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Case Report

Localized mucosal leishmaniasis caused by *Leishmania infantum* mimicking cancer in the rhinolaryngeal region

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SUMMARY

The clinical, microbiological, and histopathological findings of six patients with mucosal leishmaniasis are reported. Five of these patients were Spanish with no history of travel abroad, while the other was from Bolivia but had lived in Spain for more than 5 years. Two patients had no underlying disease, while the other four had several other medical conditions. Lesions were located in the nose in three patients and in the larynx in the other three. Symptoms included difficulty in swallowing, nasal obstruction, dysphonia, and polypoid lesions mimicking cancer. The diagnosis was based on the identification of parasites, or on PCR assay or culture. Five patients were treated with liposomal amphotericin B and the other with antimonial compounds.

1. Introduction

Mucosal leishmaniasis (ML) is characterized by mucosal destruction, with mucous membrane involvement of the nose, oral cavity, pharynx, or larynx. The symptomatology of the disease depends on the localization of lesions and may include nasal obstruction, swallowing difficulties, mucosal bleeding, and/or hoarseness.

Most ML cases are observed in Latin America and are due to *Leishmania braziliensis*,[1] and ML rarely results from infection by other *Leishmania* species. However, there have been a few reports that *Leishmania infantum* may cause localized mucosal disease in the absence of concomitant visceral or cutaneous leishmaniasis.[2] These lesions often mimic cancers, with no samples being sent for microbiological diagnosis.

Six cases of ML caused by *L. infantum* are reported, highlighting the need for a correct differential diagnosis of this entity.

2. Methods

Six patients were diagnosed with ML by the microbiology laboratory of Virgen de las Nieves University Hospital (Granada, Spain) between 2010 and 2013. All patients gave their informed consent for inclusion in this study. At the time of diagnosis, these patients were living in Granada (Andalusia), an area endemic for leishmaniasis in Spain. The initial diagnosis was made in the hospital by visualization of Leishmania amastigotes in Giemsa-stained smears in four of six patients (the results of an in-house nucleic acid amplification test (NAAT) were negative for these patients) and by in-house NAAT for the other two. Culture results were positive for all patients.

All isolates were then sent to the National Centre of Microbiology (Majadahonda, Madrid, Spain) for species identification, which was carried out with a specific nested PCR for *L. infantum*.[3] This PCR consists of a first amplification with Kinetoplastida-specific primers R221 and R332, followed by a second amplification with *Leishmania*-specific primers R223 and R333. Positive and negative controls were included.[3] For Leishmania culture, clinical samples were inoculated into minimum

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Table 1
Main characteristics of the six patients with mucosal leishmaniasis caused by *Leishmania infantum*

	Patient					
	1	2	3	4	5	6
Age (years)/sex	49/M	43/M	66/F	42/M	67/F	36/M
Country of origin	Spain	Spain	Spain	Bolivia	Spain	Spain
Travel history	No	No	No	No	Cyprus, Tunis	No
Concomitant disease or condition	Hypercholesterolemia, drinker	HIV + HCV + ex IVDU	Rheumatoid arthritis	No	Diabetes mellitus, arterial hypertension, asthma, steroid treatment	No
Location of infection	Nose	Nose	Nose	Soft palate, larynx	Larynx	Larynx
Clinical manifestation	Nasal obstruction, crusted lesions in nose	Painful lesions of the nasal vestibule	Nasal cellulitis	Odynophagia, dysphonia, weight loss	Dysphonia, dyspnoea, cough	Dysphonia, tumour lesion in the larynx
Biopsy of the lesions (stain)	Positive, Giemsa	Positive, Giemsa	Negative	Negative	Positive, Giemsa	Positive, Giemsa
Histopathology	Non-specific inflammatory infiltrate	Non-specific inflammatory infiltrate	Granulomatous inflammatory reaction	Granulomatous inflammatory reaction	Granulomatous inflammatory reaction	Granulomatous inflammatory reaction
Culture	Positive	Positive	Positive	Positive	Positive	Positive
PCR	Positive[a]	Positive[a]	Positive[b]	Positive[b]	Positive[a]	Positive[a]
Treatment	Liposomal amphotericin B	Liposomal amphotericin B	Liposomal amphotericin B, meglumine antimoniate	Liposomal amphotericin B	Liposomal amphotericin B	Meglumine antimoniate
Serology	ND	ND	ND	1/80 (IFAT)	ND	Negative (IFAT)

M, male; F, female; HIV, human immunodeficiency virus; HCV, hepatitis C virus; IVDU, intravenous drug abuser; PCR, polymerase chain reaction; ND, not done; IFAT, immunofluorescence antibody test.
[a] Positive at the National Centre of Microbiology (Madrid, Spain) by nested PCR.
[b] Positive at the authors' laboratory by in-house nucleic acid amplification tests and further confirmed at the National Centre of Microbiology.

essential medium Eagle modified (ICN Biomedicals, OH, USA) supplemented with 20% foetal calf serum.[4]

Table 1 shows the characteristics of the six patients with ML due to *L. infantum*.

3. Case reports

3.1. Case 1

A 48-year-old Spanish man required medical attention for nasal obstruction, inflammation, and crusted lesions in the nasal mucosa. He was a former smoker and current moderate drinker. He had two dogs, one of which had leishmaniasis. Physical examination revealed an erythematous lesion in the nasal wing and cheek but no other findings of interest. A complete blood count, urinalysis, and chemical profile were normal.

Histological examinations of a biopsy of the lesion revealed a non-specific inflammatory infiltrate with *Leishmania* species amastigotes in the tissue. Culture of the biopsy specimen was also positive for Leishmania. The lesions disappeared after treatment with liposomal amphotericin B (300 mg/day) for 9 days.

3.2. Case 2

A 43-year-old Spanish man with no history of foreign travel was evaluated for a painful lesion of the nasal vestibule extending to the upper lip. The patient was diagnosed with HIV infection and had positive hepatitis C virus (HCV) antibodies. He was undergoing antiretroviral treatment and had a viral load of 199 239 copies/ml (5.30 log). His CD4+ T-cell count was 181 (9%). A complete blood count, urinalysis, and chemical profile were all normal.

Histological examinations of a biopsy of the lesion revealed a non-specific inflammatory infiltrate with the presence of *Leishmania* species amastigotes in the tissue. Culture of the biopsy specimen was also positive for Leishmania. The patient received liposomal amphotericin B (300 mg/day) for 9 days, with a positive outcome.

3.3. Case 3

A 66-year-old Spanish woman with no history of foreign travel was evaluated for nasal cellulitis involving the left wing of the nose. She was receiving treatment with steroids and infliximab for rheumatoid arthritis. A complete blood count and chemical profile were normal. Histological examinations of three biopsies revealed a granulomatous inflammatory reaction with no amastigote visualization. Culture from a new biopsy grew promastigotes of Leishmania. A nested PCR was also performed, with a positive result.

Treatment started with liposomal amphotericin B (300 mg/day), but resulted in arterial hypertension and lumbar pain; the treatment was therefore changed to meglumine antimoniate (20 mg/kg per day). The lesion disappeared after 3 weeks of this treatment.

3.4. Case 4

A 42-year-old Bolivian man, a resident of Spain since 2004, was referred in 2010 for suspected cancer after the detection of a papillomatous lesion located in the soft palate and larynx. Symptoms included odynophagia and dysphonia with weight loss.

A complete blood count and chemical profile were normal. Anti-Leishmania antibodies were detected at a titre of 1:80 by indirect immunofluorescence assay test.

A granulomatous chronic inflammatory infiltrate was observed in biopsies of the oral lesions. Histological examinations with Giemsa staining revealed no intracellular amastigotes of Leishmania. A new biopsy was taken and a nested PCR was performed, with a positive result. Culture from this tissue grew promastigotes of Leishmania.

The patient was treated with liposomal amphotericin B (200 mg/week) for 5 weeks, with no improvement. A new cycle

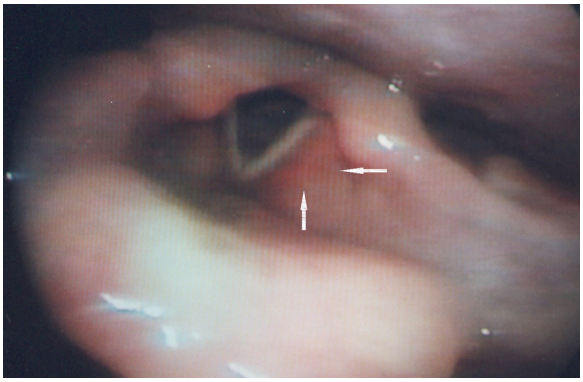

Figure 1. Indirect laryngoscopy showing a tumour-like lesion in the larynx of the patient (case 6) after a 7-month period with dysphonia.

of treatment with liposomal amphotericin B (300 mg/day) achieved total resolution of the lesions.

3.5. Case 5

A 67-year-old Spanish woman was referred with a 6-month history of dysphonia and a 1-week history of cough and dyspnoea. The patient had diabetes mellitus and was undergoing treatment with steroids for asthma. She had travelled to Tunis and Cyprus several years earlier.

Indirect laryngoscopy showed multiple lesions with a granulomatous appearance in the larynx. A biopsy of the lesions showed a granulomatous inflammatory infiltrate with Giemsa-stained Leishmania amastigotes. Culture of the biopsy specimen was also positive for promastigotes of Leishmania.

The lesions disappeared after 5 weeks of treatment with liposomal amphotericin B (300 mg/day).

3.6. Case 6

A 36-year-old Spanish man with diabetes mellitus and asthma was evaluated after a 7-month period with dysphonia. He reported no history of foreign travel. Indirect laryngoscopy showed a lesion with a tumour-like appearance (Figure 1), and a biopsy was then performed. Direct examination of the Giemsa-stained sample showed abundant Leishmania amastigotes, and culture was positive for Leishmania. A granulomatous inflammatory infiltrate was also observed.

An indirect immunofluorescence assay test for Leishmania was negative. Bone marrow aspiration for parasite examination was also negative. A complete blood count, chemical profile, urinalysis, and cell immunity study were all normal.

The lesions disappeared after treatment with meglumine antimoniate (850 mg/day) for 28 days.

4. Discussion

Mucosal leishmaniasis is a rare disease even in endemic areas. It is usually encountered in Latin America,[2] but has occasionally been recorded in other locations.[5]

L. infantum infections are endemic in the Mediterranean area and may cause localized mucosal disease, even in the absence of concomitant visceral or cutaneous leishmaniasis.

In the present study, dysphonia was observed in the three individuals with laryngeal lesions, while nasal alterations were recorded in the other three. The disease was only considered severe in one patient who suffered from acute dysphonia due to laryngeal involvement.

The diagnosis of ML is usually established by the detection of Leishmania amastigotes in Giemsa-stained lesion smears or by the observation of promastigotes in cultured tissue samples.[6] However, there are sometimes too few parasites in the lesions to be detected by microscopy or culture. In these cases, PCR-based assays offer highly sensitive and specific detection of the Leishmania parasite. In this study, PCR was only performed for the two patients with a negative microscopic examination. Leishmania amastigotes were demonstrated by Giemsa stain in the remaining cases.

Serology is rarely used as a diagnostic technique in ML, because the sensitivity and specificity can be variable and the number of circulating antibodies against Leishmania tends to be low. An indirect immunofluorescence assay for anti-Leishmania antibodies was done for two of the patients, but was positive for only one of them, at a titre of 1:80.

Histopathological study of the lesions can often contribute to the diagnosis of a Leishmania infection, even when no Leishmania amastigotes are detected by microscopy.[7] In all of the present patients, the macroscopic appearance of the lesions led to an initial clinical suspicion of cancer. These patients need to be examined carefully to rule out cancer and other diseases. However, patients with mucosal lesions that have a mixed inflammatory infiltrate should be considered potential cases of leishmaniasis in those living in or visiting endemic areas. If a Leishmania infection is suspected, a sample should be sent to the microbiology laboratory for differential diagnosis. In four of the study patients, histopathology showed a granulomatous appearance but no cancer cells were observed, and no Leishmania amastigotes were found in two of these. This emphasizes the usefulness of the histological study of lesions for the diagnosis of this infection.

Pentavalent antimonial drugs may be used as treatment, but because of several serious side-effects and their variable efficacy against ML, alternative treatment regimens can be used, such as liposomal amphotericin B or pentamidine. Five of the study patients were treated with liposomal amphotericin B, although this was changed to pentavalent antimonials in one case due to adverse effects. Treatment was successful in all patients.

In conclusion, leishmaniasis caused by *L. infantum* should be considered in the differential diagnosis of patients travelling to or residing in an endemic area and who have mucosal lesions and some of the rhinolaryngeal symptoms reported above. In these cases, a biopsy of the affected tissues should be taken for microbiological and histopathological diagnosis in order to allow the prompt initiation of anti-Leishmania treatment.

Conflict of interest: The authors declare no conflict of interest.

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