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Case Report

Cutaneous Leishmaniasis Lesion on the Ear from Kashan, Central Iran: A Case Report

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<p>Received 22 Jul 2022 Accepted 15 Oct 2022</p>	<p>Abstract</p> <p>Cutaneous leishmaniasis (CL) frequently occurs in many rural and urban areas of Iran. <i>Leishmania major</i> and <i>L. tropica</i> are principally two causative agents of CL in Iran. We report here a case of leishmaniasis of the ear in a 61-year-old man referred to the Reference laboratory, Kashan, central Iran, in Jan 2022. He suffered from a 2-month history of a 1×3 cm lesion on the left ear. In the microscopy examination, amastigotes forms of <i>Leishmania</i> spp. were observed. <i>L. tropica</i> was confirmed using a single PCR with species-specific primers. The patient was introduced to a physician to begin the treatment protocol. It is recommended that physicians, especially in an endemic area, investigate any atypical lesion for CL.</p>
<p>Keywords: <i>Leishmania</i>; Ear; Iran</p>	
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Introduction

Cutaneous leishmaniasis (CL) is an obligatory intracellular protozoon zoonotic disease caused by some *Leishmania* genus species and transmitted by the bite of female sand flies (1, 2). It is estimated, 0.7 to 1.2 million CL cases occur each year, globally. CL is more widely distributed, with about 70 to 75% of global estimated CL incidence occurring in, Afghanistan, Algeria,

Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru, (3)

CL is the most common form of leishmaniasis. It presents as singular or multiple ulcerative lesions at the site of insect exposure and is not life-threatening but causes permanent scars that occur upon healing of the lesion (4). However, up to 10% of CL cases progress to more severe manifestations known as muco-



cutaneous leishmaniasis, diffuse CL, disseminated CL, and leishmaniasis recidivans(5).

L. major and *L. tropica* are principally two causative agents of CL in the old world. CL is caused by several species, including *L. mexicana*, *L. amazoniensis*, *L. guyanensis*, *L. panamensis* and *L. braziliensis* in Central and South America (6). Iran is known as an important endemic area for CL in the world. In Iran, CL frequently occurs in many rural and urban areas. The agent of CL is *L. major* in rural areas and *L. tropica* in urban areas. CL remains an important disease and widely distributed in Iran and has been reported in central, west, southwest, south, southeast, east, and northeast region (7). It has been reported in 25 of 31 Iran provinces. The incidence of CL varied from approximately 50 to 250 cases per 100,000 of Iranian population during the 1977 to 2015 (8). Kashan is an endemic focus of urban CL, located in Isfahan Province, Central Iran (9). CL has increased in Kashan and its suburbs in recent years (9, 10).

According to the recommendation for treatment, CL lesions are divided into simple and complex infections. Complex lesions are defined as those that are more than 2-3 in number or are more than 40 mm in their maximum diameter. Some face lesions, such as lip, ear, and eyelid lesions, are regarded as complex CL because of the higher risk of deformity from the infection and lead to cosmetic problems (11).

In the present paper, we reported a case of complex CL of the ear. The microscopy and molecular results revealed *L. tropica* infection in the patient.

Case Report

A 61-year-old man weighing 65 kg was referred to the Reference Laboratory at the Kashan University of Medical Sciences, Kashan, Central Iran, in Jan 2022, with a 2-

month history of a 1×3 cm lesion on the left ear (Fig.1).

A written and signed consent to use the data and results for publication was taken from the patient.



Fig. 1: Cutaneous lesion on the left ear

The patient had diabetes and had no experience traveling outside Kashan for the past one years ago. He complained that his feature of ear was uncomfortable and scabrous, but has not any pain or any systemic complaints.

Direct microscopy examination of the scales by 10% potassium hydroxide (KOH) preparation was negative for fungus elements.

The lesion was thoroughly cleaned with 70% alcohol. Specimens were collected from the margin of the lesion by scraping with a scalpel blade. Two prepared smears were fixed by methanol, stained with 10% Giemsa stain for 30 min, and examined microscopically for the presence of amastigote forms of *Leishmania* spp. In the microscopy examination, many amastigotes forms of *Leishmania* spp were observed (Fig. 2).

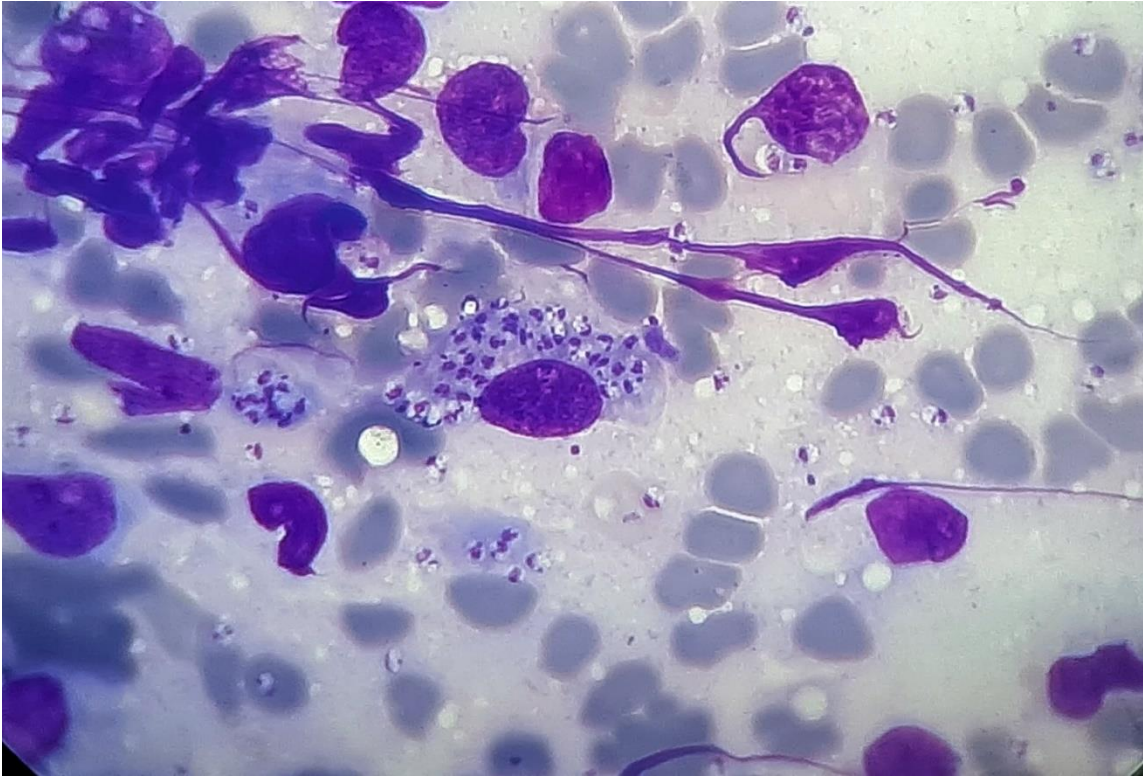


Fig. 2: Amastigote forms of *Leishmania* in Giemsa staining of smear (X 100)

The DNA extraction was performed from the stained smear samples using a DNP™ kit (Cinaclon, Iran), according to the manufacturer's instruction protocol. A single PCR was performed using species-specific primers, LINR4 (Forward: GGG GTT GGT GTA AAA TAG GG) and LIN17 (Reverse: TTT GAA CGG GAT TTC TG), to amplify the variable region of the minicircle kDNA of *Leishmania* (10, 12). These primers amplify a 760, and 650 bp fragment for *L. tropica* and *L. major* respectively.

A positive sample of *L. tropica* (MHOM/IR/89/AR2) and *L. major* (MHOM/IR/54/LV39) were used as the pos-

itive controls, and distilled water was used as the negative control. PCR product was evaluated by electrophoresis on 1.5% agarose gel, stained by ethidium bromide, and then visualized using ultraviolet light. A 760-bp fragment was amplified using the primers. The PCR products were identical in size to the positive control of *L. tropica* (Fig. 3).

Eventually, the patient was introduced to a physician to begin the treatment protocol. He was treated with glucantime, two vials of muscular, and intra-lesion injections weekly for four weeks. Unfortunately, the patient was lost for post-treatment monitoring.

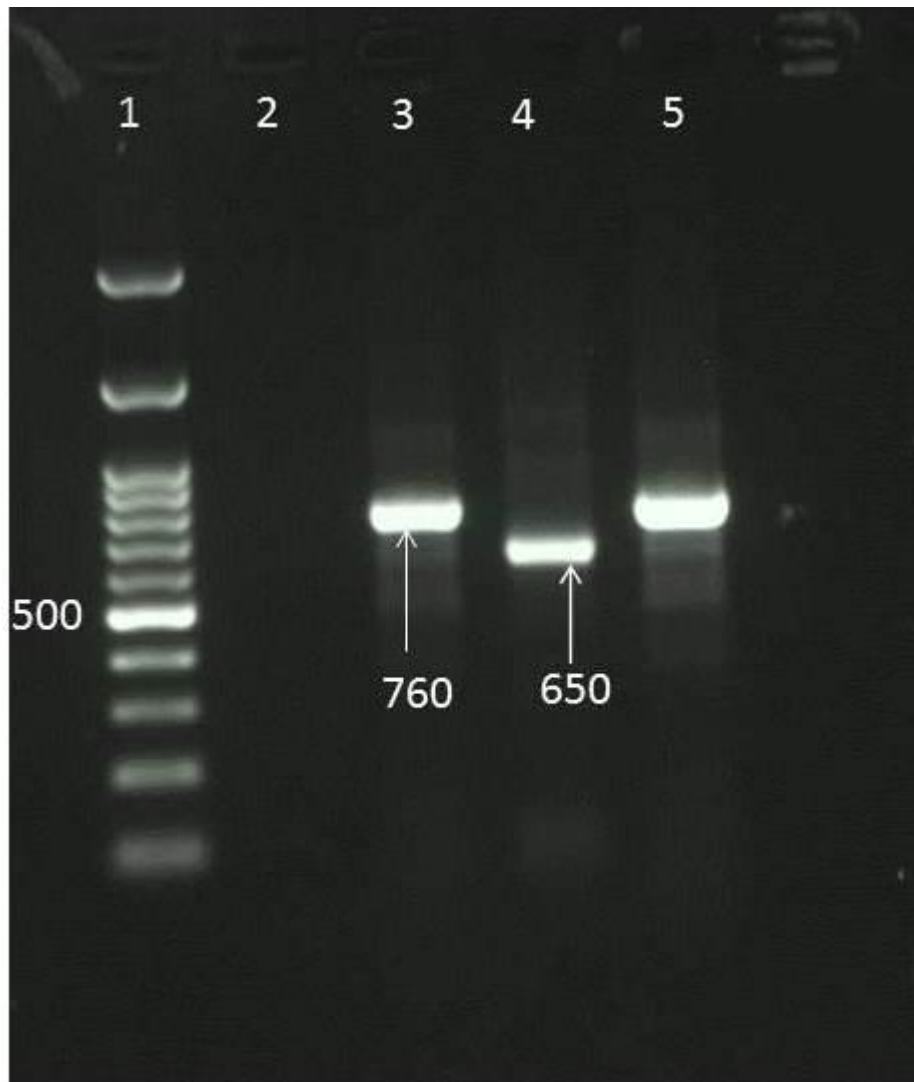


Fig. 3: 1.5% Agarose gel electrophoresis of the PCR products. Line1: 100-bp DNA ladder, lane 2 negative control, Lane 3–4 *L. tropica* and *L. major* positive control, respectively. Lane 5 ear sample

Discussion

In the old world, *L. major* is the main cause of zoonotic cutaneous leishmaniasis (ZCL) and *L. tropica* is the main cause of anthroponotic cutaneous leishmaniasis (ACL). *L. major* infection is mostly endemic in rural areas of Iran and is transmitted mostly by *Plebotomus papatasi* sandfly, different desert rodent species are reservoirs for ZCL in Iran and neighboring countries.

By contrast, *L. tropica* is endemic in some large Iranian cities, such as Tehran, Kerman, Shiraz, Isfahan, Mashhad, and Bam. *Ph. sergenti* sandfly is the main vector of ACL in Iran and throughout the Middle East. Humans and dog has been confirmed reservoirs for ACL in Iran (13).

The two main species of old- world Cl, *L. major* and *L. tropica* are frequent in Kashan. Most *L. tropica* cases occur in the center of the county, whereas *L. major* cases are found in rural and desert subareas, East and North

West of Kashan (10). A molecular and parasitological study of CL patients in Kashan (9) revealed that the main species in the region was *L. tropica* (92.1%), followed by *L. major* (7.9%).

A study carried out to identify the epidemiologic status of CL in Kashan showed a progressive increase in the CL incidence rate and revealed that most of the patients had one ulcer. The most commonly affected organs, were hands (45.8% hands, 7.1 hands with feet, and 2.9% with face), followed by feet and face (14).

The favorite sites for manifestations of CL are hands, feet, trunk, and face. CL usually does not affect the ears and eyelids, although these organs can be involved in CL rarely. Hajjarian et al reported four disseminated CL cases with multiple nodular, ulcerative and crusted lesions extended on the face, trunk, and extremities in Tehran. They isolated parasite from the active lesions and identified as *L. major* using PCR-RFLP assay and sequencing analysis (15). A rare case of a congenital form of CL around the glans penis due to *L. major* reported in a 24-years-old male patient from the central part of Iran (16).

A literature review showed only one case of primary cutaneous lesions of the ears in Iran. Bahmani et al. reported an extensive CL lesion on the ear of a 35-year-old male patient residing in Dehloran, West of Iran, in 2017 (17). Giemsa staining of resampling smears conferred diagnosis of the patient, but the species identification of parasite was not made.

Goldin et al. reported a case of complex CL of the ear of an 82-year-old Canadian woman who had traveled to an endemic area of Israeli ten months before presentation. The patient's lesion was positive in examining the smear prepared with Giemsa staining, and polymerase chain reaction (PCR) identified the organisms as *L. tropica*. She was treated with photodynamic therapy (18).

Atypical presentation lesions of CL have been reported around the world. Because

these lesions can mimic many other cutaneous diseases, confound physicians, and submit patients to unnecessary treatments, it is necessary for physicians, especially in endemic areas, to be aware that any atypical lesion must be investigated for CL. A systematic review of the literature on atypical lesions of CL, was published (19). Differential diagnosis of lip, ear, and eyelid lesions from other disorders, especially bacterial and fungal diseases, is essential for ENT specialists and dermatologists, particularly in patients living in endemic areas of leishmaniasis or with a history of traveling to these areas.

The kinetoplast minicircle (kDNA) is a suitable target for amplification and species identification because the sequence of this target is known for most species of *Leishmania* parasite and present in nearly 10,000 copies per cell (11). A combination of kDNA-PCR and microscopic methods is was ideal for diagnosing CL and determining of parasite species due to 99% sensitivity and 100% specificity (10).

Conclusion

Although CL lesions have mostly been reported in the face, trunk, hand, and feet, they sometimes have an atypical form or occur in unusual areas such as ears, nose, eyelids, and genital regions. Physicians need to be aware of unusual and atypical forms of CL, especially in people that live in endemic areas or have traveled to these regions, to reduce the misdiagnosis and control the disease progression.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

1. Pal M, Gutama KP, Steinmetz CH, et al. Leishmaniasis: an emerging and re-emerging disease of global public health concern. *Am J Infect Dis.* 2022; 10 (1):22-25.
2. Azim M, Khan SA, Ullah S, et al. Therapeutic advances in the topical treatment of cutaneous leishmaniasis: A review. *PLoS Negl Trop Dis.* 2021; 15 (3): e0009099.
3. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis Worldwide and Global Estimates of Its Incidence. *PLoS One.* 2012; 7(5): e35671.
4. Sabzevari S, Teshnizi SH, Shokri A, et al. Cutaneous leishmaniasis in Iran: A systematic review and meta-analysis. *Microb Pathog.* 2021; 152: 104721.
5. Burza S, Croft SL, Boelaert M. Leishmaniasis. *Lancet.* 2018; 392 (10151): 951–70.
6. Marra F, Chiappetta MC, Vincenti V. Ear, nose and throat manifestations of mucocutaneous Leishmaniasis: a literature review. *Acta Biomed.* 2014; 85 (1): 3-7.
7. Davoodi T, Khamesipour A, Shahabi S, et al. Geographical distribution and molecular epidemiology of cutaneous leishmaniasis in Fars Province, southern Iran. *Environ Sci Pollut Res Int.* 2022; 29: 4024–32.
8. Piroozi B, Moradi G, Alinia C, et al. Incidence, burden, and trend of cutaneous leishmaniasis over four decades in Iran. *Iran J Public Health.* 2019; 48(Supple 1):28–35.
9. Shiee MR, Mohebbali M, Doroodgar A, et al. A molecular and parasitological survey on cutaneous leishmaniasis patients from historical city of Kashan in Isfahan province, center of Iran. *Asian Pacific J Trop Dis.* 2012; 2(6):421-25.
10. Rasti S, Ghorbanzadeh B, Kheirandish F, et al. Comparison of molecular, microscopic, and culture methods for diagnosis of cutaneous leishmaniasis. *J Clin Lab Anal.* 2016; 30(5):610-15.
11. Bailey MS, Green AD, Ellis CJ, et al. Clinical guidelines for the management of cutaneous leishmaniasis in British military personnel. *J R Army Med Corps.* 2005; 151(2):73-80.
12. Aransay A, Scoulica E, Tselentis Y. Detection and identification of *Leishmania* DNA within naturally infected sand flies by seminested PCR on minicircle kinetoplastic DNA. *Appl Environ Microbiol.* 2000; 66 (5): 1933–38.
13. Ghatee MA, Taylor WR, Karamian M. The geographical distribution of cutaneous leishmaniasis causative agents in Iran and its neighboring countries, a review. *Front Public Health.* 2020; 8:11.
14. Ahmadi NA, Ghafarzadeh M, Jalaligalooosang A, et al. An epidemiological study of cutaneous leishmaniasis with emphasis on incidence rate in Kashan, Isfahan province. *J Ilam Univ Med Sci.* 2013; 21(2): 1 - 9.
15. Hajjaran H, Mohebbali M, Akhavan AA, et al. Unusual presentation of disseminated cutaneous leishmaniasis due to *Leishmania major*: case reports of four Iranian patients. *Asian Pac J Trop Med.* 2013; 6(4): 333-6.
16. Mosayebi M, Mohebbali M, Farazi A, et al. Leishmaniasis Caused by *Leishmania major* on the Glans Penis: A Case Report. *Iran J Parasitol.* 2019;14(3): 472-476.
17. Bahmani M, Shamsi M, Naserifar R, et al. A rare case report of extensive cutaneous Leishmaniasis lesions on ear from Dehloran city, West of Iran and the treatment process. *Int J Adv Bio Res.* 2017; 8(3):1747-49.
18. Goldin H, Kohen S, Taxy J, et al. *Leishmania tropica* infection of the ear treated with photodynamic therapy. *JAAD Case Rep.* 2020; 6(6):514-17.
19. Meireles CB, Maia LC, Soares GC, et al. Atypical presentations of cutaneous leishmaniasis: a systematic review. *Acta Trop.* 2017; 172:240-54.