# ORAL DISEASES

## REVIEW ARTICLE

# Mucosal leishmaniasis with primary oral involvement: a case series and a review of the literature

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OBJECTIVE: To analyze retrospectively a case series of primary oral leishmaniasis and to review the literature on head–neck primary mucosal leishmaniasis (ML) in immunocompetent patients.

SUBJECTS AND METHODS: A PUBMED search was carried out from 1950 to 2013. Clinical records of patients with primary head–neck mucosal manifestations of leishmaniasis were analyzed. In addition, clinical records between 2001 and 2012 of patients with primary oral manifestations were collected in two independent hospitals.

RESULTS: Our multicenter case series revealed seven patients with oral leishmaniasis. The most commonly affected site was the tongue (four patients, 57%), and the most common clinical presentation was an exophytic lesion (six patients, 85%). The literature review showed 11 reports published between 2005 and 2013, describing 13 patients (100% male) affected by head–neck primary ML (54% laryngeal, 31% oral, 23% pharyngeal, and 15% endonasal). The most common clinical presentation was an exophytic lesion (69%).

CONCLUSIONS: The literature analysis revealed that in immunocompetent patients, the oral mucosa is the second most frequently affected site of the head and neck region. In the oral cavity, the tongue is the most affected site. Diagnosis of oral leishmaniasis represents a challenge but must be considered in any differential diagnosis of exophytic lesions of oral mucosa.

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## Introduction

Leishmaniasis is a parasitic disease caused by several protozoan species of the genus *Leishmania*, belonging to the family Trypanosomatidae. After malaria and African trypanosomiasis ('sleeping sickness'), the leishmaniases are the third most important group of vectorborne diseases and are ranked ninth in terms of the global burden of disease of all infectious and parasitic diseases (Prabhu *et al*, 1992; Stockdale and Newton, 2013).

The leishmaniases are widely dispersed, with transmission to humans on five continents, and are endemic in 98 countries. However, the human disease burden is concentrated mainly in a few major foci. The epidemiological data on leishmaniasis are very complex, with intra- and interspecific variations in transmission cycles, reservoir hosts, sand fly vectors, and clinical forms (World Health Organization, 2010).

Travelers and soldiers who have to stay in endemic areas, foreign citizens, migrant workers, asylum seekers, refugees from endemic areas, migrants, and immunosuppressed patients are considered as risk groups (Grimaldi and Schottelius, 2001).

In humans, the clinical forms of the leishmaniases are broadly categorized into three groups: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucosal leishmaniasis (ML). However, a more complete classification encompasses 11 different clinical forms (World Health Organization, 2010). Among these, mucocutaneous leishmaniasis (MCL) or ML pure forms can occur with a latency of months, even years, after exposure in endemic areas and because of this huge latency, the diagnosis is often seriously delayed. All the clinical forms of leishmaniasis can start with primary mucosal lesions in the head–neck region, sometimes affecting the oral cavity, or certain significant symptoms detectable by the dental practitioner. Such symptoms include swallowing difficulties, dysphonia, and dyspnea. Therefore, dentists play an important role in the early diagnosis of oral leishmaniasis, to avoid the systemic spread of the disease (Pellicioli *et al*, 2012).

In any such systemic spread, the sites most commonly involved are the liver, spleen, abdominal lymphatic system, lymph nodes, and bone marrow.

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Management of leishmaniasis still represents a big challenge. Presently, the main target of any drugs used is to fight the parasite in its different physiological and biochemical aspects and to promote the immune response of the host. The therapeutic strategies currently available include pentavalent antimony derivatives, systemically or intralesionally administered, pentamidine, metronidazole, amphotericin B, azoles, and miltefosine (Masmoudi *et al*, 2013).

The primary objective of this study is to present a retrospective multicenter case series describing seven cases of primary oral leishmaniasis. The secondary objective is to review the literature on head and neck primary ML in immunocompetent/otherwise healthy patients, as no similar data are currently available in the literature, and to update the current literature with our seven new cases.

## Patients and methods

### Multicenter case series assessment

We retrospectively selected and analyzed the clinical data of leishmaniasis patients from the archives of two independent hospitals, the outpatient clinic of the Oral Medicine Unit, Department of Head and Neck Diseases, Federico II University of Naples and the Department of Dental Sciences and Surgery, University of Bari, reporting seven new cases with a history of ML with exclusive and primary oral involvement, diagnosed and treated between 2001 and 2012.

The study was approved by the Ethics Committee of University of Naples 'Federico II'.

All patients, after providing their written informed consent, were hospitalized and examined by routine hematological testing, HIV, HBV, HCV, and oral biopsies with histopathological evaluation. Abdominal CT and bone marrow biopsies were made in some cases to exclude any suspected visceral forms. The study was performed in accordance with the Declaration of Helsinki.

### Literature review

Subsequently, we retrospectively reviewed all articles from 1950 to 2013 focused on cases of ML in immunocompetent/otherwise healthy patients, by searching in the PubMed database on each of the following keywords: 'mucosal', 'oral', 'buccal', 'nose', 'nasal', 'pharynx', 'pharyngeal', 'esophagus', 'esophageal', 'larynx', 'laryngeal' in association with 'leishmaniasis', and alternatively with 'healthy' and 'immunocompetent'.

The inclusion criteria of this review encompass (i) articles published in the English language reporting primary ML for all patients described; (ii) healthy or immunocompetent patients who contracted an infection of leishmaniasis between 1950 and 2013; (iii) mucosal lesions that presented in the regions of the nasal mucosa, oral mucosa, nasopharynx, oropharynx, hypopharynx, esophageal, and laryngeal mucosa; (iv) the absence of any visceral or cutaneous manifestations; and 5) documentation on the leishmania infection.

A flow chart is shown in Figure 1.

In relation to the reviewed articles, we collected, tabulated, and depicted the following information: country and
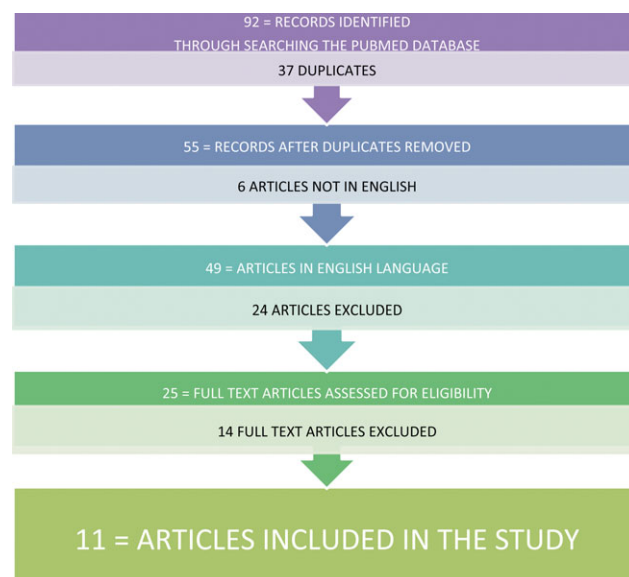


**Figure 1** Flow chart

year of publication, age, sex, underlying or past diseases, predisposing risk factors, local conditions, involved sites, symptoms, lesion description, diagnostic procedures, treatment, follow-up period, and outcomes.

## Results

The case series from our database shows seven patients, three of whom were treated at our institution.

All the patients underwent incisional or excisional biopsy (cases 2, 4, and 7), and in all cases, the biopsy showed inflammatory lesions, with the presence of parasites belonging to *Leishmania* spp., allowing the diagnosis of leishmaniasis. Subsequently, all patients underwent routine laboratory examinations, CT scanning, and bone marrow biopsy, revealing no significant findings or evidence of leishmaniasis elsewhere except for splenomegaly in case 5 (Figures 2–6).

### Case reports

Case 1 – A 55-year-old Caucasian man with a history of a 4-week painful swelling affecting the left side of the tongue. The patient had lived in Senegal for 20 years and was a smoker (25 cigarettes daily for 30 years).

Physical examination showed a nodular, whitish, and ulcerated lesion on the left edge of the tongue, 1.7 cm of maximum diameter, with some mild perilesional fissurations.

The patient was treated with 28 intramuscular injections of meglumine antimoniate at a daily dose of 5 mg kg$^{-1}$ body weight.

After 1 month, the patient had a complete remission of the tongue lesion, but after 8 months, the disease recurred with the onset of a cutaneous form of leishmaniasis. The patient was referred to the Infectious Diseases Department for treatment.

Case 2 – A 48-year-old Caucasian man with a history of a 7-week painful lesion of the tongue. The patient was

**Figure 2** Clinical aspect of the Case n. 1



**Figure 3** Clinical aspect of the Case n. 2



**Figure 4** Clinical aspect of the Case n. 5
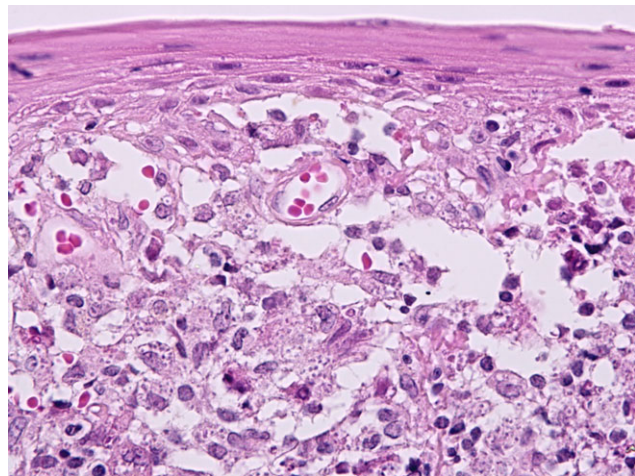


**Figure 5** Clinical aspect of the Case n. 7



**Figure 6** (Case n. 1) Histopathological examination (Hematoxylin-Eosin, X400) of the lingual lesion showed disruption of the basal-intermediate epithelial layers due to inflammatory infiltration (lymphocytes, macrophages and focal plasma cells), intra- and extra-cellular *Leishmania* spp. amastigotes and endothelial damage

a former smoker (40 cigarettes daily for 25 years), and 2 years before, he had been treated with polychemotherapy for B-cell lymphoblastic NHL.

Physical examination revealed a nodular and erythematous lesion, with a hard consistency and a mild bleeding upon palpation, affecting the left lateral border of the tongue, near the tongue tip.

Surgical treatment with an excisional biopsy was performed. The patient refused the recommended pharmacological treatment.

After the clinical healing, the patient had a 7-month follow-up period but subsequently developed a visceral form of leishmaniasis characterized at onset by fever, diarrhea, hepatosplenomegaly, and lymphadenopathy. The patient was referred to the Infectious Diseases Department for treatment.

Case 3 – A 33-year-old man from Pakistan with a history of a 7-week swelling and soreness of the tongue with a difficulty in swallowing and 4-kg weight loss.

Physical examination revealed an ulcerated, soft-to-firm, and dome-shaped mass of the dorsum of the tongue. The largest diameter was approximately 3.5 cm.

No skin lesions were observed. The patient was treated with 20 intramuscular injections of meglumine antimoniate at a daily dose of 5 mg kg$^{-1}$ body weight.

After 1 month, the patient had a complete remission of the tongue lesion; after 13 months, the disease recurred

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with the onset of a cutaneous form of leishmaniasis. The patient was referred to the Dermatological Department for treatment.

Case 4 – A 41-year-old Caucasian man with a history of an 8-week asymptomatic lesion affecting the dorsum of the tongue.

The man was a farmer, and 4 weeks before the appearance of the lesion, he had undergone two courses of antibiotics for a prostatitis.

Physical examination showed a multinodular lesion affecting the posterior dorsum of the tongue, just anterior to the circumvallate papillae, with an increased consistency and a maximum diameter of 2 cm.

After excisional surgical treatment, the patient was treated with stibogluconate 20 mg kg$^{-1}$ day$^{-1}$ for 20 days and had a complete healing with a negative 12-month follow-up.

Case 5 – A 61-year-old Caucasian woman with a history of a 5-week diffuse and sore swelling of the upper lip.

The woman was from Ischia, a well-known endemic area of leishmaniasis in Italy. She was in good health except for a reported splenomegaly, confirmed by abdominal CT scan.

Physical examination showed a diffuse swelling of the upper lip measuring approximately 4 × 1.5 cm with ulcers and erosions extending to the anterior maxillary vestibule.

Two previous biopsies, performed in others hospitals, had been negative.

We performed a third biopsy that showed an inflammatory reaction and some parasites in the cytoplasm of the histiocytes, allowing us to establish the diagnosis.

Subsequently, the patient was treated with meglumine antimoniate at a daily dose of 5 mg kg$^{-1}$ body weight. After 10 days, clinical improvement was present, and after 40 days, the patient had a complete healing.

After 2 years, she was referred to the Internal Medicine Department because of a para-ovarian abdominal mass: histopathology revealed a visceral form of leishmaniasis. The patient was referred to the Infectious Diseases Department for treatment.

Case 6 – A 66-year-old Caucasian man with a history of painful and tender lesion affecting the hard and soft palatal mucosa for several weeks. About 20 years before, he had been referred to the Department of Respiratory Diseases, Cotugno Hospital, Naples, Italy, because of a not specified pulmonary disease.

Physical examination showed a widespread, irregular, and granular plaque involving the hard palatal mucosa and extending to the soft palate, left maxillary gingiva, and alveolar mucosa. The lesion was also characterized by erythema, and multiple ulcerations mainly localized on the hard palatal mucosa.

The patient was treated with meglumine antimoniate at a daily dose of 5 mg kg$^{-1}$ body weight for 6 weeks, and subsequently, the lesions healed. The follow-up period was 30 months, and the result was negative.

Case 7 – A 31-year-old Caucasian man with a history of a 4-week painful lesion affecting the buccal mucosa.

Physical examination revealed an exophytic and well-defined lesion of the left buccal mucosa.

This mucosal-colored plaque bled easily and had an increased consistency and a largest diameter of approximately 2.2 × 1.5 cm.

The patient was treated with meglumine antimoniate at a daily dose of 5 mg kg$^{-1}$ body weight for 20 days. The follow-up period was 8 months, and the result was negative.

The case series from our retrospective study revealed seven patients with oral leishmaniasis, six of whom (85%) were men and one a woman with a mean (±SD) age at the time of diagnosis of the disease of 48 (±14) years.

The most commonly affected site was the tongue (four patients, 57%), followed by one case each on the lip, palatal mucosa, and buccal mucosa.

The clinical aspects presented were exophytic lesions in six patients (85%) and an ulcerated lesion in one patient (14%).

The analysis of the literature review revealed eleven articles published between 2005 and 2013, from five different countries, with a total of thirteen patients described. All the patients were male (100%), and their mean (±SD) age at the time of diagnosis of the disease was 56 (±14) years.

The largest number of cases was from Italy (six patients, 46%). Other patients came from Iran (three cases, 23%) and Brazil (two cases, 15%). The other two cases were from England and India. All the clinical results from the review are summarized in Tables 1–3.

The treatment was specified in only eleven of the thirteen cases. The most common treatment was amphotericin B (five cases, 45%), followed by meglumine antimoniate (three cases, 27%). One case each (9%) was treated with stibogluconate, miltefosine, and surgery. The healing times of the lesions were not available in detail, but the data on the outcomes, as well as the diagnostic criteria and follow-up, are summarized in Table 4.

Including our case series, the total number of Italian cases in literature has increased to 13.

No significant differences were found between our six cases (exclude Case 3 from Pakistan) and other cases from Italy found in the literature review in terms of gender, age, and response to treatment, except in relation to the affected sites.

In fact, in the review, there were no Italian cases of primary oral ML.

## Discussion

The leishmaniases are a group of infectious diseases caused by obligate intracellular protozoan parasites of the genus *Leishmania* that continue to be an increasing worldwide threat. Approximately 58 000 cases of VL and 220 000 cases of CL are officially reported each year. Based on assessments of under-reporting, 0.2–0.4 million new cases of VL and 0.7–1.2 million new cases of CL are estimated to occur every year (Desjeux, 1996; Alvar et al, 2012). The WHO estimates that 350 million people are at risk of contracting leishmaniasis (World Health Organization, 2010). These data are troubling considering that the disease causes significant morbidity and mortality accounting for more than 57 000 deaths per year and an estimated

**Table 1** Data of 13 patients from pubmed search: epidemiology and predisposing factors

| Reference | Country | Age | Sex | Underlying or past diseases | Predisposing risk factors | Local impairment |
|---|---|---|---|---|---|---|
| Palmeiro *et al* (2007) | Brazil | 75 | M | None | Endemic area Farmer | Deficient oral hygiene, poor tooth conservation |
| Pellicioli *et al* (2012) | Brazil | 71 | M | Hypertension (atenolol and hydrochlorothiazide treated) | Farmer | – |
| Cocuzza *et al* (2013) | Italy | 64 | M | (COPD) since 20 years. Hypersensitivity to non-steroidal treatment Anti-inflammatory | Endemic area | Former smoker (25 cig day$^{-1}$) |
| Oryan (2013) | Iran | 42 | M | None | Endemic area | – |
| Oryan (2013) | Iran | 32 | M | None | Endemic area | – |
| Pau *et al* (2009) | Italy | 52 | M | Hypertension (by ACE inhibitors treated) | Endemic area, Shepherd | None |
| Pau *et al* (2009) | Italy | 71 | M | Ischemic cardiopathy (coronary by-pass surgery 2002) | Endemic area, Farmer | None |
| Habibzadeh *et al* (2005) | Iran | 40 | M | None | Endemic area | None |
| Guddo *et al* (2005) | Italy | 59 | M | COPD, Low level of IgM (28.8 mg 100 ml$^{-1}$) | Endemic area | Smoker |
| Casolari (2005) | Italy | 53 | M | None | Endemic area | – |
| Tiseo *et al* (2008) | Italy | 64 | M | Well-compensated type 2 diabetes mellitus | Endemic area | Until 8 years previously, he had been a heavy smoker (50 cig day$^{-1}$) |
| Mathur *et al* (2006) | India | 45 | M | Hemoglobin = 11.2 G% Leukocyte tot. count: 12 000 mm$^{-3}$ | Endemic area | – |
| Kassam *et al* (2013) | England | 66 | M | COPD (diagnosed in 2002) raised ESR (34 mm h$^{-1}$) | – | Former smoker (20 cig day$^{-1}$) for 40 years, recently had three courses of antibiotics and got salbutamol, steroid inhalers and an antihistamine for the COPD |

–, Not reported/unknown; ACE, angiotensin converting enzyme; COPD, chronic obstructive pulmonary disease; ESR, erythrocyte sedimentation rate.

2–2.4 million disability-adjusted life years lost (World Health Organization, 2000; Hotez *et al*, 2004).

The parasite resides intracellularly and is transmitted between hosts by the bite of the female sand fly (genus *Phlebotomus* in the Old World localities of Europe, Africa, and Asia, and *Lutzomyia* in the New World, the Americas, and Oceania). The primary hosts are vertebrates such as humans, domestic dogs and cats, opossums, the crab-eating fox, and the common black rat. Human infection is caused by more than 20 different species that infect mammals (Balasegaram *et al*, 2012; Montalvo *et al*, 2012; Stockdale and Newton, 2013).

*Leishmania* have a digenetic life cycle consisting of the morphologically distinct promastigote and amastigote stages. Promastigotes are the flagellated, motile stage that reside extracellularly in sand fly vectors of the genera *Phlebotomus* or *Lutzomyia*. Virulent metacyclic promastigotes are inoculated into the dermis of mammalian hosts during sand fly blood meals. Macrophages rapidly internalize the promastigotes forming a parasitophorous vacuole that then fuses with the lysosomes to generate an acidic compartment containing hydrolytic enzymes, where they further differentiate into amastigotes (Sacks *et al*, 1994; Murray *et al*, 2010).

Human VL is mainly caused by two species of *Leishmania* parasites, each having a characteristic regional distribution: *L. infantum* is the causative agent in the Mediterranean, Middle-east, Central Asia, China, and Central and South America; *L. donovani* in India and East Africa. VL may also be caused by *L. tropica* in the Old World and *L. amazonesis* in the New World and is fatal in 85–90% of untreated cases and up to 50% of treated cases (Gill and Beeching, 2009).

Visceral leishmaniasis is characterized by weight loss, fever, cytopenia, and hepatosplenomegaly (Carranza-Tamayo *et al*, 2010).

Approximately 90% of CL occurs in Afghanistan, Pakistan, Syria, Saudi Arabia, Algeria, the Islamic Republic of Iran, Brazil, and Peru (Kassi *et al*, 2008), with an incubation period ranging from 2 weeks up to several months (Grimaldi and Schottelius, 2001) and a wide spectrum of clinical presentations ranging from cutaneous ulceration to various degrees of mucosal involvement.

The symptoms and the extent and localization of the lesions depend both on the characteristics of the parasite and on the host immune response.

Cutaneous leishmaniasis is the most prevalent and is characterized by the presence of ulcers with a well-defined erythematous border and a central crust that is often hemorrhagic, located in exposed areas of the body and ranging in number from one to ten (Nogueira *et al*, 2008; Goto and Lindoso, 2010). MCL is the most severe form and presents clinically a few years after the manifestation of the cutaneous form, affecting the upper aerodigestive tract, with lesions mainly in the oral and nasal mucosa and occasionally in the laryngeal and pharyngeal mucosa (Palmeiro *et al*, 2007; Nogueira *et al*, 2008). These lesions are generally associated with pain, edema, halitosis, bleeding, and sialorrhea (Palmeiro *et al*, 2007).

Mucosal leishmaniasis of the upper respiratory tract is usually associated with the VL form or is found in immunosuppressed individuals like those with HIV infection or

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**Table 2** Data of 13 patients from pubmed search: Clinical features

| Reference | Involved sites | Symptoms | Lesion description |
|---|---|---|---|
| Palmeiro et al (2007) | Palatal mucosa (hard and soft palate) Uvula gingiva | Swallowing difficulties | Granular area with presence of coarse granulation  Fine granulation |
| Pellicioli et al (2012) | Palatal mucosa (hard and soft palate) oropharynx | Weight loss (approximately 8 kg). Slight pain and no bleeding upon palpation | Largest diameters measuring approximately 5 × 4 cm. Multiple ulcerated nodules, with granulomatous appearance and fibro-elastic consistency |
| Cocuzza et al (2013) | True vocal cords | 8 months history of hoarseness and discomfort | Two focal hard and whitish lesions |
| Oryan (2013) | Laryngeal mucosa | Several months history of dysphonia, dyspnea, hoarseness, and odynophagia | Single lesion |
| Oryan (2013) | Epiglottis, cricoarytenoid muscle, laryngeal mucosa | Several months history of: dysphonia, dyspnea, hoarseness, odynophagia. Vocal cords paralysis; swelling, erythema and edema of the epiglottis and aryepiglottic folds | Deep mucosal damaging processes |
| Pau et al (2009) | Endonasal mucosa (anterior part of left cartilaginous septum) | Epistaxis | Bright red, 1 cm nodule bled easily, moderately infiltrated, covered by serohematic crusts |
| Pau et al (2009) | Endonasal mucosa (left nasal vestibule) | Epistaxis, rhinorrhea, sense of obstruction, and respiratory difficulty. Moderate labial edema | Hard erythematosus, polypoid, non-ulcerated and painless, 1.5 cm diameter nodule |
| Habibzadeh et al (2005) | Right side of the tongue (against the first molar tooth) | None | Fleshy mass, measuring 5 × 7 × 5 mm |
| Guddo et al (2005) | Larynx (subglottic mucosa) | Cough, exertional dyspnea, mucus production, bronchiectases | 3 mm mucosal polypoid-like lesion |
| Casolari (2005) | Right epiglottis region Infiltrating the pharyngo-laryngeal wall | Dyspnea, Dysphonia Odynophagia | Whitish, fungating, swelling Lesion |
| Tiseo et al (2008) | Left wall of the pharynx, vocal cords | Dysphonia | – |
| Mathur et al (2006) | Hemilarynx (bilaterally) Epiglottis, involving posterior commissure | Hoarseness, cough and a history of fever for 18 months | Fixed ulcerative growth, With edematous surrounding areas |
| Kassam et al (2013) | Tongue (left mid-dorsum) | 3-month history of swelling and soreness of the dorsum of the tongue because of painful ulceration, and some difficulty in swallowing | Lymphoid-like tissue swelling |

–, Not reported/unknown.

**Table 3** Comparative data on age, gender and sites of involvement

| | Our cases (n = 7) | Review cases (n = 13) | Total (n = 20) |
|---|---|---|---|
| Overall age range (years) | 31–66 | 32–75 | 31–75 |
| Men | 6 | 13 | 19 |
| Women | 1 | 0 | 1 |
| Mean (±SD) age | 48 (±14) years | 56 (±14) years | 53 (±14) years |
| Larynx | 0 | 7 | 7 |
| Tongue | 4 | 2 | 6 |
| Pharynx | 0 | 4 | 4 |
| Palate | 1 | 2 | 3 |
| Nasal mucosa | 0 | 2 | 2 |
| Buccal mucosa | 1 | 0 | 1 |
| Gingiva | 0 | 1 | 1 |
| Lip | 1 | 0 | 1 |

SD, Standard deviation.

a solid organ transplant. In these clinical settings, leishmaniasis shows the most severe signs and symptoms, characterized by fever, chills, hepatosplenomegaly, pancytopenia, gastrointestinal involvement, and/or ascites.

In immunocompetent patients, the primary and exclusive mucosal involvement of the head–neck region is very uncommon and the exclusive and localized oral mucosa leishmaniasis is an even more rare event.

Most of the cases reported in literature, and excluded from our study, describe a mixed form of leishmaniasis, in which the mucosal lesions are contemporary with or secondary to the visceral or cutaneous forms.

Diagnosis is very often a challenge with several reports in the literature of a significantly delayed diagnosis or even an erroneous clinical diagnosis of malignancy (Casolari et al, 2005; Mathur et al, 2006; Tiseo et al, 2008; Pellicioli et al, 2012; Cocuzza et al, 2013; Oryan et al, 2013). Differential diagnosis encompasses other infectious or non-infectious diseases such as fungal infections (blastomycosis), tertiary syphilis, sarcoidosis, paracoccidioidomycosis, histoplasmosis, tuberculosis, leprosy, lethal midline granuloma, pemphigus vulgaris, pemphigoid, plasmacytic gingivitis, (deficiencies) anemia, leukemia, antineutrophilic cytoplasmic antibody (ANCA)-associated (Wegener) granulomatosis, and squamous cell carcinoma (Casolari et al, 2005; Palmeiro et al, 2007; Pellicioli et al, 2012).

**Table 4** Data of 13 patients from pubmed search: diagnosis and treatment

| Reference | Diagnostic tests | Treatment | Follow-up (months) | Outcome |
|---|---|---|---|---|
| Palmeiro et al (2007) | Montenegro skin test<br>Serology (IIF)<br>Histopathological (hematoxylin–eosin, Ziehl-Neelsen, Silver), Culture | Meglumine antimoniate (5 mg kg$^{-1}$ day$^{-1}$) i.m. for 20 days | 30 | Healing |
| Pellicioli et al (2012) | Montenegro skin test<br>4 Biopsies (inconclusive diagnoses)<br>Histopathological (hematoxylin–eosin, Ziehl-Neelsen, Silver, Pas)<br>Immunohistochemistry | Liposomal amphotericin B for 5 weeks | – | Healing with residual fibrosis |
| Cocuzza et al (2013) | 12-2009<br>Fibroscopy and laryngeal biopsy (inflammation and hyperplasia) | Amoxicillin | | Lack of clinical improvement |
| | 07-2010<br>Second laryngeal biopsy<br>PCR | Liposomal amphotericin B (3 mg kg$^{-1}$ day$^{-1}$) for 7 days, then (3 mg kg$^{-1}$ per once a week) for 5 weeks | | Achieved complete recovery |
| | 05-2011<br>Third laryngeal biopsy | Liposomal amphotericin B (3 mg kg$^{-1}$ day$^{-1}$) for 7 days, then (3 mg kg$^{-1}$ per once a week) for 5 weeks | 20 | Resolution of symptoms and good health |
| Oryan (2013) | Laryngoscopy<br>Cytologic (Wright and Papanicolaou)<br>Nested PCR | – | – | – |
| Oryan (2013) | Laryngoscopy<br>Cytologic (Wright and Papanicolaou)<br>Immunocytochemistry, Nested PCR | – | – | – |
| Pau et al (2009) | Endoscopy<br>Facial X-ray<br>Histopathological (Giemsa) | Meglumine antimoniate 300 mg ml$^{-1}$: intralesional infiltration (1 ml per weekly) for 4–5 weeks | 36 | Full resolution |
| Pau et al (2009) | Rhinoscopy<br>Cytological and histopathological (Giemsa)<br>Isoenzymatic characterization | Meglumine antimoniate 300 mg ml$^{-1}$: intrales. infiltr. (1 ml per weekly) for 4 weeks | 12 | Healing |
| Habibzadeh et al (2005) | Histopathological<br>Indirect immunofluorescence | Surgical | 48 | Healing |
| Guddo et al (2005) | Bronchoscopy<br>Histopathological (hematoxylin–eosin, Ziehl-Neelsen, Gram, Grocott, Giemsa)<br>PCR, ELISA test on serum<br>Microbiological | Ciprofloxacin (without clinical improvement) then Amphotericin B: (0.5 mg kg$^{-1}$ day$^{-1}$) | - | Clinical improvement |
| Casolari et al (2005) | Laryngoscopy<br>Multiple Histological (Giemsa), Culture<br>Nested PCR | Liposomal amphotericin B 2 courses, 10 days apart, (3 mg kg$^{-1}$ day$^{-1}$) for 5 days | 12 | Healing |
| Tiseo et al (2008) | CT scan<br>Direct laryngoscopy<br>Histological (Giemsa)<br>Antibody title (weakly positive) | Liposomal amphotericin B: starting with 0.5 mg kg$^{-1}$ day$^{-1}$, Overall dosage: 950 mg | 2 | Healing Lesion decrease |
| Mathur et al (2006) | Direct laryngoscopy, Histopathological (hematoxylin–eosin, Pas, Giemsa), Serology | Stibogluconate (20 mg kg$^{-1}$ day$^{-1}$) For 2–3 weeks | 3 | Significant symptom improvement |
| Kassam et al (2013) | Histopathological (hematoxylin–eosin, Giemsa) PCR | Miltefosine: 150 mg BD for 28 days | 10 | Healing |

–, Not reported/unknown; IIF, indirect immunofluorescence; PCR, polymerase chain reaction; CT, computed tomography.

One further important aspect to be discussed is the difficulty of establishing the diagnosis of infectious diseases with a biopsy. Two cases from this literature review, and one in our case series, had previously been submitted to several earlier biopsy procedures, with inconclusive histopathological diagnoses. This is probably due to the similarity of all the granulomatous lesions in the histopathological analysis, the morphological differentiation of the parasites being the only way to make a histopathological diagnosis. This aspect highlights the need to complete the examination in all uncertain cases with a more specific diagnostic procedure like ELISA or PCR.

The literature shows a wide spectrum of diagnostic criteria which encompasses clinical signs, culture of smears of samples, and PCR. Serological testing has also been reported, such as the direct agglutination test, conventional ELISA test, and rk39 rapid diagnostic ELISA test (World Health Organization, 2010; Pellicioli et al, 2012; Masmoudi et al, 2013; Stockdale and Newton, 2013). Serology can be helpful when other diseases have to be considered in a differential diagnosis or if there are uncertain clinical or histopathological data. PCR can be used on skin mucosa biopsy samples or slit-skin specimens. The sensitivity of this test varies depending on the PCR methods

and the clinical features of the lesions (World Health Organization, 2010; Oryan *et al*, 2013). For this reason, the identification of amastigotes in biopsy specimens from the skin or mucosa is the most common diagnostic tool (World Health Organization, 2010).

The cases described from our database confirm that the most common clinical presentation of primary ML in the head and neck region is an exophytic lesion found in 85% of our cases as compared to 69% in the literature review.

The most commonly involved oral site is the tongue, followed by the palatal mucosa.

Comparing our results with the data from the literature, we found an 8 year difference in the mean age of the patients, with the same standard deviation of 14 years. Our data confirm a near-exclusive dominance of the male gender.

Predisposing local conditions have to be highlighted: As reported in the review and in our case series, more than 50% of the patients had a local impairment, such as a smoking habit or inflammatory conditions such as chronic obstructive pulmonary disease, and recurrent respiratory tract infections (Ferlito *et al*, 1986; Benítez *et al*, 2001; Aliaga *et al*, 2003; Guddo *et al*, 2005; Tiseo *et al*, 2008; Teemul *et al*, 2013).

It is important to emphasize that, in our experience, localized ML can be considered in some cases as an aspect of a not yet detectable systemic infection. In fact, in our case series, during follow-up, two patients showed cutaneous forms of the disease and two patients a visceral form.

The relapse in some cases is probably due to under-treatment. We suppose that in these patients, there may remain a hidden reservoir of parasites in a quiescent state.

A limited number of drugs are available for the treatment of leishmaniasis, and these face challenges including the development of drug resistance, the limited efficacy for different strains and species, and the cost.

The treatment depends on the causative *Leishmania*; however, species identification is fastidious, time-consuming, and not always available. In such cases, the choice of the drug should be inferred from the geographical setting of the patient, and the epidemiological data of *Leishmania* distribution as well as the clinical symptoms.

The treatment of choice for mucosal forms of leishmaniasis is based on the administration of pentavalent antimonial drugs, such as meglumine antimoniate, or stibogluconate (Masmoudi *et al*, 2013).

To conclude, leishmaniasis of the mucous membranes of the head–neck region must be considered in the differential diagnosis of mucosal lesions. It is becoming an emerging infectious disease not only in immunocompromised patients but also in those geographical settings where the parasite is endemic, because of the increasing modern habit of people to travel often abroad.

The diagnosis remains a challenge because of the varying clinical presentation and its ability to emulate different diseases. The dentist plays an important role in the early diagnosis of leishmaniasis which has systemic repercussions.

Any patient affected by leishmaniasis should be managed by a professional with expertise in this disease, preferably an infectious disease specialist. Furthermore, after a complete healing and a long follow-up period without signs or symptoms, the possibility of relapses has to be considered.

## Author Contributions

Michele Davide Mignogna and Antonio Celentano have made the research design, drafting the paper and revising it critically. Stefania Leuci, Marco Cascone, Daniela Adamo and Elvira Ruoppo have made the acquisition of data. Michele Mignogna, Antonio Celentano and Gianfranco Favia have selected and classified patients. Michele Mignogna and Gianfranco Favia revised critically the paper.

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