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JCIMCR OPEN ACCESS Journal of Clinical Images and Medical Case Reports

ISSN 2766-7820

Case Report

Open Access, Volume 5

First reported case of old world leishmaniasis in Greece affecting the oral mucosa

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Received: Sep 03, 2024

Accepted: Sep 20, 2024

Published: Sep 27, 2024

Archived: www.jcimcr.org

DOI: www.doi.org/10.52768/2766-7820/3272

Abstract

Background: Leishmaniasis is an important infectious disease caused by kinetoplastid protozoan parasites of the genus *Leishmania*, transmitted to mammalian hosts by the bite of infected female phlebotomine sandflies. The clinical manifestations of the disease are broadly classified into three types: Visceral (VL), Cutaneous (CL), and Mucocutaneous (MCL) leishmaniasis, depending on the Leishmania species. Mucosal Leishmaniasis (ML) is a rare metastatic complication of *Leishmania* infection, presenting destructive and disfiguring outcomes of the nasal architecture and an airway compromise. Oral manifestations of leishmaniasis usually occur from infection by New World *Leishmania* species, while is an uncommon form of the disease in the Old World.

Case presentation: We present the first to our knowledge report of leishmaniasis with oral mucosa symptoms, in Greece. The causative species was identified as *Leishmania infantum.* The patient presented concurrent visceral symptoms and was a renal-transplanted patient with a previous medical history of leishmaniasis.

Conclusions: Overall, leishmaniasis with oral manifestations merits awareness towards differential diagnosis of oral lesions, in regions of the Old World where *Leishmania* spp. are endemic, including the Mediterranean basin where *Leishmania infantum* is prevalent.

Keywords: Oral mucosal leishmaniasis; Leishmania infantum; Greece; Diagnosis.

Introduction

Leishmaniasis is a vector-borne complex of diseases caused by the bite of *Phlebotomus* and *Lutzomyia* female sandflies transmitting *Leishmania* (L.) parasites, the causative agents of the disease. Leishmaniasis is a growing public health concern as it has a worldwide distribution, being endemic in circumscribed geographic areas in Africa, Southern Europe, Middle East, and Central and South America [1]. An estimated 1 million new cases are reported annually from nearly 100 endemic countries, while about 15 million people are infected and 350 million are at risk of acquiring the disease [1]. Major risk factors are associated with the increase in incidence and prevalence of leishmaniasis, such as environmental conditions, socio-economic status, demographic and human behaviors like great migration, deforestation, urbanization and immunosuppression [2].

The clinical features of the disease include a wide range of manifestations with various degrees of severity that depend on the involved parasite species and the host's immune response [3]. Despite the variety of clinical symptoms, there are three main manifestations. Cutaneous Leishmaniasis (CL), is the most common form of the disease, which causes self-curing skin lesions [3]. Visceral Leishmaniasis (VL) is the most severe form which affects host's visceral organs, mainly the liver and the spleen, and is associated with high fatality if left untreated [3]. At last, Mucosal Leishmaniasis (ML) is a rare and serious condition typically affecting the larynx, pharynx, inner nostril wall and oral cavity [3]. The lesions may cause severe facial disfigurement and can lead to severe deformity if left untreated [4]. Several factors promote the ML onset, such as malnutrition, male sex and immunosuppression [5]. It is largely confined to regions of Central and South America, where more than 90% of ML cases are reported, and parasites that belong to the New World *L. Vianna* subgenus *(L. braziliensis, L. panamensis, L. guyanensis, L. peruviana)* are considered the main aetiological agents [4,6]. Even though the great majority of ML cases occur in America, an increased incidence is very likely to occur in other regions of the world, due to environmental and anthroponotic changes. The Old World *Leishmania* subgenus, notably from *L. donovani* complex (*L. donovani* and *L. infantum*) and *L. tropica* complex parasites (*L. tropica* and *L. major*), causing Visceral (VL) and Cutaneous (CL) Leishmaniasis respectively [6], is rarely suspected as a causative agent of ML, leading to remarkable delays in diagnosis and treatment.

In this manuscript, we report the first case of leishmaniasis in Greece, caused by *Leishmania infantum*, which affected the oral mucosa of an immunocompromised patient. *Leishmania infantum* is the basic autochthonous species of southern Europe, including Greece, causing VL. ML is a rare and unusual clinical presentation of an *L. infantum* infection and should be considered in the differential diagnosis in endemic regions [6-9].

Case presentation

In April 2023, a 61-year-old male patient presented symptoms of gingival hyperplasia in the oral cavity, and a deciduous febrile movement. At the time of the symptoms, the patient was receiving immunosuppressive therapy with mycophenolic acid (560 mg twice a day), tacrolimus (5-6 mg) and methylprednisolone (4 mg). His medical history included end-stage renal disease due to chronic glomerulonephritis, and renal transplantation in 2011. In 2014, the patient had displayed gastrointestinal manifestations and a colon biopsy test revealed a *Leishmania* infection. Blood PCR, bone-marrow biopsies and antibody rK39 dipstick test were negative. The patient recovered after treatment with Liposomal -Amphotericin B (L-AMB) administered for three weeks (cumulative dose 20 mg/kg).

In order to investigate the patient's gingival hyperplasia, a biopsy was performed. The histological examination of gingival tissue revealed intense inflammatory infiltration and the presence of Giemsa-positive microorganisms within the cytoplasm of macrophages. Thus, a possible *Leishmania* infection was suspected and the patient was hospitalized. Blood tests also revealed mild pancytopenia, while abdominal ultrasound confirmed splenomegaly (~14.3 cm diameter). A blood sample and a tissue sample from the gingival lesion were sent to the Hellenic National Reference Laboratory for Leishmaniasis for further diagnosis and confirmation of leishmaniasis and for molecular investigation of the causative species. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). Samples were handled under sterile conditions and were analyzed by quantitative real-time PCR targeting kinetoplast DNA [10]. *Leishmania* typing was performed by sequencing of the amplified Internal Transcribed Spacer 1 (ITS-1) product of PCR, as previously described [11]. The causative *Leishmania* species was identified as *Leishmania infantum* (Figure 1). In addition, the diagnosis of leishmaniasis was also performed by histology methods with detection of the parasites in the gingival lesion, were they were visualized as amastigotes, by variable pressure scanning electron HG microscopy (Figure 2A and 2B). Based on the leishmaniasis diagnosis, the patient was assigned to an intravenous L-AMB regimen, with a cumulative dose of 40 mg/kg for five weeks, followed by a reduction of the immunosuppressive therapy (360 mg mycophenolic acid, twice a day, 4 mg tacrolimus).

At the end of the antileishmanial treatment, fever regression was noticed and the spleen was determined with normal dimensions. Bone marrow histopathology test did not reveal pathological findings and the imaging of the chest and abdomen with a CT scan, was normal. As part of the checkup, a colonoscopy was performed, which revealed findings of colitis. Histological lesions were associated with a positive Cytomegalovirus (CMV) diagnostic test, negative for *Leishmania* infection. After the CMV diagnosis, the patient was additionally treated with valganciclovir for three weeks, while mycophenolic acid was reduced to 250 mg twice a day, and the dose of methylprednisolone was increased (6 mg) to protect the transplant. Due to persistent gingival hyperplasia (Figure 2C and 2D), the treatment with L-AMB continued for up to one year.

Discussion and conclusions

To the best of our knowledge, this is the first reported case of the rare *Leishmania infantum* leishmaniasis in a kidney-transplanted patient, with oral mucosal manifestations, in Greece. The patient presented also concurrent visceral manifestations. His medical history involved leishmaniasis affecting the colon nine years before the onset of the mucosal symptoms, although it is not clear if the subsequent mucosal manifestations represent a relapse or a new infection. It is noteworthy that immunosuppressed patients are at the greatest risk of leishmaniasis infection and reactivation [12]. *Leishmania in-*

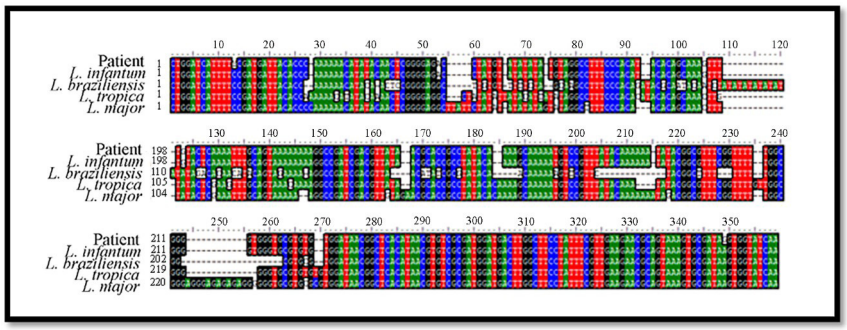

Figure 1: Partial sequence alignment of the patient's amplified Leishmania ITS1 region and sequences from different Leishmania species. The patient's *Leishmania* ITS1 sequence was amplified from genomic DNA isolated from the blood, and was aligned by ClustalOmega to ITS1 sequences of *L. infantum* (JPCM5 strain), *L. braziliensis* (MHOM/BR/75/M2904_2019 strain), *L. major* (Friedlin strain) and *L. tropica* (strain MHOM/KE/84/NLB297) DNA. The alignment was visualized with BioEdit software. Letters not corresponding to standard DNA bases (A, T, C, G) represent a set of bases according to the IUPAC code [Genome Browser IUPAC Codes (ucsc.edu)].

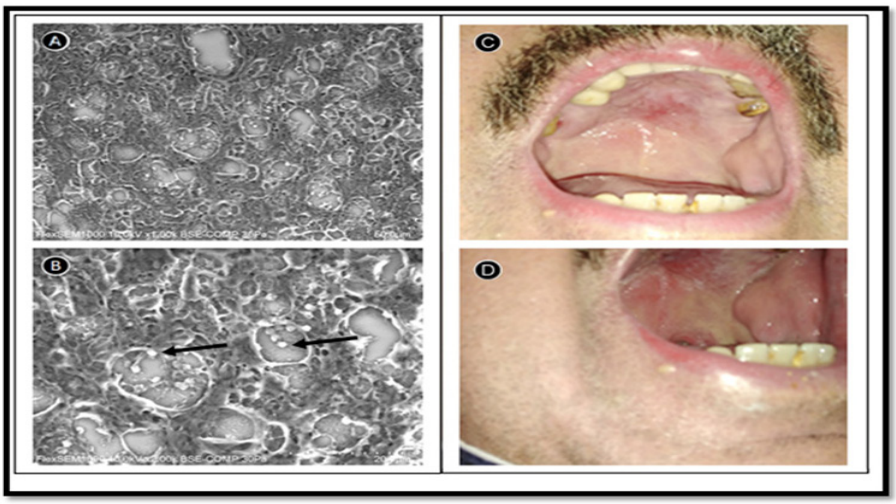

Figure 2: Leishmania infection of the oral cavity.
(A, B) Variable pressure (VPSEM) scanning electron microscopy images of paraffin-embedded sections. Patient's tissue from the gingival lesion was fixed and embedded in paraffin, sections of 3-µm-thickness were cut and mounted on glass slides, and deparaffinized. The observation was performed at low-vacuum mode using FlexSEM-1000II without metal coating (30Pa, 10kV acceleration voltage, backscattered electron detector [BSE]; Hitachi Hitech, Japan). (A) Low magnification image of the section (x1.00k, bar = 50 µm) showing intracellular *Leishmania* amastigotes. (B) Higher magnification of the central part of image (x2.00k, bar = 20 µm) showing intracellular *Leishmania* amastigotes (black arrows). (C, D) Gingival hyperplasia with the involvement of lesions in the soft and hard palate. Forward view (C) and side view (D).

fantum leishmaniasis affecting the mucosal cavity is extremely rare in Europe and a recent surveillance of the disease in 15 European centers revealed that 5% of autochthonous infections caused ML [13]. In addition, there are reviews, which describe predisposing factors, including male sex and immunodeficiency that match this case report [14,15]. The majority of *Leishmania infantum* ML cases present localized lesions with no concurrent visceral disease [14,15], unlike the presented case report where the patient combined both mucosal and visceral symptoms. Factors allowing this concurrency of mucosal and visceral symptoms [16] may be attributed either to parasite factors and/or to the immune system of the host. Consequently, doctors in the Mediterranean basin, due to the presence of viscerotropic *L. infantum,* should suspect both forms of the disease. In this particular case, apart from the existing immunodeficiency, the CMV infection may have additionally affected the patient's immune system, contributing to this rare manifestation. Overall, this report should raise the awareness of clinicians in the Mediterranean region for including ML in the differential diagnosis of oral mucosal lesions and for investigating the coexistence of visceral disease, especially in immunocompetent and immunocompromised individuals living in endemic areas or reporting travels to endemic areas.

Declarations

Acknowledgments: Not applicable.

Author contributions: Conceptualization was performed by D. S. and E.A. E.T., V.F., and Sm. M. treated the patient. D.S., E.T., V.F., So. M., M.E., E.A., and O.S.K. performed the data collection. O. S. K., So. M., and M. E. performed laboratory work and analysis. D.S., O.S.K., and D. P. analyzed the data. O.S.K. and D.S. contributed to the writing of the manuscript. All the authors drafted and approved the published version of the manuscript.

Funding: *Leishmania* molecular diagnosis and typing were funded by the Hellenic National Public Health Organization. The views and opinions expressed in this publication are solely those of the authors, and they do not purport to reflect the opinions, views or positions of the Hellenic Public Health Organization.

Data availability: The data that support the findings of this report are available from the corresponding author, upon reasonable request.

Ethical approval: The patient has given his written consent for publishing this case report with photographs.

Conflicts of Interest: The authors declare that they have no conflicts of interests.

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