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Case Reports

A multidisciplinary approach to an uncommon case of laryngeal leishmaniasis in Turkey

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Leishmaniasis is a zoonotic disease caused by the genus *Leishmania*. A sandfly vector called *Phlebotomus* is the agent responsible for transmitting the pathogen to humans. The disease may be found in 1 of 3 forms, visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (ML), depending mainly on the infecting species and the host immune response. Leishmaniasis is endemic in 88 countries, particularly localized in areas of the tropics, subtropics, and southern Europe[1]. In most of the ML cases, the nasal cavity is affected, but the localization of *Leishmania* spp in the laryngeal mucosa is unusual. Furthermore, laryngeal mucosa involvement can appear in patients with previous CL as well as being part of a widespread VL in immuno-supressed patients who suffer infections beyond the mononuclear phagocyte system.

Here we report a rare case of treated laryngeal ML due to *Leishmania infantum* in an 81-yr old male patient resident of Adana, Turkey which is a subtropical area. The case was investigated with a multidisciplinary approach.

Case report

The first application of patient was in February 2011 who is a former heavy smoker with no any other systemic diseases or risk factors with lesions referring leishmaniasis on left hand and left foot. Physical examination findings were as follows: patient fever at 37°C, TA (blood pressure) at 130/80 mmHg with no hepatosplenomegaly. After physical examinations, the tissue samples were subjected to routine microbiological *Leishmania* screening with Giemsa-stained smear testing and molecular tests including PCR-RFLP and DNA sequencing. Giemsa-stained smear test was found negative for *Leishmania*.

A Qiagen DNA extraction kit (QIAGEN Inc., Valencia, CA, Spain) was used according to the manufacturer's instructions for extraction purpose. The extracted whole genome DNA was used to amplify miniexon and *ITS-1* genes with specific primers as follows: LITSR:5'-CTGGATCATTTTCCGATG-3', L5.8S:5'-TGATACCACTTATCGCACTT-3' for ITS1 region and Fme5'-ACAGAAACTGATACTTATATA GCG-3', Rme 5'-TATTGGTATGCGAAACTTCCG-3' for miniexon region[2–3]. The amplified miniexon and *ITS-1* genes were restricted by restriction enzymes *EaeI* and *HaeIII*. After amplification, sequence analysis was performed with the same primers on both strands of the miniexon PCR products. The sequence analysis was done using the dye terminator cycle sequencing method and an ABI Prism Big Dye Terminator kit (Applied Biosystems, Foster City, USA). The assay was carried out according to the standard protocol. Data were collected on an ABI 3100 automated fluorescence sequencer (Applied Biosystems). The types of miniexon and *ITS-1* genes were identified by comparing the sequences of the database of G. Jacoby and K. Bush (*http://www.lahey.org/studies/*) with the sequences in GenBank. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) testing resulted with restriction profiles specific to *L. infantum* and sequencing comfirmed the results (Fig. 1). Sodium stibogluconate was used for treatment and patient was discharged on Day 21.

The second application of patient was in May 2013 with hoarseness complaint for the last three months. Routine examination was followed with a computed tomography (CT) scan and endoscopic examination which biopsy materials were taken from lesions around vocal cord. Irregularity informing lesions with a papillomatous mass holding the ventricle and extending into the ventricle was seen after CT examination. Endoscopic examinations re-

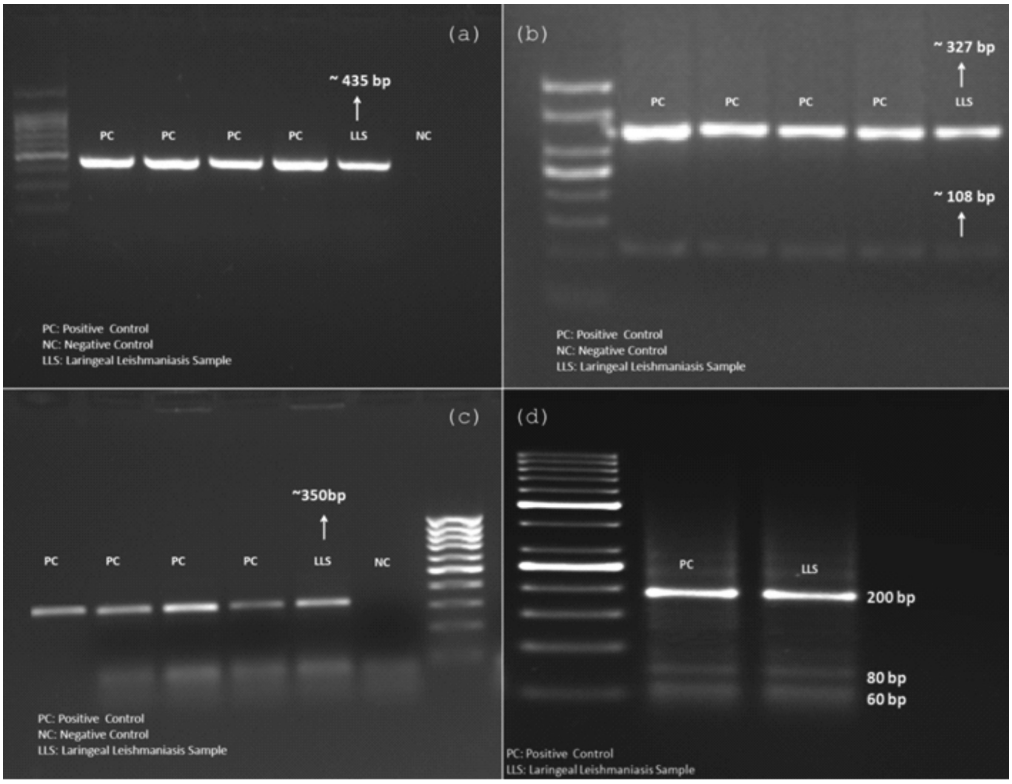

Fig. 1: Amplicons obtained after PCR and restriction processes: (a) miniexon gene region; (b) *EaeI* restricted miniexon gene region; (c) *ITS-1* gene region; and (d) *HaeIII* restricted *ITS-1* gene region.

vealed non-specific lesions with edema and erythema below vocal folds. The obtained paraffin-embedded laryngeal biopsy materials were examined with microbiological methods which were employed on first application.

Common ulcerative lesions, histiocytosis reactions, reactive changes in some places of squamous epithelium and *Leishmania* amastigotes in histiocyte cytoplasm were observed upon pathological examinations of biopsy ma-

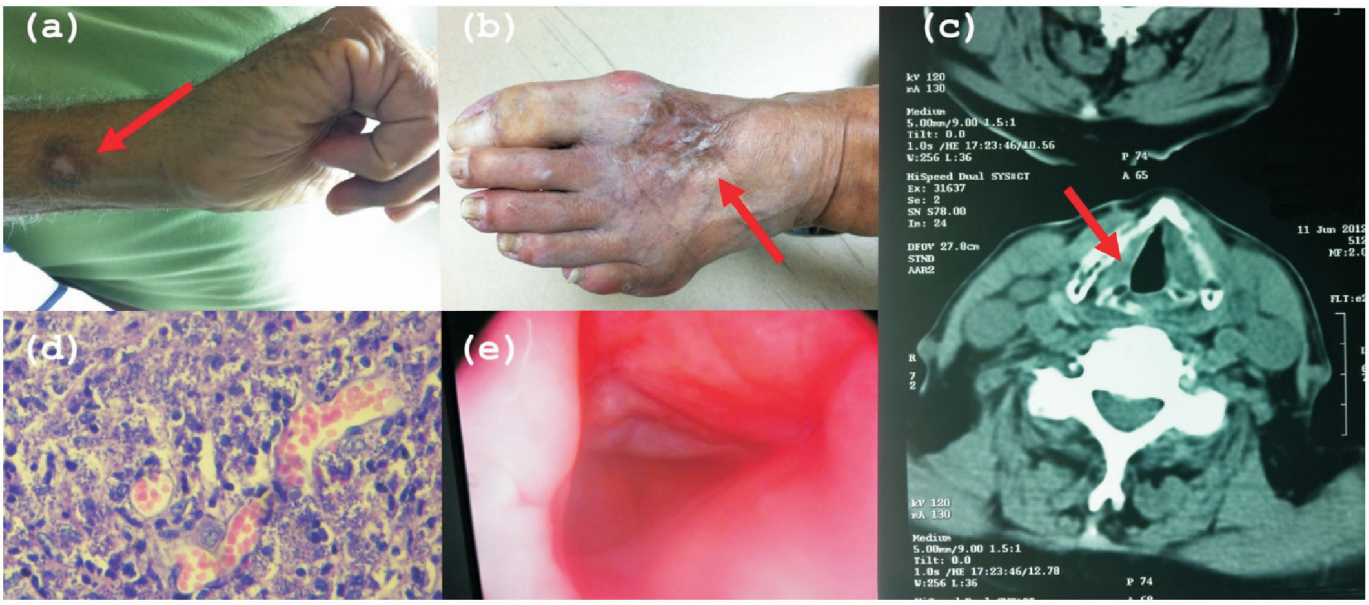

Fig. 2: (a) CL lesions on patients hand on his first application; (b) CL lesions on patient's foot on his first application; (c) BT of neck showing papillomatous push elongating into ventriculus; (d) Histological examination of laryngeal biopsy specimen (May-Grunwald Giemsa staining; original magnification, 1000 ×) showing intracellular amastigotes of *Leishmania* species and histiocytes with vacuolated cytoplasm and corpuscles inside; and (e) Laryngoscopic examination showing lesions with edema and erythema.

terial. Furthermore, eosinophil granulocytes in addition to amastigotes inside macrophages were found in Giemsa-stained smear preperations. Eventually, both PCR-RFLP and sequencing methods identified the agent as *L. infantum* (Fig. 2). Treatment of the patient was handled with 3 mg/kg dosed, liposomal and intavenous Amphotericin B on 1, 2, 3, 4, 5, 14 and 21 days. Patient was discharged by the end of treatment with no complication.

DISCUSSION

Although leishmaniasis is an endemic disease in Mediterranean countries, laryngeal involvement is a very uncommon case. Reported laryngeal leishmaniasis cases are so rare throughout the world since mid-1900s to now-a-days[4–10]. A literature (Medline plus local databases) search did not reveal any reported cases in Turkey.

Most common syndrome in laryngeal leishmaniasis is dysphonia characterized by a muffled voice as in this report but dysphonia is not a syndrome-specific to laryngeal leishmaniasis. Diseases such as laryngeal tumors or other larynx infections may as well lead to dysphonia too. Furthermore, sources of laryngitis syndromes can be infectious caused by many bacterial or parasitic agents like *Mycobacterium tuberculosis*, *Paracoccidioides* spp and *Leishmania* spp, so differential and definitive identification is the key to treat the infection. In many cases, laryngeal leishmaniasis can occur in association with VL, CL or with any other infections like HIV and this makes its diagnosis easier. Immunodeficiency, travel to endemic areas, use of steroids, chronic alcoholism, and smoking are the known risk factors for laryngeal leishmaniasis[8–11]. Herein, probable risk factors for our case were that he had CL lesions on left hand and left foot, former he was a heavy smoker and was 81-yr old. It can be said that damage of larynx due to heavy smoking at the past coupled with age caused CL lesions to develop laryngitis.

In this case, a multidisciplinary approach was used to diagnose the disease with endoscopic, radiologic and microbiological examinations. At first step CT and endoscopy revealed lesions at the disease site but it was unable to diagnose and start the right choice of treatment with such findings. Microscopic diagnosis of leishmaniasis is widely used and can be made by either PAS or Giemsa staining by detecting the parasite in the aspiration, biopsy or a defacement material as intra- and/or extramacrophagic granules[1]. Though, microscopy is the most common and the cheapest technique, amastigotes are usually scarce so definitive diagnosis at species level can be done only by molecular microbiological methods[12]. Moreover, sensitivity of Giemsa is not clear. Motta *et al*[13] reported the sensitivity of PCR to be in the range of 47.4 to 83.3%. In our case, Giemsa staining did not give any result at first application but showed amastigotes in the biopsy material. Amplification procedure used to identify the *Leishmania* specific ITS1 and miniexon regions succesfully on both the applications. On the other hand, PCR-RFLP technique identified the agent as *L. infantum* succesfully twice and DNA sequencing confirmed the results.

Mucocutaneous leishmaniasis traditionally refers to a metastatic sequelae of cutaneous infection, which results from dissemination of parasites from the skin to the naso-oropharyngeal mucosa usually by a species called Viannia subgenus (*L. braziliensis, L. Panamensis* and *L. guyanensis*) or less commonly by *Leishmania amazonensis*. *L. infantum* and *L. donovani* are the agents known to be responsible from VL for the New World[14–15]. On the other hand *L. infantum* and *L. donovani* are common agents for ML in the Old World[16]. In Turkey, CL and VL are common and the responsible agents for CL are usually *L. tropica* and *L. infantum*[17], whereas it is *L. infantum* for VL[18]. To the best of our knowledge this case is atypical because dissemination of *L. infantum* from a CL lesion to develop ML is the first in Turkey. Identification of *L. infantum* which is a typical agent of VL in a ML case is a new finding for our area and a worrisome situation for the future.

CONCLUSION

There is still a need for quick and cheap detection of ML and VL infections especially in endemic areas so that the studies focusing on molecular microbiological methods can help to develop new diagnostic methods in addition to elucidate the etiology and epidemiology of leishmaniasis. This case should emphasise the importance of considering leishmaniasis in the differential diagnosis of granulomatous lesions especially in the endemic regions and patients with risk factors since it is a treatable infection.

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