disclosed scarce amastigotes and the definitive diagnosis was facilitated by immunohistochemical analysis. The clinical presentation of this lesion is unusual and underlies the importance of considering leishmaniasis in the differential diagnosis of oral lesions, especially in endemic areas. A literature review of the cases of mucosal leishmaniasis with exclusive primary lesions of the oral mucosa was also performed.

Keywords Immunohistochemistry · Infections · Leishmaniasis · Oral pathology · Parasites

Introduction

Leishmaniasis is a vector-borne disease caused by *Leishmania* species and transmitted by the bite of infected sandflies belonging to either *Phlebotomus* spp. (in Europe, North Africa, the Middle East, and Asia) or *Lutzomyia* spp. (from southern USA to northern Argentina) [1, 2].

Leishmaniasis is classified as cutaneous, mucocutaneous, and visceral or kala-azar, with a wide spectrum of clinical manifestations. *Leishmania peruviana*, *L. guyanensis*, and *L. braziliensis* cause the majority of cases of cutaneous leishmaniasis (CL), whereas *L. braziliensis*, followed by *L. panamensis* and *L. amazonensis*, are the principal etiological agents of mucosal leishmaniasis (ML), a chronic infiltrative disease of delayed onset that affects the upper airways and appears in up to 5.0 % of CL cases [3]. Thus, mucosal involvement by leishmaniasis is uncommon and results from hematogenous or lymphatic dissemination of amastigotes from the skin to the nasal, oropharyngeal, laryngeal, and/or tracheal mucosa. Moreover, there are a very few reported cases showing mucosal lesions alone. These latter cases can be difficult to diagnose, even when clinically active, because amastigotes are usually scarce [3, 4].

Oral leishmaniasis without cutaneous involvement is rare. We found only 19 cases of leishmaniasis, with involvement reported exclusively in the oral area in the English-language literature [3–13], and 6 cases in immunocompromised patients [3, 5–7]. These lesions usually appear as erythema and ulceration or as plaque, papules, and/or exophytic nodules, usually affecting the hard or soft palate and tongue. However, they can affect any site such as the lip, uvula, gingiva, tonsil, and retro-molar region [5, 6, 9, 11].

The case reported here presents an exclusive primary lesion of ML in the mouth and highlights the difficulties of this diagnosis, especially when only a small amount of parasites is detected by microscopic examination. In these cases, immunohistochemistry can be a valuable tool in order to establish the diagnosis. A literature review for cases of ML with exclusive primary lesions of the oral

✉ Tatiana Fernandes Araujo Almeida
tatiana.fernandes@hotmail.com

¹ Department of Dentistry, Clinical Stomatology, Federal University of the Jequitinhonha and Mucuri Valleys, Rua da Glória 187, Diamantina, Minas Gerais CEP: 39100-000, Brazil

² Oral Pathology, Department of Stomatology, Public Oral Health and Forensic Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

mucosa was also performed, addressing the methods of diagnosis and treatment.

Case Report

A 41-year-old Brazilian man was referred to the Oral Diagnosis Clinic of the Dental School, University of Diamantina, Minas Gerais, Brazil, complaining of lack of adaptation of an upper prosthesis over the last 4 months. The medical history was noncontributory and the extra-oral examination did not reveal alterations. The oral examination showed painless irregular erythematous and edematous plates with granulomatous surface on the upper alveolar ridge/gingiva and hard palate (Fig. 1a, b). The main differential diagnoses included paracoccidioidomycosis and leishmaniasis. Under local anesthesia, an incisional biopsy sample was obtained. The histopathologic examination on Hematoxylin & Eosin stain (H&E) slides showed pseudoepitheliomatous hyperplasia, associated with intense inflammatory cellular infiltrate containing lymphocytes, plasma cells and numerous histiocytes occasionally arranged in discrete granulomas (Fig. 2a, b). However, no microorganisms were detected on staining with periodic-acid Schiff, Grocott-Gomori, and Giemsa stains. After meticulous revision of the H&E slides, small foci of histiocytes containing scarcely apparent encapsulated microorganisms consistent with amastigotes of leishmaniasis in the subepithelial location were visualized (Fig. 2c), being confirmed after immunohistochemical (IHC) analysis for *L. braziliensis* (mouse monoclonal antibody, dilution 1:5000; Medical School of the University of São Paulo) which disclosed numerous parasites that were strongly stained (Fig. 2d). Moreover, Montenegro's reaction was positive. Together, the data led to the diagnosis of oral leishmaniasis. The treatment with N-methyl-glucamine (20 mg/kg/daily for 28 days) by an intramuscular route was started; the lesion improved within a few days, and remarkably so within 4 weeks. No adverse effects of treatment were observed. After 4 years of follow-up, the patient showed no signs of recurrence (Fig. 1c).

Discussion

Mucosal involvement is the most serious complication in *L. braziliensis* infections and it can lead to disfiguring and life-threatening disease in a varying proportion of patients. In most endemic areas, 1–10 % of localized CL result in ML 1–5 years after localized CL had healed, but reports do exist for which ML presented at the same time with CL [2]. The reason why few patients with CL subsequently develop ML is not known [14]. Leishmaniasis with exclusive and primary involvement of the mucosa is very rare, and in the oral cavity is an even more rare event [9], but it can occur by the fact that *L. braziliensis* spreads to distant sites during an early phase of infection, before the appearance of skin lesions [15].

The current case had only oral mucosa involvement, since he denied prior history of ulcerative lesion in skin or other mucosal sites, and the clinical examination did not find any scars in other sites of his body which could indicate prior leishmaniasis. Furthermore, there were no signs or symptoms of systemic involvement. Other authors have also reported cases of oral leishmaniasis without cutaneous involvement, and most of these cases were from Italy (5 cases, 26.0 %) and Spain (4 cases, 21.0 %). According to our review, of the 19 cases so far published of ML with oral involvement only, 16 (84.0 %) occurred in males with a mean age of 53 years, similar to the current case (Table 1).

In our review, including the current case of exclusive primary lesion of oral leishmaniasis, most occurred in tongue (35.0 %), followed by palate (20.0 %) and buccal mucosa (10.0 %). Furthermore, in 4 cases (20.0 %), multiple lesions involving different sites on the oral mucosa were observed. Ulcerated lesions were most frequent (11 cases, 55.0 %), which differs from the clinical feature of plates shown in the present case.

Mignogna et al. [9] reported that ML is rare in immunocompetent patients. However, in the current review (Table 1), we found that leishmaniasis with exclusive primary involvement of the oral mucosa was more frequent in immunocompetent patients (14 cases, 70.0 %). In

Fig. 1 **a** Clinical features of the lesion showing red plates in upper alveolar ridge, and **b** hard palate. **c** Clinical view after 4 years of follow-up

Fig. 2 **a** Microscopic features showing pseudoepitheliomatous hyperplasia and intense and diffuse lymphocytic infiltration. **b** Plasma cells and numerous histiocytes occasionally arranged in discrete granulomas. **c** A few microorganisms in the clear cytoplasm

of macrophages (*red arrows*), suggestive of amastigotes. **d** Immunohistochemical analysis identifying the species of *Leishmania* (*V. brasiliensis*; *inset* shows in detail the amastigotes strongly stained

immunocompromised patients, we found 6 cases (31.6 %), with 3 (15.8 %) in HIV-positive patients [3, 6] and diabetes, kidney transplant, and corticotherapy with one case each [5–7]. Interestingly, in all cases that were difficult to diagnosis, the patients were HIV-negative, whereas in HIV-positive patients the parasite was easily visualized in the tissues. This can be explained by the fact that immunological disturbances caused by HIV, such as nonfunctional T-lymphocytes, are particularly favorable for the uncontrolled multiplication of the parasite [16].

The diagnosis of leishmaniasis is reliably made by visualization of the parasites in tissue biopsy samples. On the histopathologic examination, leishmaniasis is characterized by a subepithelial granulomatous reaction containing variable amounts of lymphocytes, plasma cells, and neutrophils. Inclusion within the cytoplasm of histiocytes seen in H&E staining may suggest *Leishmania*; however, other organisms must be considered such as *Histoplasma*

and *Toxoplasma*. ML can be difficult to diagnose, even when clinically active, because amastigotes usually are scarce and they may not be seen in both H&E and Giemsa stains [17]. Although few cases of oral leishmaniasis without skin lesions have been reported, the majority of them had no difficulties in establishing the diagnosis. However, in the current case, even with the patient belonging to an endemic area, few parasites were detected. These findings may reflect the variability of the morphological features and immune response to *Leishmania* species.

Other methods that can be used to confirm the diagnosis include polymerase chain reaction (PCR), immunohistochemistry, immunofluorescence, parasite culture, animal inoculation, direct smear, skin test, and serologic assay [14]. Montenegro's test can be helpful; however, it fails to distinguish between past and present infections mainly in endemic areas [2]. Immunohistochemical analysis using

Table 1 Characteristics of 20 patients with exclusive primary lesion of oral leishmaniasis, literature review and present report case

Reference	Country	Age	Sex	Sites	Clinical features	Concomitant diseases	<i>Leishmania</i> species	Diagnostic method	Treatment
Aliaga et al. [5].	Spain	40	F	Gums and palate	Nodule	Kidney transplant	<i>Leishmania infantum</i>	H&E	Itraconazole and allopurinol
Van Damme et al. [13].	Netherlands	85	M	Palate	Ulcer	–	<i>Leishmania infantum</i>	H&E and Giemsa	NI
Habizadeh et al. [4].	Iran	40	M	Tongue	Fleshy mass	–	NI	H&E	Surgical excision
García de Marcos et al. [6].	Spain	70	M	Floor of mouth	Ulcer	Diabetes mellitus, corticotherapy	NI	IMF	Meglumine antimoniate, (20 mg/kg/day), for 28 days
	Spain	41	M	Buccal mucosa	Ulcer	HIV positive, tuberculosis	NI	H&E	Meglumine antimoniate, (20 mg/kg/day) for 28 days; HAART
	Spain	50	M	Upper alveolar ridge	Papules granular surface	HIV positive, hemophilia, duodenal ulcer	NI	H&E	NI
Motta Lopes et al. [3].	Guatemala	47	F	Hard and soft palate	Ulcer	HIV positive	NI	H&E	NI
Palmeiro et al. [11].	Brazil	75	M	Uvula and gums	Ulcer granular surface	–	<i>Leishmania braziliensis</i>	H&E	Meglumine antimoniate (5 mg/kg/day) for 20 days
Leitner et al. [8].	Austria	49	M	Tongue	Ulcer	–	<i>Leishmania donovani</i>	Giemsa and PCR	Liposomal amphotericin B (3 mg/kg/day) for 5 days
Pellicoli et al. [12].	Brazil	71	M	Hard and soft palate	Ulcer	Hypertension	NI	H&E and IHC	Liposomal amphotericin for 21 days
Kassam et al. [7].	England	66	M	Tongue	Ulcer	Airways disease corticotherapy	<i>Leishmania donovani/infantum</i>	H&E and PCR	150 mg miltefosine for 28 days
Passi et al. [10].	India	51	M	Gums and palate	Ulcer and nodule	–	NI	H&E and Giemsa	Oral miltefosine (100 mg day) and systemic sodium stibogluconate (20 mg/kg) for 28 days
Mignogna et al. [9].	Senegal	55	M	Tongue	Ulcer and nodule	–	NI	H&E	Meglumine antimoniate (5 mg kg/day) for 28 days
	Italy	48	M	Tongue	Nodule	–	NI	H&E	NI
	Pakistan	33	M	Tongue	Ulcer	–	NI	H&E	Meglumine antimoniate (5 mg kg/day) for 20 days
	Italy	41	M	Tongue	Multinodular	–	NI	H&E	Surgical excision and stibogluconate (20 mg/kg/day) for 20 days
	Italy	61	F	Upper lip	Swelling	–	NI	H&E	Meglumine antimoniate (5 mg/kg/day) for 40 days
	Italy	66	M	Hard and soft palate	Plates	–	NI	H&E	Meglumine antimoniate (5 mg/kg/day) for 40 days
	Italy	31	M	Buccal mucosa	Exophytic lesion	–	NI	H&E	Meglumine antimoniate (5 mg/kg/day) for 20 days
Present case	Brazil	41	M	Gums and palate	Plates	–	<i>Leishmania braziliensis</i>	H&E, IHC	N-methyl-glucamine (20 mg/kg/day) for 28 days

F Female, H&E Hematoxylin & Eosin, IHC Immunohistochemical analysis, NI no information, IMF immunofluorescence, M male, PCR polymerase chain reaction

Leishmania specific monoclonal antibodies is more accessible in our region. Studies have demonstrated a higher level of sensitivity of this technique in the identification of amastigotes. The percentage of positivity ranges between 51.0 and 88.0 % [18]. Beena et al. [19] studied 50 cases of postkala-azar dermal leishmaniasis with either H&E stain or IHC analysis, and amastigotes were identified in 50.0 % of samples with the H&E stain, whereas IHC study detected them in 80.0 % of cases. It has been suggested that IHC analysis seems to be more useful than conventional H&E stain for identifying amastigotes. In the present case, IHC analysis was helpful for the diagnosis of leishmaniasis in earlier stage allowing correct and prompt treatment, as well as a better prognosis.

The treatment of choice for ML is the administration of pentavalent antimonial drugs, such as meglumine antimoniate, or stibogluconate [20]. Here, we find that most cases (55.0 %) were treated with pentavalent drugs for 20 to 40 days, but other treatments were used including amphotericin B and miltefosine. According to Mignogna et al. [9], patients affected by ML should be managed to achieve complete healing and a long follow-up period without signs or symptoms, the possibility of relapses has to be considered. In the present case, there was no recurrence after a follow-up of 4 years.

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. IHC analysis can be used as a supplementary tool in order to confirm the diagnosis.

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