Review
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Changes in the Epidemiology of Cutaneous Leishmaniasis in Northeastern Iran

Kuzeydoğu İran'da Kutanöz Leishmaniasisin Epidemiyolojisindeki Değişimler

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ABSTRACT

The province of Khorasan-Razavi in the North East of Iran is an endemic area for anthroponotic cutaneous leishmaniasis (ACL caused mainly by *Leishmania tropica*) and zoonotic cutaneous leishmaniasis (ZCL caused mainly by *Leishmania major*). Based on clinical signs, some cities were considered as ACL foci while others were considered to be endemic for ZCL.

This paper reviews studies performed on patients diagnosed with cutaneous leishmaniasis (CL) via the use of direct slide examination, ELISA, electrophoresis isoenzyme, RAPD PCR and PCR in Mashhad; the study also includes cases of CL in other cities of the Khorasan-Razavi province where only PCR used as a diagnostic tool. The data show that both *Leishmania tropica* and Leishmania major caused CL in most of the cities investigated. Our review shows that *Leishmania major* was found in areas where ACL is prevalent and *Leishmania tropica* was observed in areas with high incidence of ZCL. This distribution represents a major change in the epidemiological pattern of Leishmania in the Khorasan-Razavi province.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, *Leishmania tropica*, molecular and immunological techniques, Khorasan-Razavi province.

ÖZ

Khorozan-Razavi eyaleti, Kuzey Doğu İran'da yer alan, anthroponotik kutanöz leishmaniasis (AKL'nin sebep olduğu *Leishmania tropica*) ve zoonotik kutanöz leishmaniasis (ZKL'ye sebep olan *Leishmania major*) için endemik bir bölge niteliğindedir. Bu çalışmada klinik önem bakımından bazı şehirler AKL olarak nitelendirilmesine karşılık bazı şehirler ZKL olarak tanımlanmıştır.

Bu araştırma, kutanöz leishmaniasis (KL) olarak teşhis edilmiş hastalardan elde edilen örneklerin direkt mikroskobik bakıyla teşhisleri ile ELISA, elektroforez, izoenzim, RAPD PCR ve PCR yardımı ile Meşhed'te gerçekleştirilmiştir. Bu araştırma aynı zamanda Khorozan-Razavi eyaletinde sadece PCR kullanılarak yapılan KL örneklerini de içerir. Yapılmış olan bu çalışmanın sonuçları araştırma yapılan şehirlerin çoğunda *Leishmania tropica* ve *Leishmania major*'un KL enfeksiyonlarına sebep olduğunu göstermiştir. Bizim çalışmalarımızda AKL olgularının yaygın olarak görüldüğü bölgelerde *Leishmania major*'a rastlanırken, ZKL olgularınun yüksek oranlarda görüldüğü yerlerde de *Leishmania tropica* saptanmıştır. Bu dağılım Khorosan-Razavi eyaletindeki Leishmania'nın sebep olduğu olduğu hastalıkların dağılımını göstermektedir.

Anahtar Kelimeler: Kutanöz leishmaniasis, *Leishmania major*, *Leishmania tropica*, moleküler ve immünolojik teknikler, Khorasan-Razavi bölgesi

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Leishmaniasis is a vector-borne disease caused by protozoa belonging to the *Leishmania* genus. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis in the Middle East. CL is transmitted by the bite of sand flies (a subfamily of *Phlebotomine*) (1). Humans and dogs are the main reservoirs of *Leishmania tropica* causing anthroponotic cutaneous leishmaniasis (ACL) with chronic, dry form, delayed ulceration and plentiful amastigotes. Wild rodents have an important role as reservoirs of *Leishmania major* causing zoonotic cutaneous leishmaniasis (ZCL) with acute, moist form, early ulceration and few amastigotes (2).

According to the World Health Organization (WHO) approximately 0.6-1 million new CL cases are estimated to occur worldwide annually; two-thirds of cases are in Afghanistan, Algeria, Brazil, Colombia, Iran and the Syrian Arab Republic (3).

Over the last decades a significant increase in the number of cases of leishmaniasis has been reported worldwide (4). This rise may be due to behavioral and environmental changes such as new settlements, deforestation, large migrations from rural to urban areas, fast and unplanned urbanization, building of dams and new irrigation projects that increase exposure of humans to sand fly vectors (5).

Prevention and control programs are directly dependent on targeting the right species of the parasite (6). However, the species of *Leishmania* that cause different forms of CL are morphologically similar, making differentiation between species by microscopy alone impossible. For this reason, alternative methods such as isoenzyme analysis and immunological approaches such as fluorescent dye-conjugated monoclonal antibodies have been recently used for identifying *Leishmania spp.* (4). Furthermore, molecular methods based on PCR have also been used routinely by many laboratories to detect *Leishmania* in carrier hosts and to map parasite reservoirs (7,8).

Several studies have been performed in the Khorasan-Razavi province to diagnose leishmaniasis from observation of clinical symptoms (9). However, information on the incidence of leishmaniasis based on new immunological or molecular methods is poor in this area. To improve diagnosis and eventually approaches to treatment, the University of Medical Sciences in Mashhad performed immunological and molecular studies to identify those *Leishmania* species that cause CL and to design plans for targeted epidemiological intervention in endemic cities of the Khorasan-Razavi province.

METHODS

This paper reviews clinical and epidemiological studies on leishmaniasis performed in the province of Khorasan-Razavi. The paper briefly reviews current global and Iranian epidemiological data on CL. The review compares previous data on CL in different cities of Khorasan-Razavi province with current epidemiological data. The paper is based on PubMed and Google Scholar literature searches from 1979 to 2018 using the following keywords: "CL", "prevalence OR epidemiology", "causative agents of CL OR molecular detection of *Leishmania spp.*". These key words were then, combined with "Iran" and "Razavi Khorasan OR Khorasan-Razavi province". Both papers in English and Persian were considered. We also considered data from graduate theses on epidemiology of CL and on molecular detection of *Leishmania* species in Khorasan-Razavi.

RESULTS

Epidemiology of Cutaneous Leishmaniasis in Iran

CL is one of the 10 most important parasitic diseases in tropical regions of the world, being present in all continents except for Australia (10). Investigations on various aspects of this disease have been prompted and supported by WHO and global burden of disease (GBD). According to GBD, the prevalence of CL has risen from 2.1 million in 2002 to nearly 4 million cases in 2015 (11-13). The number of individuals with latent CL is estimated at about 12-20 million (10).

Disability-adjusted life years (DALY) due to CL is estimated at about 2 million years. Considering that only patients who are positive for *Leishmania* spp. are included in the DALY formula, and the psychosocial impact of scars due to CL (approximately 40 million cases) has not been taken into account, the real DALY is likely to be higher than the current estimated figure (13).

CL is the second most prevalent arthropod-transmitted disease in Iran (14). Despite the attempts of Iranian health officers to prevent and control leishmaniasis, the disease is still spreading and new foci of CL are reported every year. For example, 17 previously infection-free provinces of Iran are now endemic for leishmaniasis (15).

According to the data published by the Iranian Ministry of Health in 2011, about 20000 new CL cases are reported in Iran annually, while the actual number of patients with CL is estimated to be four to five-fold higher. Patients with either moist or dry lesions are observed in both rural and urban areas in Iran (16). The rural form or ZCL with moist lesions is observed in 15 provinces of Iran including Isfahan, Sarakhs, Lotfabad, Khuzestan, Kashmar, Kashan, Dehloran and Damghan, whereas the urban form or ACL with dry lesions has been reported in Tehran, Shiraz, Mashhad, Neishabur, Kerman, Bam, Rafsanjan and Khomeyn Shahr (16,17).

Epidemiological studies in Iran indicate more than 90% of CL cases occur in 88 cities and transmission occurs in 17 provinces in Iran with a prevalence rate ranging from 1.8% to 37.9% (18). Isfahan, Fars, Bushehr, Ilam, Khuzestan, Yazd, Semnan, Kerman, and Khorasan were high incidence areas between 1983-2013. A lower burden of infection was observed in Gilan, Kurdistan, Azerbaijan-West, Ardabil, Tehran, Qazvin, Hamedan, Azerbaijan-East, Kermanshah, Kohgiloyeh-Boyer-Ahmad, Lorestan, Markazi, and Chaharmahal- Bakhtiari. The last data about number of CL patients in different province of Iran during 2011-2013 has been shown in Table 1 (16,19).

The incidence rate of CL follows a cyclical pattern of rise and fall between 20 to 40 cases per 100,000 people and a periodic trend lasting 5-6 years, which shows the limited effect of control measures that are transiently implemented in response to episodes of increased incidence of the disease (20).

Therefore, improved and permanent measures for epidemiological control of CL in Iran must be rationally designed and implemented with the participation of health authorities and relevant governmental agencies in order to reduce the incidence and prevalence of CL (16).

Table 1. The number of CL patients in different province of Iran during 2 years (2011-2013) (16)

Provinces	The number of CL patients
Razavi Khorasan	13 379
Fars	13 356
Isfahan	8 785
Kerman	4 435
Khuzestan	3 615
Ilam	2 027
Golestan	1 960
Northern Khorasan	1 286
Sistan and Balouchestan	1 144
Yazd	1 069
Qom	1 012
Tehran	848
Semnan	786
Bushehr	385
Kermanshah	355
Hormozgan	286
Lorestan	270
Chaharmahal and Bakhtiari	242
South Khorasan	226
Hamadan	209
Kohgiluyeh and Boyer-Ahmad	195
Mazandaran	150
Markazi	115
Alborz	115
West Azerbaijan	90

The Epidemiology of Cutaneous Leishmaniasis in Khorasan-Razavi

The present study reviews the CL status in different districts of Khorasan-Razavi.

CL has been present for a long time as an important endemic disease in the Khorasan-Razavi province in the North East of Iran (21). The Khorasan-Razavi province has the highest prevalence of CL infection in Iran (16). Since this province has shared borders with Afghanistan and Turkmenistan, changes in the incidence rate and epidemiological pattern of CL might be linked to neighboring countries. In fact, gerbil reservoirs and vectors of ZCL have been confirmed along the border between Iran and Turkmenistan as well as in the Afghan city of Heart which is in an area endemic for ACL and close to Iran (22,23). Most of infected domestic and wild Canidae have been affected by *L. infantum* in Iran (24,25). However, infections with with *L. tropica* were reported from canidae in Syria, Morocco, and Israel (26-30). Dogs are therefore a secondary reservoir host for *L. tropica* and for its transmission to human.

Mashhad, the second most populous city in Iran and the capital of Khorasan-Razavi province is located in the North East of Iran and close to the border with Turkmenistan and Afghanistan. Mashhad is one of the main endemic areas for ACL. During the last two decades, CL cases have significantly risen in Mashhad causing concerns in the health authorities (6). According to the Khorasan-Razavi Health Center in 2001, the highest prevalence of CL was seen in the region of Ab-o-Bargh, where population density, stray dogs, cultural and economic poverty in suburbs are considered as the main socio-epidemiological risk factors. Haj Norouz is another high disease area in Mashhad due to predisposing factors such as, the existence of building debris, old building with wooden roofs and areas where waste material is accumulated (17).

Neishabur, the former capital of Khorasan-Razavi province, has also been considered as an important endemic city for ACL with the rate of infection in some regions such as Sarcheshmeh and Kariz-no being higher than in others (5). ACL is present in the city of Sabzevar in Khorasan Razavi, while the disease has not been detected in Mazinan in Sabzevar (13).

Sarakhs in the North East of Iran is a high prevalence region for *L. major*. It is possible that its location on the Silk Road, the proximity to Turkmenistan and the high volume of commercial traffic could underpin the increased incidence of leishmaniasis in this city (31). Torbat-e Jam is another county where many CL cases have been observed annually. This district is located in the South West of Mashhad and north of Taybad, West of the Afghanistan border (32,33).

Based on the clinical appearance of CL lesions, it has been inferred that the patients in the towns of Chenaran and Quchan probably suffer from *L. tropica*, while *L. major* has been known as dominant strain of CL in Dargaz and Kalat, that are located in the North and North East, respectively. It is noticeable that Quchan, in the North East of Iran, has a border with Turkmenistan and is connected by road with Chenaran city half way to Mashhad.

Based on the clinical appearance of CL lesions, several investigations have indicated that Torbat-e Heydarieh and Fariman, in the South of Mashhad, are endemic for *L. tropica*. However, conclusive data on the identification of *Leishmania* species in this area based on immunological or molecular tools is still lacking. Since these towns are situated on the way of travel to Mashhad, accurate identification of CL agents can be effective to determine the control and prevent strategies (34).

It is still unclear which parasite species cause CL in the cities of Gonabad, Kashmar and Bardaskan in the southwest and Khaf and Taybad districts in the South East of Khorasan-Razavi province.

Identification of the Parasite Species Responsible for Cutaneous Leishmaniasis in Endemic Areas of North East Iran

The identification of *Leishmania* species is essential to monitor the spread of the disease as well as to design and implement effective control methods. Since control and prevention of ZCL and ACL are different, several studies have focused on the correct identification of *Leishmania species* in Khorasan-Razavi during the past two decades (6).

Mohajery et al. (35), used isoenzyme electrophoresis for the identification of *Leishmania spp.* and showed that Mashhad is the main focus for *L. tropica*, but *L. major* was present in other regions, particularly in the suburbs.

In another study, Valizadeh et al. determined the causative species of *Leishmania* in 72 cases of CL in Mashhad using an ELISA method based on species-specific monoclonal antibodies. 52 cases were identified as *L. tropica*, 16 as *L. major* and 4 cases were caused by unidentified species (35).

Between 2002 and 2005, Hajjaran et al. (4) collected 87 parasite samples from patients with CL who were referred to Mashhad health centers and determined the species of *Leishmania* by RAPD-PCR. 94.2% of the isolates were identified as *L. tropica*, whereas 5.8% were identified as *L. major*.

Mahmoudi in 2005 used PCR to determine the causative agent of CL in Mashhad and demonstrated *L. major* in 2/21 cases and the other samples were identified positive for *L. tropica* (36). Similar results were reported by Fata et al. (37) in 2007 using the Whatman paper and PCR methods.

The epidemiological pattern of leishmaniasis in Mashhad and the increased incidence of CL in Khorasan-Razavi prompted further studies to identify the main causative agents of CL in other counties of this province.

A study including 84 CL patients in the county of Sabzevar in 2006-2007 detected 32 cases of *L. tropica* and 52 cases of *L. major*. Another study on 52 patients in Neishabur using RAPD-PCR only detected *L. tropica* (38,39). The cities of Sabzevar and Roudab were the foci in the county of Sabzevar where *L. tropica* was detected, whereas the cities of Hokm Abad, Ghezelgharshi, Joghatay and Mohamad Abad were endemic foci of both *L. tropica* and *L. major* (39).

Molecular investigations by Hossein Farash et al. and Saadabadi et al. (40) on 144 patients with CL identified only *L. tropica* in the districts of Torghabeh-Shandiz (2010) and Kharv (2007), which are close to Mashhad and Neishabur, respectively (6,40). Pagheh et al. reported similar findings in 2013 in Torbat-e Jam in a cohort of 504 patients with *Leishmania* lesions (32).

In 2013, Shamsian et al. (31) identified *L. tropica* in 12 patients and *L. major* in 52 cases in Sarakhs that had previously been known as an area where *L. major* was prevalent as the cause of ZCL (31).

Despite Khaf and Taybad had been considered as ACL foci (*L. tropica*) until 2015, Abdolmajid et al. (41) found 20 cases for *L. major* among 66 positive persons with CL by PCR method (41). Using a similar method between 2015 and 2017, Shamsian et al. diagnosed 7 patients with ZCL out of 60 subjects with CL in Torbat-e Heydarieh and 16 with *L. major* out of 84 patients from Kashmar (11 cases), Bardaskan (2 cases) and Gonabad (3 cases) (34,42).

On the contrary, using PCR in 2015 Fata et al. (36) demonstrated 22 patients infected by *L. tropica* from 85 cases of CL patients in the ZCL foci of Dargaz and Kalat 2015 (29).

13 cases of *L. tropica* out of 42 patients were diagnosed by Shamsian et al. (43) in Fariman, another known focus for *L. major*, in 2017.

Finally, Shamsian et al. (44) found 29 out of 86 samples positive for *L. major* in CL infected patients in Quechan and Chenaran despite *L. tropica* had been considered as the only causative species of CL in this area.

It is noticeable that all the studies reported here, except for the ones in Neishabur, Kharv, and Torbat-e Jam, were performed using the same PCR protocol and same primers that give a 615 bp band for *L. major* and a 744 bp band for *L. tropica* (Figure 1,2). These studies are therefore directly comparable (6).

DISCUSSION

The global epidemiology of CL has changed, mainly due to population movements, individual risk factors and environmental changes. CL is one of emerging parasitic diseases in recent years, posing a public health problem in many parts of the world and is widespread in Iran (39,40). The epidemiology of CL has changed in the past two decades in Iran and both *L. tropica* and *L. major* have being present in Khorasan-Razavi province (17,45).

The first step in any control and prevention program for leishmaniasis is the identification of the infectious agents, reservoirs and vectors (35). In recent years, many studies have been conducted in Khorasan-Razavi province with the aim to refine the immunological and molecular diagnosis of the causative *Leishmania* species (4,6,34,35,39-42,46).

The aim of this review was to design a new epidemiological picture of CL, using data from several studies performed in Khorasan-Razavi province to identify the boundary shifts for causative agents of CL during a twenty-year period as the basis to new insights for control and prevention measures.

The investigations carried out in Mashhad demonstrated that ACL and ZCL co-exist. Furthermore, *L. tropica* has been known the dominant agent causing CL in this city (4,35,37,46). Mashhad is the main focus for ACL, but also is a center for *L. major*, particularly in suburbs.

Sabzevar, has two foci of CL, one with dry lesions and the other consisting of patients with wet lesions. Until now Sabzevar has been known just as an ACL focus while now both forms (ACL and ZCL) of the disease have been found to exist at this location (39).

These epidemiological changes may be due to migration of

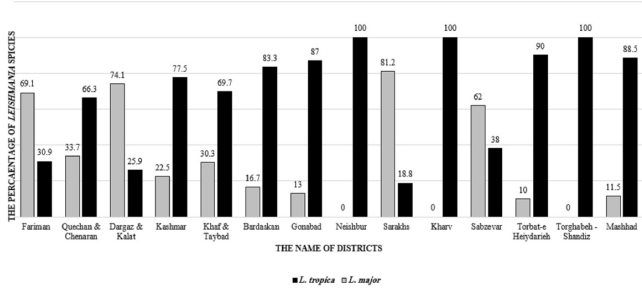

Figure 1. The percentage of *Leishmania* species frequency in different districts of Khorasan-Razavi

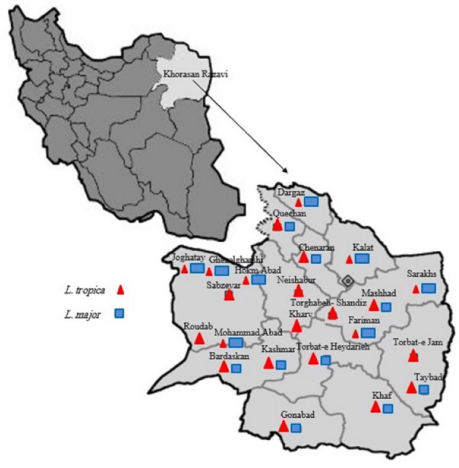

Figure 2. Cutaneous leishmaniasis map of Razavi Khorasan province in 2017

people from neighboring countries such as Afghanistan and Iraq, rural to urban fluxes which provide suitable conditions for transmission, piling of building debris and litter near residential areas, which lead to expansion of vector reservoirs for both species. Agriculture also causes an increase of *L. major* reservoirs and vector population. Moreover changes in the climate and in people lifestyles or the lack of immunity in people who immigrate to endemic areas are other risk factors that can increase the incidence of CL in a paerticular area (19,34,35). Similar reasons for the epidemiological changes listed above are likely to underpin the variations in the distribution of *Leishmania* species observed in Torbat-e Heydarieh, Bardaskan, Kashmar, Khaf, Taybad, Quechan and Chenaran (34,41,42,44).

The presence of both *L. tropica and L. major* species as causes of CL in Sarakhs might be related to its location near an international railroad and in proximity to the border with Turkmenistan; increased movement of people through this region since 2013 is likely to be an additional factor in the observed epidemiological pattern of leishmaniasis (31). The diffusion of *L. tropica* in Fariman, Kalat, and Dargaz could result from changes in health programs to control the vectors and development of industry and tourisms. Therefore, some of labor migrants or travelers who are infected by *L. tropica* could be reservoir hosts for this emerging agent (36).

Torbat-e Jam, Neishabur, Kharv, Torghabeh-Shandiz are located in the vicinity of Mashhad and similarly to Mashhad have been considered as ACL foci (6,32,39,40). Despite all cases in these regions were identified as *L. tropica* with molecular method, the number of lesions, their morphological characteristics and the season of occurrences in some patients corresponded to the characteristics typical of *L. major* infections; therefore, it may be desirable to extend and refine the examinations to ensure that cases of ZCL do not go undetected.

CONCLUSION

CL is a major health problem in Khorasan-Razavi province where most of the regions are affected by both *L. tropica* and *L. major* species.

Several measures can be suggested to reduce the incidence of the disease in the urban endemic areas. Classes on health education for inhabitants, reducing the numbers of stray dogs, identification and urgent treatment of clinical cases could be effective. Reduction in rodent and carrier population, removal of waste and environmental modifications should be considered as key health policies in areas with *L. major* (6,19).

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Authorship Contributions

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