



Microscopical Diagnosis of Oral Leishmaniasis: Kinetoplast

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Received: 21 January 2021 / Accepted: 5 February 2021 / Published online: 18 February 2021
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To the Editors,

Recently, we have read with interest the article intitled “tongue nodule as primary manifestation of american cutaneous leishmaniasis in an immunocompetent patient” [1], which describes an unusual case of lingual leishmaniasis affecting a 57-year-old man. After incisional biopsy, microscopy showed a dense chronic inflammatory reaction with macrophages containing scarce round-shaped leishmania-like intracytoplasmic inclusions, suggesting the diagnosis of leishmaniasis. Histochemical analysis (Grocott methenamine silver stain [GMS], periodic acid-Schiff [PAS] stain) was not reported. DNA extraction was performed from paraffin-embedded tissue, and subjected to PCR assays with Leishmania-specific PCR primers, which confirmed the diagnosis of leishmaniasis.

Here, we would like to contribute with an additional case of leishmaniasis, which affected the buccal mucosa of a 65-year-old man. The medical history was noncontributory, including the absence of insect bite report, skin lesion and systemic disease. After incisional biopsy, an exuberant chronic granulomatous inflammation was observed, in which numerous macrophages with clear cytoplasm contained round-shaped intracellular inclusions. The main histopathological differential diagnoses were histoplasmosis and leishmaniasis. However, PAS and GMS stains were negative, excluding histoplasmosis. A detailed microscopic study revealed several amastigotes containing the diagnostic kinetoplast (Fig. 1). Similar with the case reported by Botelho

et al. (2020) [1], the PCR assays confirmed the diagnosis of leishmaniasis. The patient was referred to infectious disease service. Currently, he is well, with no alterations in the lesional area.

Leishmaniasis with exclusive involvement of the oral mucosa has been reported [2]. This clinical presentation is very rare. However, some studies indicate that during an early phase of *Leishmania braziliensis* infection, the parasite spreads to distant sites, before the appearance of cutaneous lesions [2], which can explain the clinical presentation of the current case.

The kinetoplast is a diagnostic structure of the Kinetoplastida order, which encompasses the Trypanosomatidae family. This family comprises species of several genera, including *Angomonas*, *Strigomonas*, *Crithidia*, *Leishmania*, *Trypanosoma*, among others. The kinetoplast is a specialized region of the mitochondria harboring the most complex and unusual mitochondrial DNA [3]. The kinetoplast DNA (kDNA) represents approximately 30% of the total cellular DNA [3].

An interesting study of mucosal leishmaniasis [4] reported that the diagnostic kinetoplast of amastigotes are rarely identifiable in histopathological slides. Similarly, the kinetoplast of trypanosomal amastigotes of Chagas disease are difficult to see in histopathological sections [5]. However, if identified, it becomes an important microscopic finding with diagnostic impact, such as observed in the current case. Relevantly, the amastigotes of leishmaniasis and Chagas disease are identical in morphology, being differentiated by cellular location. *Leishmania* usually concentrates within macrophages, whereas *Trypanosoma cruzi* has a predilection for myocyte fiber. Moreover, oral involvement by Chagas disease is extremely rare, a clinical finding that aids in the differential diagnosis.

Such as above commented, the diagnosis of histoplasmosis was initially considered as a strong possibility in the current case. However, PAS and GMS stain were negative, with PCR assays confirming the diagnosis of leishmaniasis. In addition to PCR assays, the immunohistochemistry, using

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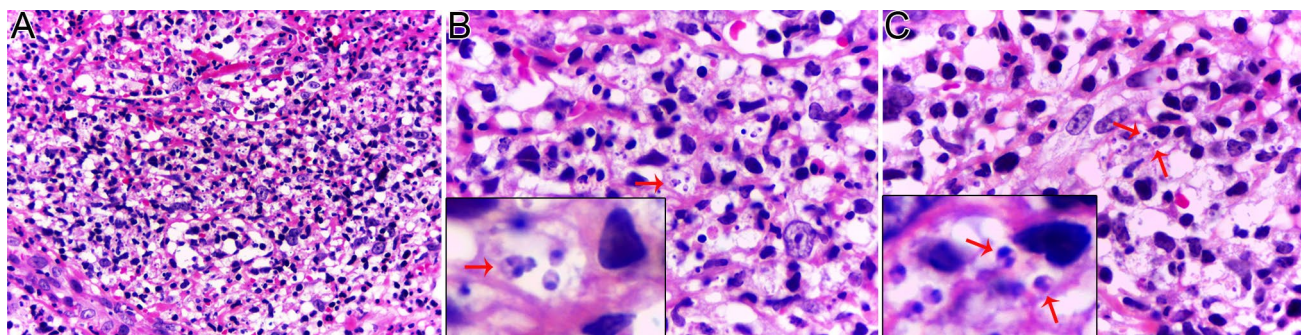


Fig. 1 Histopathological findings of oral leishmaniasis. An exuberant granulomatous inflammatory response was observed, showing numerous macrophages containing round-shaped leishmania-like intracytoplasmic inclusions (**a** $\times 40$). In high-power view, notice sev-

eral round-shaped intracytoplasmic inclusions exhibiting the diagnostic kinetoplast (arrows) (**b** and **c** $\times 100$, oil immersion lens). The areas indicated by the arrows (**b** and **c**) are shown in detail in the respective inset. The inset shows (arrow) typical kinetoplast

Leishmania specific monoclonal antibodies, is also another important tool that supports the diagnosis of leishmaniasis with high sensitivity and specificity [2]. Furthermore, it is important to mention that Histoplasma shows lack of kinetoplast, similar to that observed with Toxoplasma [5]. In addition, unlike histoplasmosis, oral involvement by toxoplasmosis is rare, and GMS stain positivity excludes the diagnosis of toxoplasmosis.

In our experience, the Giemsa and Toluidine blue stains often show equivocal results, when assessing histopathological sections of suspected leishmaniasis cases.

In summary, the current case shows that the detection of kinetoplast in intracytoplasmic round-shaped inclusions inside macrophages, when assessing histopathological sections of paraffin-embedded tissue, strongly assists in the diagnosis of leishmaniasis.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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