Original Article

Epidemiological and Clinical Study on the Cutaneous Leishmaniasis in Aran and Bidgol, Center of Iran

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Abstract

Aims: Cutaneous leishmaniasis (CL) is a skin infection that causes various forms of ulcers and also remains scars even after treatment. This disease is prevalent in many countries of Middle East including Iran. Since determining the species of the parasite is important for prevention and control programs, this study was conducted to identify *Leishmania* species in Aran and Bidgol, Isfahan province, center of Iran. **Materials and Methods:** This cross-sectional study was carried out on 112 CL suspected patients who referred to health centers of Aran and Bidgol. Serosity of the wound was collected, and amastigote form was detected by microscopic method. After extraction of DNA from serosity, kDNA-polymerase chain reaction (PCR) was used to identify *Leishmania* species. **Results:** Fifty-four of all suspected CL samples (48.2%) were positive microscopically, while 55 (49.1%) were positive using kDNA-PCR. The results of PCR revealed that 51 isolates (92.7%) were *Leishmania major* and 4 (7.3%) *Leishmania tropica*, respectively. The most lesion form caused by *L. major* was papular or volcanic-like, while all of wounds caused by *L. tropica* were papular/nodular forms. **Conclusion:** The results of the study indicated that the predominant species was *L. major* and zoonotic CL is more prevalent in this region.

Keywords: Aran and Bidgol, cutaneous leishmaniasis, kDNA

Introduction

Leishmaniasis, as vector-borne disease has global prevalence. Despite scientific advances, various clinical manifestations of this disease have great importance in public health.[1-3] Leishmaniasis is known as a neglected infectious disease and has been reported from almost 100 countries.[2] The cutaneous leishmaniasis (CL) is the most common form of disease. Zoonotic CL (ZCL) and anthroponotic CL (ACL), which caused by *Leishmania major* and *L. tropica*, respectively, are the different forms of disease.[4] Of all reported cases, more than 70% have been reported from Brazil, Costa Rica, Afghanistan, Ethiopia, the Islamic Republic of Iran, Peru, Algeria, Sudan, Colombia, and the Syrian Arab Republic.[3] The rate of CL varies in different parts of Iran from 1.8% to 37.9%, the annual incidence of the disease in Iran is about 20,000 per year.[5-8] Fars, Isfahan, and Khuzestan provinces are the most important endemic area of ZCL in Iran.[9-12] Mashhad, Bam, and Khoram Abad city are the endemic area of anthroponotic CL in Iran.[13-15]. Both forms of CL is reported in Kashan.[16] Aran-o-Bidgol is situated in northeast part of Kashan city in Isfahan province, center of Iran. Mosquitoes Phlebotominae (sand fly) are known as vector of parasite. Rodents are a major reservoir of this disease.[17,18] Leishmania diagnosis depends on clinical observations, region epidemiology, and laboratory methods. Direct smear is known as first and common method for the diagnosis of CL.[19,20] Due to the accuracy and sensitivity of molecular techniques, they are the best diagnostic methods for detection of CL. In different studies, molecular methods, such as polymerase chain reaction (PCR), random amplified polymorphic DNA-PCR, restriction fragment length polymorphism, nested PCR, real-time PCR, and DNA sequencing have been used to diagnosis of leishmania.[10,13,21,22] Different molecular targets, including internal transcribed spacer1 (ITS1), ITS2, gp63 (63-kd glycoprotein), mini-exon, kDNA, small subunit ribosomal RNA genes have been used for the identification of parasites from various samples.[10,23-27] Although there is no standard target molecule for the diagnosis

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of leishmania, some researchers due to multiple copies per parasite have introduced kDNA gene as most sensitive molecules for diagnostic purposes.[25,28] In the Aran-o-Bidgol region, reservoir and main vector of disease are Rhombomys opimus and Phlebotomus papatasi.[29,30] The aim of this study was to evaluate the epidemiological and clinical situation of the CL in the Aran-o-Bidgol region.

Materials and Methods

Study population

This cross–sectional study was carried out on 112 patients suspected to CL, referred to Aran and Bidgol healthcare center, Isfahan province, Iran, during January 2014 until September 2015. The demographic, symptoms, and signs of patients were recorded in questioner's forms by interview. Skin scrapings from the ridge lesions were taken for microscopic study; furthermore, serosity added in 1.5 microtubes containing 0.5 ml of sterilized normal saline and stored in −20°C for DNA extraction. Slides were stained using Giemsa. All slides were viewed under oil immersion for confirming of amastigotes inside or outside macrophages.

DNA extraction

The genomic DNA of samples was extracted by specific Kit (Bioneer; South Korea) according to the manufacturer's instruction and stored in −20°C.

Polymerase chain reaction amplification

The PCR was performed on 112 DNA samples. Species-specific primers, LINR4 forward (GGG GTT GGT GTA AAA TAGGG), and LIN17 reverse (TTT GAA CGG GAT TTC TG) were used by previous study[31] to kDNA gene amplification. The expected bands were 650 bp and 760 bp for *L. major* and *L. tropica*, respectively. PCR was carried out in a 20 µl reaction mixture including 1.5 mM MgCl2 (Amplicon, Denmark), 10 pMol LINR4, 10 pMol LIN17, and 100 pg DNA (2 µl). The PCR cycling program was 95°C for 5 min, followed by 30 cycles of 94°C for 30 ss, 52°C for 30 s, and 72°C for 40 seconds, and then, a final extension at 72°C for 5 min in a thermocycler (Analytik Jena's FlexCycler2). Reference strains *L. tropica* (MHOM/IR/89/AR2) and *L. major* (MHOM/IR/54/LV39) and DW instead of DNA template included in all PCR runs as positive and negative controls, respectively. PCR products were analyzed on 1.2% agarose gel by electrophoresis and visualized by ultraviolet transilluminator after stained with 0.5 µg/mL ethidium bromide. Diagnosis of CL was based on microscopic and kDNA PCR results.

Statistical analysis

All the data were recorded in SPSS software version 16.5 (SPSS Inc., Chicago, IL, USA) and were analyzed using the Chi-square and Exact significant Chi-squared test.

Results

Out of 112 suspected CL cases, 48.2% (54/112) were positive by microscopic method. The overall infection rate was 49.1% (55/112) by amplification of *Leishmania* using kDNA primers [Table 1 and Figure 1]. One sample was negative microscopically, but was positive by PCR. *Leishmania* species were identified in 51 isolates (92.7%) as *L. major*, followed by *L. tropica* in 4 isolates (7.3%), respectively [Figure 1 and Table 1]. The rate of infection in female and male were 36.4% (20/55) and 63.6% (35/55), respectively ($P = 0.88$). The mean age of the patients was 40.3 ± 21.6.The highest rate of positive PCR (40%) was observed in age group 20–39 years, but no positive cases were seen in more than 80 age groups ($P = 0.21$).The rate of infection peaked in 2014, and was diminished in 2015. From the 55 positive PCR, the highest rate and the lowest rate of CL 47.3% (26/55) and 3.6% (2/55) were seen in individuals with elementary and college education, respectively ($P = 0.6$). The main characteristics of CL according to *Leishmania* species were shown in Table 1.

In regard to the form of CL lesion, out of 51 L. *major*, the most form was papular or volcanic-like and the lowest rate 3.9% ulcer, but in *L. tropica*, all of the four isolates were papular/nodular [Table 1 and Figure 2] ($P = 0.34$). Distribution of CL according to season was shown in Table 2.

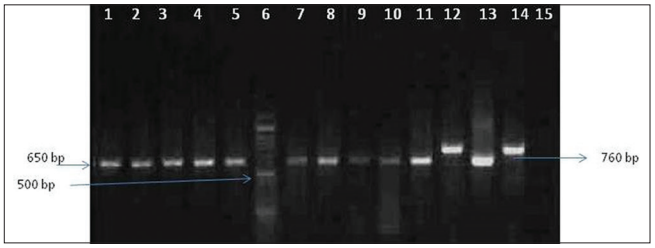

Figure 1: Electrophoretic patterns of KDNA-PCR products of *Leishmania* species in cutaneous leishmaniasis patients. Lanes 1–5 *L. major*, Lane 6: ladder markers 50 bp, Lanes 7–11: L. major, Lane 12: *L. tropica*, Lane13: Reference strains of *L. major* (650 bp), Lane 14: Reference strains of *L. tropica* (760 bp). Lane 15: Negative control. PCR: Polymerase chain reaction, *L. major*: *Leishmania major*, *L. tropica*: *Leishmania tropica*

Table 1: The main characteristics of cutaneous leishmaniasis according to *Leishmania* species

CL characteristics	*Leishmania major, n* (%)	*Leishmania tropica, n* (%)	Total positive	*P*
Leishmania sp. identification	51 (92.7)	4 (7.3)	55 (100)	0.00
Kind of lesion				
Dry	26 (51)	2 (50)	28	0.97
Wet	25 (49)	2 (50)	27	
Total	51 (100)	4 (100)	55	
Lesion forms				
Papular/nodular	23 (45.1)	4 (100)	27	0.34
Volcanic-like	21 (41.2)	0	21	
Pustule	5 (9.8)	0	5	
Ulcer	2 (3.9)	0	2	
Lesion location				
Face	10 (19.6)	1 (25)	11	0.57
Hand	24 (47.1)	3 (75)	27	
Feet	16 (31.4)	0	16	
Body	1 (1.9)	0	1	

CL: Cutaneous leishmaniasis

Table 2: Distribution of cutaneous leishmaniasis according to season

Season	*Leishmania major*, *n* (%)	*Leishmania tropica*, *n* (%)	Total positive	*P*
Fall	32 (62.7)	2 (50)	34	0.004
Winter	3 (5.9)	0	3	
Spring	0	1 (25)	1	
Summer	16 (31.4)	1 (25)	17	
Total	51 (100)	4 (100)	55	

Discussion

Leishmaniasis prevention and control in endemic areas are dependent on several factors, which determination of the dominant causal agent is one of the most important factors. The finding of this study revealed that the prevalent species of *Leishmania* was *L. major*, and this region is a ZCL focus. The previous epidemiological finding on vector and reservoirs indicated that CL in Aran and Bidgol was mainly zoonotic diseases.[29,30] The results of the previous study in Kashan revealed that from 130 patients suspected for CL 71.4% and 26.6% were infected with *L. tropica* and *L. major*, respectively.[28] The results of another study showed *L. major* was the main causative agent in Isfahan region.[11] Based on the results of the previous study, the dominant species in Natanz and Isfahan and Ahvaz were *L. major*.[12,32] The result of a study in Mashhad using ITS-PCR showed that *L. tropica* is predominant agent of CL.[13]

Molecular method is suitable for CL accuracy diagnosis, as well as for species characterization, fast treatment and monitoring of relapses. The small number of parasites in the wound of ZCL may cause false results;[10] however, kDNA PCR with 10,000 copies of gene and high sensitivity give positive results.[28,33-35] The sensitivity of kDNA-PCR, KDNA-nested PCR, and microscopic methods were 99%, 97%, and 87.9%, respectively.[28] CL infection was more prevalent among males (63.6%) than female (36.4%), respectively (P = 0.88). Moreover, the highest rate of infection was seen in the age of 20–39 years because occupational situation they are more exposed to mosquito bites, which is agreement with previous report.[28,36] The most rate of ZCL was observed in autumn 62.7%, but no case was seen in spring [Table 2] (P = 0.004), which statistically was significant. The most distribution of CL lesions caused by *L. major* was observed on hands 47.1%, feet 31.4%, and the lowest lesions seen in trunk 1.9%, respectively, [Table 1] (P = 0.57), which is agreement with results of previous studies.[36,37] *L. major* cause moist wound in body extremities and quickly becomes infected, which creates many problems such as movement limitation so that they should be hospitalized. Since this area due to beautiful deserts has the tourist attractions; therefore, to diminish incidence of this disease, effective control programs are need.

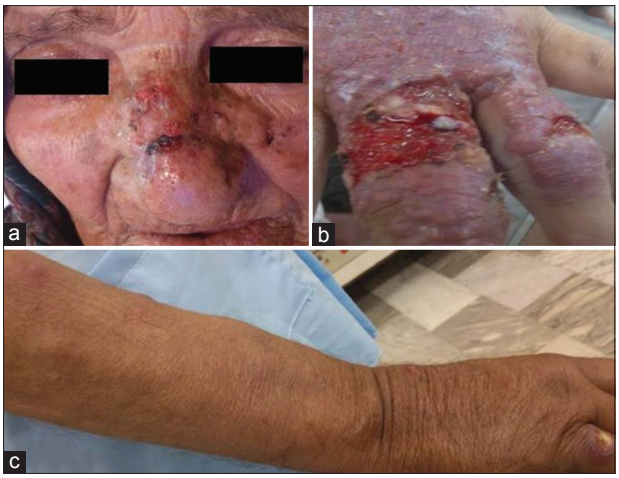

Figure 2: Some clinical forms in patients with cutaneous leishmaniasis. (a and b) Ulcer, (c) sporotrichoid

Conclusion

kDNA-PCR is one of the most sensitive methods for the diagnosis of CL. *L. major* is main causative agent of ZCL in Aran and Bidgol region. A remarkable point is that Kashan is an endemic area for ACL and ZCL, while Aran and Bidgol (a city in the vicinity of Kashan) ZCL is more prevalent. These results can be used in health control programs and treatment systems.

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Conflicts of interest

There are no conflicts of interest.

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