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Short Communication

Molecular Identification of Cutaneous Leishmaniasis Species in the Northcentral Iranian Province of Alborz: Is There a New Focus on Cutaneous Leishmaniasis in the Province?

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Abstract

Background: Cutaneous leishmaniasis (CL) is an endemic infection in the Middle East, including Iran that is also spreading to new foci. We aimed to determine the leishmaniasis species causing CL in Alborz province.

Methods: Overall, out of 55-suspected CL patients referred to health centers in Alborz Province, north central Iran in 2019, 40 patients had positive smear for CL based on optical microscopy. The internal transcribed spacer 1 (ITS1) of nuclear ribosomal DNA (rDNA) was amplified by PCR. *Leishmania* species were identified by PCR–restriction fragment length polymorphism (PCR-RFLP) using BshF I (Hae III) enzyme.

Results: Out of the 40 positive patients with CL, 34 cases (85%) had been caused by *Leishmania* (*L*) *major* and six (15%) by *L. tropica*. Fifteen patients had no history of traveling to the disease endemic areas, of which nine were Iranians. Skin lesions and scars caused by CL were mostly observed on the hands and face. Moreover, more than two skin lesions were observed in 22 cases (55%), all of which were infected with *L. major*. A single skin ulcer was seen in 18 (45%) of the CL patients.

Conclusion: Climate change, reduced rainfall, and demographic changes such as migration into Alborz Province and the increasing marginalization of the population and their entry to settle in new areas might have caused natural transmission of both *L. tropica* and *L. major* in this province.

Introduction

Leishmaniasis is a globally significant, vector-borne, parasitic disease with a wide range of clinical symptoms that can be classified as a neglected disease (1-3). Cutaneous leishmaniasis (CL) is a disease reported in the tropical and subtropical regions of the world (4). In Iran, more than 20,000 cases of CL are reported annually (5). CL is also prevalent in neighboring countries of Iran, including Iraq, Afghanistan, Pakistan and Turkey (6).

CL is considered an important disease in Iran due to its spread in different parts of the country, the difficulty and length of its treatment and the reported resistance to Meglumine antimoniate (Glucantime) as the drug of first choice for treatment, the possibility of secondary infection and the imposition of economic burden on the country's health system (7,8).

Although microscopic and culture methods are involved in the diagnosis of CL, these methods are not able to identify *Leishmania* species. For this purpose, molecular methods such as RFLP-PCR are used to determine the species of *Leishmania* (9). The advantages of this method can be 92% sensitivity and 100% specificity, low error probability, the need for a very small amount of nucleic acid in the organism and the ability to examine many samples per unit of time (1).

According to our data, despite the prevalence of CL in most parts of Iran, and the spread of CL to new foci and neighboring provinces including Tehran (10) there were no parasitic and molecular studies on the prevalence of CL and *Leishmania* species involved in CL in Alborz province, located to the northwest of Tehran, the capital of Iran. Previous studies have also reported visceral leishmaniasis in humans and owned dogs in rural regions of Alborz Province (11,12).

Therefore, we aimed to determine the prevalence of CL and identify *Leishmania* species that were isolated from CL patients by parasitological and molecular methods in Alborz Province.

Materials and Methods

A sample of skin lesions suspected of CL was obtained from 55-suspected patients referred to health centers located in Karaj, Savojbolagh, Nazar Abad and Fardis in Alborz Province, northcentral Iran in 2019. In total, 40 patients had positive microscopic smear examination for Cl. The DNA was extracted from *Leishmania* positive microscopic slides using commercial kit (Favorgen Biotech Corp, Taiwan). The internal transcribed spacer 1 (ITS1) of nuclear ribosomal DNA (rDNA) was amplified by PCR. The sequences of primers used in this study were LITSR (forward: 5'-CTGGATCATTTTCCGATG3') and L5.8S (reverse: 5'-TGATACCACTTATCGCACTT) (20). Positive and negative controls were used for *Lishmania* (*L*) *major* and *L. tropica* in PCR (Fig. 1). PCR and RFLP-PCR were performed to determine the species of *Leishmania* according method described by Kazemi Rad et al. with minor modifications (13). The PCR reaction was amplified for 35 cycles at 94°C for 30 sec, 50°C for 30 sec and 72°C for 1min.

RFLP-PCR

The enzyme used in this step was BshF I (Hae III), produced by Takapouzist Company (licensed by Jena Bioscience); this enzyme could help find the desired sequence and identify the *Leishmania* species. For this purpose, 8 µl of the PCR products were incubated with the 1 µl enzyme and 2.5 µl enzyme buffer at 37 °C for 2 hours. The PCR-RFLP products were then run on agarose gel 1% and the electrophoresis gel was photographed with an ultraviolet transilluminator.

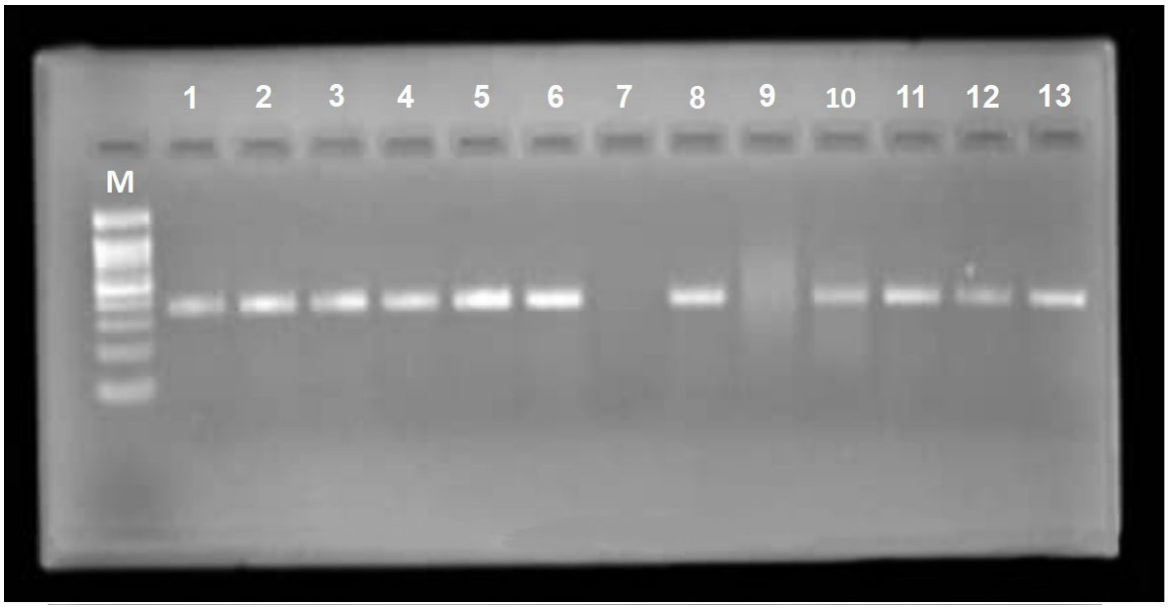

Fig. 1: Electerophoresis findings of ITS1-RCR from *Leishmania* slide smears. Lane 1 positive control, Lane 7 Negative control. M: 100 bp size marker. Lanes 2-6 ,8 and 10-13 (about 350 bp) are Positive for CL

***Statistical analysis*:** Chi-square was used for statistical analysis of data.

***Ethical considerations*:** This study was approved by the Ethics Committee of Alborz University of Medical Sciences (IR.ABZUMS.REC.1397.084). Informed consent was obtained from all the participants prior to participation in the research.

Results

Out of 40 positive samples, 34 cases (85%) showed *L. major* and six (15%) showed *L. tropica* (Fig. 2). Most of CL cases were over 18 years old (Table 1). Thirty-one CL patients (77.5%) were male and nine (22.5%) were female. There was no statistically significant difference in age and sex between the patients with respect to urban and rural CL (P=0.1). This infection had a relatively different pattern with seasonal changes, as its prevalence was higher in fall (Table 2). There was no statistically significant difference between *Leishmania* species and season (P=0.3).

Most cases of Cl were observed in the central part of Karaj (Table 3). As for the nationality of the infected patients, 19 (47.5%) were Afghans and 21 (52.5%) were Iranians. In the patients of Afghan nationality, 16 cases were *L. major* and three were *L. tropica*, while in the patients of Iranian nationality, 18 cases were *L major* and three *L. tropica*. Overall, 15 of the participants had no history of traveling to endemic areas of the disease, of which nine were Iranians.

As for the site of lesions caused by CL, 52.5% were observed on the hands, 35% on the face, 10% on the feet and 2.5% over the body. A total of 22 cases (55%) had more than one lesion, and all those in this group were infected with *L. major*. A single lesion was observed in 18 (45%) CL cases. Overall, 30 (75%) of the CL patients had less than high school diploma while ten (25%) had university education.

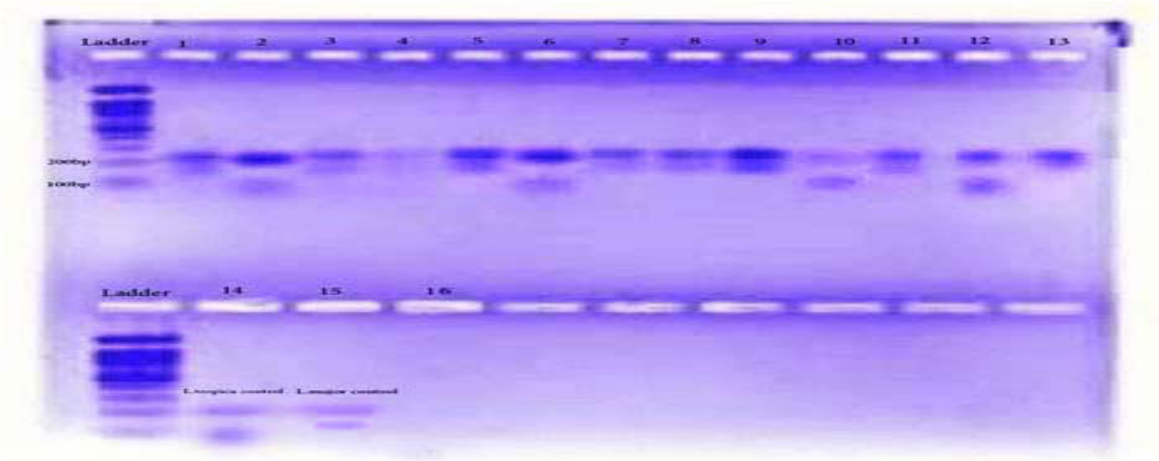

Fig. 2: RFLP-PCR gel electrophoresis pattern, using 100bp Ladder, Lanes 2, 6, 10 and 12: *L. tropica* and other lanes belong to *L. major*. Lanes 14 and 15 are positive controls for *L.tropica* and *L.major* respectively. Lane 16 is Negative control

Table 1: Prevalence of Leishmania species according to the age in patients with CL referred to Health Centers in Alborz Province in 2019

Species	***Age (yr)***			***Total***
	<5	5-18	>18	
L. major	5	6	23	34
L. tropica	0	1	5	6
Total	5	7	28	40

Table 2: The prevalence of Leishmania species according to the season of infection in patients with CL referred to health centers in Alborz Province in 2019

Species	***Season***				***Total***
	Spring	Summer	Autumn	Winter	
L. major	9	11	11	3	34
L.tropica	1	1	2	2	6
Total	10	12	13	5	40

Table 3: Prevalence of Leishmania species based on place of residence in patients with CL referred to health centers in Alborz Province in 2019

Species	***Region***				***Total***
	Karaj	Fardis	Savojbo-lagh	Nazar Abad	
L.major	17	11	5	1	34
L.tropica	3	3	0	0	6
Total	20	14	5	1	40

Discussion

Despite the decrease in cases of CL throughout the world, including Iran, over the past decades, the spread of this disease has undergone changes that have made CL spread to new foci. The new foci in Tehran province, including Pakdasht and Varamin, which are located 60 km away from Karaj, have reported cases of CL caused by *L. major* (10) .

Alborz province is not considered an endemic region for CL in Iran; however, the demographic and climatic changes in this province along with the referral of patients with CL to the health centers of the province inspired these researchers to study this subject. The development of CL in residents of this province who had no history of traveling to endemic areas of the disease over the two years before the lesions appeared was very interesting.

In the present study, 15 (37.5%) of the subjects had not travelled to endemic areas of CL, including nine (22.5%) of Iranian nationality; this observation provides valuable information in terms of epidemiological status and probability of local transmission of CL in Alborz Province.

A study conducted on the frequency of vectors of CL and VL in Alborz Province found that the *Phlebotomus sergenti* vector of *L. tropica* was the predominant species isolated from the genus of *Phlebotomus* (14). In our study, however, six cases were infected by *L. tropica*, including four who had no history of travel to endemic regions of CL in Iran or neighboring countries. Despite the decrease in the burden of urban Cl caused by *L. tropica* throughout the world (15), the possibility of disease spread is increased due to factors such as climate changes, decreased rainfall, population displacement and settlement, increased marginalization of populations and high number of immigrants from other endemic regions as well as from Afghanistan into Alborz Province. These factors have enabled the growth and multiplication of leishmaniasis vectors and reservoirs. This finding is in line with the reported spread of *L. tropica* foci to new areas in Iran, Afghanistan and Syria (15-16).

In a study performed in Kerman in the southeast of Iran using microscopy and PCR, migration, poverty and environmental distress were found to contribute to the epidemic of anthroponotic CL (17).

Phlebotomus papatasi was also reported in Alborz province as the main vector of *L. major* (14). Moreover, vectors of cutaneous leishmaniasis have grown and multiplied in Alborz Province, and along with climate change and increased marginalization of populations, the probability of local transmission of the disease is raised. One of the reasons for the increase in ZCL might be the rising temperature in the Middle East, which causes *Phlebotomus papatasi* to grow and spread wider (16). Even in Turkey, which has long been one of the primary sources of *L. tropica* in the Middle East, cases of *L. major* have recently been reported with the arrival of Syrian refugees into that country (16).

In the present study, both sexes were susceptible to the disease, but most cases occurred in men, which is consistent with other reports from Iran (18,19). Men seem to spend more hours outdoors because of their jobs as well as differences for clothing worn by men and women, making men more exposed to vector bites. Furthermore, high levels of infection in adulthood (over 18 years) also indicates prolonged adult exposure.

In our study, *L. major* was the predominant species, as similar to other studies conducted in Iran, such as in Bushehr, Fars, Qom, Kermanshah and Sistan-Baluchistan provinces; these studies have examined cutaneous leishmaniasis by both molecular and parasitological methods (7, 20-22). In the neighboring countries of Iran, including Iraq, Pakistan and Afghanistan, *L. major* is also the predominant

species (6,23). Moreover, because a significant part of patients have a history of traveling to endemic areas of CL and *L. major* has played an essential role in the development of CL, most cases of CL in Alborz province are caused by *L. major*. Therefore, the high rate of *L. major* compared to *L. tropica* in the present study seems to be consistent with the higher prevalence of *L.major* in the country (24).

In line with the literature, lesions were more common on the exposed areas of the body, such as the hands and face, as these parts are more prone to sandfly bites.

In all societies, the level of health is commensurate with the community's level of awareness and knowledge. In our study, the vast majority of the patients had no university education and were people who had to spend more time outdoors.

One of the limitations of this study was that the number of samples was not large enough to explain easily the epidemiological factors of CL

Conclusion

Climate change, reduced rainfall and demographic changes such as migration into Alborz province and increasing marginalization and entry of people to settle in new areas, have contributed to the local transmission of cutaneous leishmaniasis in this province. Therefore, in addition to the need to fight the carrier and reservoir of CL, the public should also receive health tips and be instructed on ways to prevent cutaneous leishmaniasis. Further studies should be conducted in this province on the carriers and reservoirs of CL.

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Conflict of interest

The authors declare that they have no conflict of interest.

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