Abstract

Background: The Islamic Republic of Iran is one of the most important endemic foci of cutaneous leishmaniasis (CL) in the world. Annually, a large number of Iranian Shia pilgrims travel to Iraq from this area in order to participate in one of their most important religious ceremonies. This trip has coincided with the seasonal activity of sand flies in recent years. So, CL could be a serious threat for pilgrims on this pilgrimages trip.

Aims: To report cases of CL among Iranian Shia pilgrims attending a religious ceremony in Iraq during 2017.

Methods: Sixteen patients were referred to our laboratory in the Department of Parasitology and Mycology at Qazvin University of Medical Sciences. Dermal scrapings and stained slides prepared from skin lesions were used for morphological diagnosis. DNA extraction and polymerase chain reaction were optimized for identification of Leishmania species.

Results: Leishmania bodies were microscopically diagnosed in all patients. L. major was detected by molecular approach. The number of lesions observed in patients was 1 (31%), 2

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(25%), and ≥ 3 (44%). A maximum of 6 lesions was seen in 1 patient.

**Conclusions:** It seems that CL will be a potential risk for Iranian pilgrims who participate in pilgrimage trips in future years. Since a large number of Shia Muslims participate in the annual religious ceremonies, serious measures must be taken to prevent the disease.

Keywords: cutaneous leishmaniasis, Islamic Republic of Iran, Mehran, pilgrims, travellers

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**Introduction**

Leishmaniasis is an important parasitic disease with a diverse spectrum of clinical syndromes, including cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis and visceral leishmaniasis (1). Some cutaneous lesions can be self-healing. CL is an important public health problem when the lesions present for a long period, with persistent ulcers and secondary bacterial infections, incurring high treatment costs and complications of drug therapy (2–4). CL is caused by species of Leishmania genus, an obligate intracellular protozoan parasite of mononuclear phagocytes in vertebrate hosts (5). To date, more than 30 species of Leishmania have been detected and at least 20 can infect humans (6,7). Female phlebotomine sand flies are vectors of the parasite in tropical and subropical regions. About 95% of CL is distributed in South and Central America, the Mediterranean, Middle East and Central Asia (8,9). Leishmaniasis has been reported in 98 countries within 5 continents, among those, the most important endemic foci of CL in the old world include Afghanistan, Algeria, Islamic Republic of Iran, Saudi Arabia and Syrian Arab Republic, and in the new world Brazil, Colombia and Peru.
Cutaneous leishmaniasis as an increasing threat for Iranian travellers attending religious ceremony in Iraq

CL is common in the Islamic Republic of Iran and comprises both anthropogenic CL and zoonotic CL, caused by Leishmania tropica and Leishmania major, respectively (3,12). To date, CL is endemic in 17 of 31 provinces in the Islamic Republic of Iran, with the majority being zoonotic CL (11). Nevertheless, the prevalence of human parasitic infections, especially those caused by intestinal helminths, has remarkably decreased in the Islamic Republic of Iran (13), but zoonotic CL is spreading in the country and new foci of infection have been reported in recent years (14–19). It seems that migration of rodent reservoirs to new areas is a possible major cause of the spread of CL in the Islamic Republic of Iran. Annually, about 20 000 of new cases of CL are recorded in the country (20). Also, CL epidemics have been reported from the Islamic Republic of Iran in recent decades (21,22).

Residents of nonendemic areas for CL in the Islamic Republic of Iran are the population at risk for this disease when they travel to endemic areas during seasonal activity of sand flies. Ilam Province is one of the 31, and also an important endemic focus of CL that is located in the West of the Islamic Republic of Iran in the neighbourhood of Iraq. Mehran is a county in west of the province and categorized as a hyperendemic area of CL (14, 23–25). This county is the shortest route from the Islamic Republic of Iran to Karbala City in Iraq. Karbala is a holy city for Shia Muslims, and a large number travel to Karbala to participate in one of the most important religious ceremonies, called Arbaeen Husayni. In subsequent years, this massive pilgrimage will coincide with peak seasonal activities of sand flies (final months of summer and first months of autumn); therefore, there is an increased risk of CL among the participants.

Methods

This cross-sectional study was performed from January to April 2018. Sixteen patients (15 male, 1 female, aged 28–60 years) were referred to the Parasitology and Mycology Laboratory, Qazvin University of Medical Sciences, Qazvin, Islamic Republic of Iran. None of the pilgrims had a history of CL. Except for the woman from Qazvin (a nonendemic region for CL) who was among the pilgrims to Karbala, all the patients were bus company employees from Qazvin. These people were responsible for transferring pilgrims from Mehran to border regions of the Islamic Republic of Iran and Iraq. These people rested and slept in a building located on the outskirts of Mehran, where they stayed for 20 days. Mehran is in Ilam Province in the West of the Islamic Republic of Iran.

Initially, cutaneous lesions were disinfected by 70% ethanol. Two to three smears were prepared from each lesion. The smears were fixed in methanol and then stained by Giemsa stain. The slides were viewed under light microscopy at 1000× magnification to detect Leishmania bodies (amastigotes).
DNA extraction was performed on the slides. The immersion oil on each smear was removed with xylol, and then the entire smear was scraped into a 1.5-ml microcentrifuge tube. DNA of the smear was extracted using a Qiagen QIAamp DNA Mini Kit. Polymerase chain reaction (PCR) was performed, targeting with specific primers the kinetoplast DNA pattern of 615 bp for L. major and for 744 bp for L. tropica. Conventional PCR was carried out in a 25-μl reaction volume containing, 5 µM template DNA, 0.1 μM each primer and 0.5–4 µM MgCl2 (26). Negative and positive controls were used to monitor all reactions. After electrophoresis, The PCR products were stained with ethidium bromide and specific bands were seen under UV light.

The local Ethics Committee ruled that no formal approval was required in this particular study. The cost of testing was free for the patients. The test results were provided to the patients and they were admitted to university medical centre for treatment. Photographs were taken of the patients’ wounds with their oral permission.

**Results**

Leishmania bodies were microscopically diagnosed in all 16 patients referred to our laboratory (Figure 1). The microscopic results were confirmed by molecular approach, and L. major was detected among all specimens (Figure 2). The incubation periods of CL in our patients were not clearly defined, but the onset of the first signs of disease (boils) appeared 2 weeks to 2 months after departure from Mehran area and arrival in Qazvin province. An incubation time of almost 6 months was observed in 1 patient. A maximum of 6 lesions was observed in 1 patient. The frequency distribution for the number of lesions was: 1 wound (31%; 5/16); 2 wounds (25%; 4/16); and ≥ 3 wounds (44%; 7/16). A rare case of CL was seen in a patient who had 2 ulcers, 1 in the upper eyelid and another in the middle and front of the neck (Figure 3). Surprisingly, 1 patient attempted self-treatment by burning lesions with a cigarette (Figure 4).

**Discussion**

Traveling to endemic areas of vector-borne diseases is a risk factor for people who travel from nonendemic areas. CL is a sand-fly-borne disease that has been reported among travellers entering endemic foci of this protozoan parasitic disease (26–30). In our study, CL occurred among bus company employees who were responsible for transporting the Arbaeen pilgrims from Mehran to the border areas between the Islamic Republic of Iran and Iraq and vice versa. The pilgrims were located in a building on the margin of Mehran City, which is considered to be
an important endemic focus of zoonotic CL in the West of the Islamic Republic of Iran (23).

In the present study, L. major was the only species of Leishmania that was isolated from the patients and identified by PCR amplification, as reported in a previous study (23). The findings indicated that the area in question is a focus of zoonotic CL in the Islamic Republic of Iran, and that human infections with Leishmania have zoonotic origin in this region. Reservoirs of the parasite in the western regions of the Islamic Republic of Iran are rodents of the Gerbilidae family, including Rhombomys opimus, Meriones libycus, Meriones persicus, Tatera indica and Nesokia indica, with T. indica categorized as the main reservoir (28, 29). Phelobotomus papatasi was identified as a major vector for L. major in the region and neighbouring regions (30).

Evidence from 92 patients shows that CL has increased in the Mehran area in recent years (23). CL could be a potential risk for Iranian religious pilgrims who are eager to participate in the Arbaeen Husayni ceremony in Iraq in future years. This ceremony is one of the most important for Shia Muslims. One of the most holy cities to Muslims is Karbala in Iraq, where the burial ground of the third Imam of the Shiites (Imam Husayn) is located. One of the most important days to commemorate Imam Husayn is the 40th day after his martyrdom, known as Arbaeen. Around this time, large crowds of Shia Muslims travel to Karbala every year (31). Mehran is the shortest route for Iranian pilgrims and most go to Karbala this way. In 2017, the transfer of some Iranian pilgrims to the Iranian–Iraqi border region was carried out by employees of the bus company in Qazvin. The pilgrims were settled in a building around Mehran City that was close to the habitat of rodents and vectors of leishmaniasis. Therefore, they were at risk of CL. Sixteen individuals were referred to our laboratory. Other patients may have been referred to other laboratories or had small lesions and preferred not to visit a doctor for their treatment.

In this study, only 1 of the patients was among the Arbaeen pilgrims. All the patients were free of immunodeficiency and lived in Qazvin, which is considered to be a nonendemic region for CL. However, the number of pilgrims was higher than the number of persons involved in transportation. Probably, the low frequency of CL among the pilgrims was related to the short-term stop of a few hours in comparison to the 1-month stay of staff in the area, so the pilgrims were at lower risk of sand fly bite.

It seems that the risk of CL will be an increasing threat to Iranian pilgrims involved in the Arbaeen ceremony in future years, even those with short-term residency in the endemic area, because this pilgrimage will gradually coincide with the peak biting activity of sand flies in the study area. The ceremony is based on lunar months with 354 days in a year (355 days in the third year), whereas it is 365 days in a year according to solar (Shamsi) and Gregorian
calendars. Therefore, the Arbaeen ceremony will occur 10 days earlier than in the previous year, based on Shamsi and Gregorian calendars. The ceremony was held on November 9, 2017 and October 30 2018. The second peak activity of sand flies in the West of the Islamic Republic of Iran is around the end of summer to early autumn, so it is predicted that if essential preventive measures are not established within the next few years, an outbreak of CL may occur. The eagerness of Shia Muslims for the pilgrimage is that even the risk of CL will not discourage them.

In conclusion, there is an increasing risk of CL for Iranian pilgrims who wish to participate in Arbaeen pilgrimage trips in future years. Therefore, it is recommended that healthcare providers take serious preventive measures to secure good health conditions for the pilgrims, such as providing insect repellents to pilgrims.

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References


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