



The Present Status of Cutaneous Leishmaniasis in a Recently Emerged Focus in South-West of Kerman Province, Iran

Ahmad KHOSRAVI¹, Iraj SHARIFI^{1*}, Eshagh DORTAJ², Abbas AGHAEI AFSHAR¹, Mahshid MOSTAFAVI¹

1. Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran
2. Faculty of Health, Kerman University of Medical Sciences, Kerman, Iran

*Corresponding Author: Tel: +98 341-3239843 Email: iraj.sharifi@yahoo.com

(Received 26 Sep 2012; accepted 18 Dec 2012)

Abstract

Background: The objective of this study was to assess the magnitude of the cutaneous leishmaniasis (CL) disease and identification of the causative agent by nested-PCR for current control strategy.

Methods: This study was carried out as descriptive house-to-house visits in Orzoieh district in Kerman Province, south-east Iran, during 2011-2012. A questionnaire was completed for each individual consisting of demographic and clinical data. Suspected individuals were examined by direct smear microscopy and subsequent identification by nested-PCR. X²-test was used for any significance ($P < 0.05$).

Results: A total of 18308 inhabitants (mean age; 22.7 yr) consisting of 9011 males (49.2%) and 9297 females (50.8%) were examined for the presence of active or chronic lesions. The overall prevalence was 4.7%, including 30 cases of active and 839 cases of scar, distributed more significantly ($P < 0.01$) in females (5.2%) than males (4.3%). Individuals <10 years of age showed the highest (6.3%) and >50 years the lowest rate of CL disease, respectively ($P < 0.001$). The proportion of infection was the highest in Soltanabad (14.7%), followed by Vakilabad (6.8%), Dolatabad (3.2%) and Shahmaran (2.8%). The majority of cases had 2 lesions (mean; 2.1 lesions). Hand was the most common site of involvement (35%), and then face (26%), and multiple locations (39%). Nested-PCR displayed 29 isolates as *Leishmania major* and one isolate *L. tropica*. The CL disease first emerged in 1998 as epidemic in the area and appeared endemics, thereafter.

Conclusion: *L. major* was the sole species caused ZCL. These findings are necessary for future control programs and strategic planning.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, Epidemiology, Iran

Introduction

Cutaneous leishmaniasis (CL) caused by *Leishmania* species represents a complex disease with wide spectrum of clinical features (1). It is endemic in over 88 countries, mainly in tropical and sub-tropical areas (2), where over 90% of the cases occur in Afghanistan, Brazil, Algeria, Peru, Iran, Saudi Arabia and Syria (3). Surveillance data indicate the number of cases has sharply increased in recent years, as documented in several countries (1).

Cutaneous leishmaniasis is endemic in over 50% of the provinces of Iran (4, 5); anthroponotic CL (ACL) caused by *L. tropica*, exists mainly in urban areas of many large and medium size cities (6-9). The main host is human (2, 5, 10). Whereas, zoonotic CL (ZCL) due to *L. major* presents in rural areas (11) where the major reservoir host is gerbil (12-14). Several factors which contribute to the spread of CL throughout the world are urbanization, migration, war situation, environmental modification

and also to some extent improved diagnosis and case notification (15). Outbreaks of CL have currently occurred throughout Iran, where a suitable environment is present for breeding of the vector, propagation of the organism and transmission of the causative agent (16-18).

The primary objective of this study was to assess the present status of CL in endemic areas of Baft, currently Orzoieh district. First emergence was started as epidemic in 1998 and the disease became endemic, thereafter (17). After the massive earthquake, it seemed that the feature of CL has been changing in this area, mainly because of the recent expansion of villages and towns, as well as movement of the population for various occupational reasons. Due to the spread of the CL cases to the neighboring regions and reports by the physicians and health authorities in the area, the CL lesions present different clinical and epidemiological characteristics.

Therefore, the aim of the present study was to assess the magnitude of the CL infection and to identify the causative agent by a molecular method for future control strategy and current therapeutic measures.

Materials and Methods

Study areas

This study was carried out in Orzoieh district, in south-west Kerman Province, south-east of Iran. This district is recently segregated from Baft and consists of 4 main regions including, Vakilabad, Soltanabad, Dolatabad and Shahmaran. Orzoieh surrounds 4600 km² area, located 125 km south to the city of Baft, adjacent to Jiroft district and Hormozgan Province. The climate is warm in the summer, while mild in the winter. Due to the productivity of soil in this region, 6000 hectares of the land is cultivated by all kinds of tropical crops including orchard of oranges, summer crops and various vegetables. Its irrigation system is provided by 700 water wells. This region has recently been attracted by residents of Isfahan, Yazd and people from other provinces for agricultural purposes. Thus, numerous labors moved to the region for occupational reasons.

Population and sampling

This study was carried out as descriptive and prospective census in house-to-house visits during March 2011 to February 2012 for a period one year. The entire population of the affected villages was screened and followed-up actively and passively. A questionnaire was completed for each individual, recording demographic characteristics (sex, age, location and residence) and clinical status of CL (location of lesions, number and type of lesions and time of contraction). The entire body was clinically examined by two health personnel and those with suspected lesions were referred for further clinical examination by an experienced physician and parasitological examination.

Smear preparation

Suspected lesions were subjects for smear preparation. Skin scrapings were taken by a scalpel and blade from the edge of each active lesion, smeared on a glass slide, fixed with methanol and stained by Giemsa.

Molecular identification

Direct skin smears obtained from the borders of lesions were used for nested-PCR. All smears were transferred to the Leishmaniasis Research Center at Medical School in Kerman for further molecular study. DNA was extracted by proteinase K (Roche, Germany) from the smear preparations following the method of Noyes et al (19). Briefly, two types of primers were used in two sequential steps. First, two external general primers; CSB2XF 5'-CGAGCAGCA-GAACTCCCGTTCA-3' and CSB1XR 5-'ATT'TTTCGCGAT'TTTCGCAGAACG-3' and second, two other internal specific primers; 13Z 5'-ACTGGGGGTTGGTGTAAAATAG-3' and LiR5'TCGCAGAACGCCCT-3' were used. These primers amplified a variable minicircle k DNA of *Leishmania* species.

The PCR products were visualized by 1.5% agarose gel electrophoresis (Uvitech, Cambridge, UK), using a DNA size marker (100bp) at 260nm wavelength.

Ethical issues

The protocol was reviewed and subsequently approved by the Ethic Committee of Kerman University of Medical Sciences. The consent of patients was obtained and the confirmed cases were referred to a local physician for treatment of CL.

Data analysis

A SPSS software was used for data entry and χ^2 -test was performed for any significant differences at $P < 0.05$.

Table1: Age and sex distribution of the study population in Orzoieh district, Kerman province, south-eastern Iran, 2011- 2012

Age groups (yr)	Male		Female		Total	
	n	%	n	%	n	%
≤10	1591	17.6	2004	21.6	3595	19.6
11-20	1917	21.3	1724	18.5	3641	19.9
21-30	2264	25.1	2258	24.3	4522	24.7
31-40	1240	13.8	1281	13.8	2521	13.8
41-50	809	9.0	804	8.6	1613	8.8
>50	1190	13.2	1226	13.2	2416	13.2
Total	9011	100.0	9297	100.0	18308	100.0

Table 2: Distribution of cutaneous leishmaniasis in Orzoieh district, Kerman Province, south-eastern Iran by sex, 2011-2012

Gender	No. examined	No. infected (%)
Male	9011	384(4.3)
Female	9297	485(5.2)
Total	18308	869(4.7)

All age groups were infected (Table 3), however subjects <10 years of age indicated the highest (6.3 %) and >50 years the lowest rate of CL infection (1.1%), respectively. Overall, there was a significant difference between <10 years old children and other age groups ($P < 0.001$).

The proportion of infection was the highest in Soltanabad (14.7%), followed by Vakilabad (6.8%), Dolatabad (3.2%) and Shahmaran (2.8%). The majority of cases (38.2%) had 2 lesions (mean; 2.1). The hands (35%) were the most common place of involvement, followed by face(26%), hands and

Results

A total of 18308 inhabitants, aged 1-73 years (mean age; 22.7 years) including of 9011 males (49.2%) and 9297 females (50.8%) were examined for presence of active or chronic lesions (Table 1). Most of the individuals were < 30 years (64.2%) and the smallest age group was >50 years (13.2%). The overall prevalence rate was 4.7% including 30 cases of active lesions and 839 cases of scar, distributed among males (4.3%) and females (5.2%) with a significant difference ($P < 0.01$, Table 2).

legs (21.7%), hands and face (10.9%), legs (5.7%) and other parts of the body (0.7%, Fig. 1).

Table 3: Distribution of cutaneous leishmaniasis in Orzoieh district, Kerman Province, south-eastern Iran by age, 2011-2012

Age groups (yr)	No. examined	No. infected (%)
≤10	3595	228(6.3)
11-20	3641	209(5.7)
21-30	4522	206(4.6)
31-40	2521	112(4.4)
41-50	1631	88(5.5)
>50	2416	26(1.1)
Total	18308	869(4.7)

DNA extracted from direct smear preparations showed 29 cases consisting of fragments of 560 bp, while only one case displayed a fragment of 750 bp, corresponding to *L. major* (99.9%) and *L. tropica* (0.1%), respectively (Fig. 2).

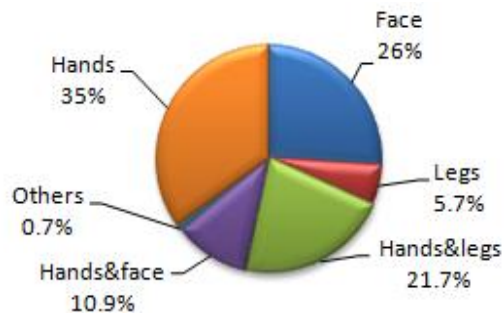


Fig. 1: Distribution of cutaneous leishmaniasis in Orzoieh district, Kerman Province, south-eastern Iran by location of lesion, 2011-2012

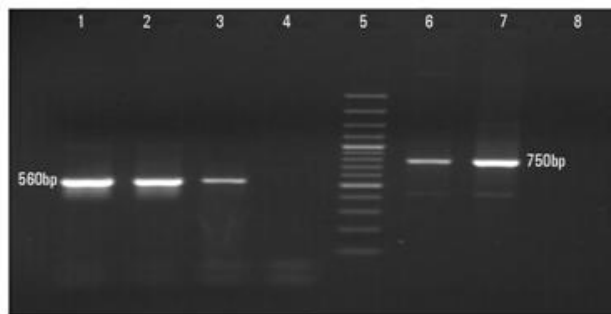


Fig. 2: Electrophoresis gel fractionation of nested-PCR for identification of *Leishmania* isolates. Lane1, *L. major* (positive control 560 bp); Lanes 2, 3, *L. major* isolates; Lanes 4,8, distilled water (negative control); Lane 5 DNA size marker 100bp; Lane 6 *L. tropica* (positive control 750 bp) ; Lane 7 single *L. tropica* isolate obtained from a patient in Orzoieh district, Kerman Province, south-eastern Iran, 2011-2012

Infected individuals were entirely natives. The CL cases first appeared during 1998, but the majority occurred thereafter.

Discussion

Leishmaniasis is still a significant cause of morbidity and mortality in numerous countries across the world, including Iran (2, 3). CL also affects a considerable portion of human beings with a major health impact in recent years (1).

Although, *L. tropica* is the main species in endemic foci of various districts in Kerman Province (8), the district of Orzoieh is determined to be purely of ZCL nature. The only case detected as *L. tropica*

was a native resident who had history of travelling to Jiroft, an area adjacent to this district with a known ACL focus (20).

The CL cases were commonly found in females more significantly than males. However, there is no definite explanation for such a sex difference. It might be attributed to individual and behavioral risk factors which could play a role as previously reported by others (16, 21).

All age groups were infected, although there was a strong tendency for cases to be prevalent more significantly in children of <10 years than other age groups. This is likely expected because children in this age play outdoors and are more exposed to the source of infection than others. Similar findings are found by others (11,12,17,21).

The clinical manifestations of CL were in general that of multiple lesions, with a predominant location on hands, face, hands-legs or hands-face (11, 12). These extrinsic factors are consistent with the molecular identification by nested – PCR which showed that *L. major* was the only causative agent in the area as previously reported in other places (5, 8, 11, 12). In this study nested PCR with high specificity and sensitivity was applied to identify the causative parasite species among the inhabitants. This method is currently the most commonly used technique in discrimination of species (8, 11, 13, 18, 19).

The present status of the CL cases indicates that the study area is designated a recently emerged focus occurred for the first time in 1998 (17) and sustained as endemic, thereafter (12-13). It appeared that the source of infection was introduced by the new settlements that arrived in the area from endemic regions for agricultural purposes (17). In addition, the environmental and ecological changes favored an increase in the vector population and reservoir host, gerbils (12-13) which might also have played some roles in propagation of the life cycle and transmission of the causative organisms.

The semi nested-PCR was positive for 0.05% of *Phlebotomus papatasi* in the region, a further evidence for a ZCL focus (13). Furthermore, another investigation indicated that *P. papatasi* was the predominant species and they reported prevalence of

1.1% for ulcers and 10.4% for scars among inhabitants in 2003 (12). The same study indicated that *Tatera indica* was the common gerbil in this area. They concluded that this gerbil might have played a role as the reservoir host in the focus.

Conclusion

L. major was the principle species caused ZCL in the study areas. This kind of information clearly has therapeutic significance and helpful in selection of optimal therapy. These findings are also necessary for designing future control programs and strategic planning.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

Acknowledgment

This study was supported by Kerman University of Medical Sciences (project no 89/254). We thank the health personnel in Orzoieh Health System for their kind assistance. The authors declare that there is no conflict of interest.

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