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First Report on Various Aspects of a Focus of Cutaneous Leishmaniosis in Southwestern Iran

Hamid Kassiri[1]*, Mahmoud Mehr-Aghaei[2]

[1] Department of Medical Entomology and Vector Control, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2] Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

ABSTRACT

Leishmaniasis is a neglected and re-emerging disease which exists in three types worldwide, including mucocutaneous, visceral, and cutaneous. The disease is among the ten most important parasitic diseases in the tropics. It is widely distributed in ninety countries. The disease is distributed in new foci due to influence of many risk factors, including environmental factors. Evidence indicates an increase in the incidence of disease in the New and Old Worlds at the early years of this century. At present, Cutaneous Leishmaniasis (CL) is one of the most important vector borne diseases in Iran. In Iran, the disease is commonly manifested in 17 out of the 31 provinces of the country, with estimated annual cases exceeding 20000 within the country. There are two dominant types of leishmaniasis in Iran namely kala and azar, and two forms of CL namely urban type and rural type. Considering the current significant situation of CL in the Karun County, the present study was aimed at assessing the epidemiology of the disease and analyzing its potential risk of infection. This is a cross-sectional, descriptive-analytical study conducted during the period of 2013 to 2017 in Karun County. Suspected patients with skin lesions were referred to the Health Center of this county. The diagnosis was dependent largely on clinical examination and Giemsa stain. All slides were viewed under oil immersion for confirming amastigote forms inside or outside macrophages. Patients were examined to evaluate the CL lesion (gender, age, site, size, type, whether lesion is with secretion or without secretion, job, history, number, month, season, method of medicine injection and residential area). A questionnaire was filled through a direct interview with the patients. A P value of $p \leq 0.05$ was considered statistically significance. The present study clarified that the highest frequency of the disease had been documented in 2014 with 26 cases. Out of suspected CL cases, 81 were positive by microscopic method and clinical examination. The rate of infection in female and male were 42% and 58%, respectively. The difference in the numbers of patients of both genders was significant. The highest rate of positive (40%) was observed in age group of 20–29 years. In regard to the form of CL lesion, most of them (81.5%) were with secretion. Distribution of CL cases according to residential areas was 58% in villages and 42% in city. The highest rate (19.8%) and the lowest rate (1.2%) of CL were seen in January and August, respectively. The highest rate of CL was observed in winter (44.5%), but 8.4% was seen in summer, which statistically was significant. The highest distribution of CL lesions was observed on hands (43.2%), feet (16%), faces (14.8%), and on few limbs (20.1%). Considering lesions number, the prevalence was as such: single lesion (38.3%), two lesions (24.7%) and three or more lesions (37%), which were significantly different. This study clarifies that CL is a public health problem in districts of Karun County and its preventing must be one of the priorities of health directorate. Therefore, to reduce incidence of this disease, effective control programs are needed.

Keywords: Cutaneous Leishmaniasis, Incidence, Epidemiology, Iran.

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Corresponding author: Hamid Kassiri
E-mail ✉ Hamid.Kassiri @ yahoo.com
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INTRODUCTION

The World Health Organization (WHO) has recommended comprehensive studies on different aspects of leishmaniasis, as one of the six important diseases in tropical regions. Epidemiologically, Cutaneous Leishmaniasis (CL) is presently endemic in 98 countries worldwide, including Iran; The Cutaneous Leishmaniasis (CL), as a zoonosis, is one of the endemic parasitic diseases in Iran. Its causal agent is a mastigophora protozoan, family Trypanosomatide and genus *Leishmania*. The

disease is transmitted by sand fly vectors of the Psychodidae family, subfamily Phlebotominae, genus *Phlebotomus*, from animal reservoirs that often have lesions on their bodies. These lesions may remain for one year [1-5].

Health is on a continuum—one does not arrive at good health accidentally [6]. Due to the public health importance of this disease, it has been emphasized by WHO over time. Recently, the Special Program for Research and Training in Tropical Diseases (TDR) has classified leishmaniasis as a group of emerging or uncontrolled diseases (Category I). Approximately, 90% of CL cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia, Syria, Algeria and Sudan; whereas, about 90% of Muco-Cutaneous Leishmaniasis (MCL) cases occur in Bolivia, Brazil, and Peru [7]. The most common type of leishmaniasis is CL with the estimated prevalence of 1-1.5 million cases per year, accounting for 50.75% of the total new cases of leishmaniasis every year; however, only 600,000 cases are officially reported [8, 9]. According to a WHO report, Iran is now an important focus of leishmaniasis in the world. Leishmaniasis in many regions is limited to urban areas and is seen in many parts of Asia, Africa, the Mediterranean region, and the South of the Soviet Union. Recent epidemiological studies in different parts of the world show the emergence of new foci and a significant increase in the disease rate in various parts of the world, including the Middle East [10, 11].

Leishmaniasis has two common forms in Iran, CL and Visceral Leishmaniasis (VL); whereas, the MCL has not been reported in Iran [12]. The total prevalence of CL was estimated 37, 27 and 22 per 100000 populations in 2008, 2011 and 2013, respectively. According to the reports, the new cases of VL or kala-azar was 71 in Iran in 2013, of which 37 cases (52.1%) were male and 34 cases (47.9%) were female [13]. studied epidemiology of cutaneous leishaniasis in Sabzevar (Iran) from 2009-2013[14].

In spite of global progress, there is not any approved vaccine for it [15, 16]. Fighting the disease has always been a concern in our country's national plans. Despite extensive efforts and national and international investments, not only this disease has not been eradicated, it has also become more prevalent with the emergence of new foci in different parts of the country. This disease, as a major problem, accounts for an important part of health and social issues and caused irreparable damage to the community by creating economic problems. The national leishmaniasis control program has emphasized epidemiological properties of the disease in its foci [17, 18]. The adoption of an appropriate method for combating the disease to enhance control program achievements requires the identification of its epidemiological characteristics in the disease foci. This epidemiological study of CL was conducted in Karun County from 2013 to 2017.

MATERIALS AND METHODS:

Khuzestan Province is one of the 31 provinces of Iran. It is in the southwest of the country, bordering Iraq and the Persian Gulf. Its capital is Ahvaz and it covers an area of 63238 km^2. Karun County (33°45′N 46°34′E) is a county in Khuzestan Province in Iran. The county of Karun with a population of 180000 is comprised of 20 towns and villages, extending along both sides of Ahvaz-Abadan road. Today, Kut-e Abdollah, as a major route to Ahvaz metropolis, is regarded as the capital city of this county. The county is subdivided into three districts namely the Central District, Soveyseh District and Karun District. The county has one city, Kut-e Abdollah. This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Informed consent was obtained from all participants.

The ridge lesion was disinfected by 70% alcohol. Serosity of the wound was collected and smeared on a glass slide and left to dry, afterward fixed for 30 seconds using 70% alcohol and next left to dry. The fixed, dried smears were stained for 20 minutes with Geimsa stain and after that rinsed with tap water. The stained smears were left to dry, subsequently examined under oil immersion lens by microscopic method. amastigote form (Leishman body) was detected inside or outside

macrophages as spherical or round shape with specified kinetoplast. The epidemiologic, demographic and symptoms of patients were recorded in questioner's forms by direct interview. The data analysis was performed using descriptive statistics, including frequency and frequency percentage. The analysis was performed using SPSS version 18. Comparisons were made using chi-square and t tests. A P value of $p \leq 0.05$ was considered statistically significance.

RESULTS:

In this five-year study from 21 March 2013 to 20 March 2018, eighty-one patients were identified. In addition, the incidence of this problem in this county was estimated one per 10000 people/population per year. Among the patients, 47 cases (58%) were men and 34 cases (43%) were female with 21 housewives. The t-test results showed a significant relationship between the gender and CL ($p<0.05$). Results showed that the age group of 20-29 years old with 28 cases (34.6%), followed by the age group below 10 years with 19 cases (23.5%) had the highest incidence of the disease. The incidence rate of the disease was lower in patients more than 30 years old. The chi-square test results suggested a significant relationship between the incidence of the disease and age ($p<0.01$).

Results also showed that the rural areas with 47 cases (58%) had the highest incidence rate. The t-test results indicated a significant relationship between the incidence of this disease and place of living ($p<0.01$). The highest incidence of active lesion with 25.9% was observed among the housewives, followed by students with 23.5%. The chi-square test results showed a significant difference in incidence between different jobs ($p<0.02$). Based on the lesion site on the body, hands, legs, face, and on few limbs accounted for 35 (43.2%), 13 (16%), 12 (14.8%), and 17 (20.1%) cases, respectively. The chi-square test results showed a significant difference in incidence between different anatomic lesion sites ($p<0.05$).

The incidence rate varied from month to month. In that, the highest incidence rate was observed in January with 16 cases (19.8%) and in February with 13 cases (16.1%), followed by a decline, which reached 1.2% in August. The highest incidence rate was observed in the winter with 36 cases (44.5%). The lowest rate was observed in the summer with 8.4%. The chi-square test results showed a significant relationship between the incidence rate and season ($p<0.001$). Results showed that 32.1% of the patients had a lesion with a diameter of more than 3 cm, 27.1% had a lesion with a diameter of 3 cm, 29.7% had a lesion with a diameter of 2 cm, and 11.1% had a lesion with a diameter of 1 cm. There was a significant relationship between the lesion size and choosing a therapy method ($p<0.001$). In that, intralesional injection and cryotherapy accounted for 74.2% of lesions with a diameter of 1 cm.

Regarding the number of lesions, 38.3% of patients had one lesion, 24.7% had two lesions, 12.3% had three lesions and 24.7% had more than three lesions. Data analysis showed a significant difference between the incidence of the disease and number of lesions ($p<0.001$). The appearance of the lesions showed that 81.5% of patients had dry lesion without discharge, 17.3% had a lesion with discharge, and only one patient had a lipoid lesion. Data analysis showed a significant difference between the incidence of the disease and frequency of lesion shapes ($p<0.001$). Although this study did not investigate the *Leishmania* parasite, the lesion appearance showed higher incidence of its rural type in this county.

Table 1: Distribution of cutaneous leishmaniasis cases based on age groups, gender, lesion frequency, residential area, lesion sites, medicine injection methods, season and history in Karun County, southwestern Iran (2013-2017)

Year	Age Groups					Gender		
	0-9	10-19	20-29	>30	Total	Male	Female	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
2013	4 (30.8)	1 (7.6)	4 (30.8)	4 (30.8)	13 (100)	6 (46.1)	7 (53.9)	13 (100)
2014	6 (23.1)	6 (23.1)	10 (38.4)	4 (15.4)	26 (100)	19 (73.1)	7 (26.9)	26 (100)
2015	2 (15.4)	3 (23.0)	4 (30.8)	4 (30.8)	13 (100)	5 (38.5)	8 (61.5)	13 (100)
2016	3 (18.8)	2 (12.5)	6 (37.5)	5 (31.2)	16 (100)	11(68.8)	5 (31.2)	16 (100)
2017	4 (30.8)	1 (7.6)	4 (30.8)	4 (30.8)	13 (100)	6 (46.1)	7 (53.9)	13 (100)
Total	19 (23.5)	13 (16.0)	28 (34.6)	20 (24.7)	81 (100)	47 (58.0)	34 (42.0)	81 (100)
Year	Lesion frequency					Residential Area		
	1	2	3	>3	Total	Urban	Village	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
2013	2 (15.4)	4 (30.8)	1 (7.7)	6 (23.1)	13 (100)	6 (46.1)	7 (53.9)	13 (100)
2014	9 (34.6)	5 (19.2)	4 (15.4)	8 (30.8)	26 (100)	6 (23.1)	20 (76.9)	26 (100)
2015	7 (53.8)	5 (38.5)	0 (0.0)	1 (7.7)	13 (100)	8 (61.5)	5 (38.5)	13 (100)
2016	5 (31.3)	3 (18.7)	3 (18.7)	5 (31.3)	16 (100)	7 (43.7)	9 (56.3)	16 (100)
2017	8 (61.5)	3 (23.1)	2 (15.4)	0 (0.0)	13 (100)	7 (53.9)	6 (46.1)	13 (100)
Total	31 (38.3)	20 (24.7)	10 (12.3)	20(24.7)	81 (100)	34 (42.0)	47 (58.0)	81 (100)
Year	Lesion sites					Medicine Injection		
	Hands	Feet	Faces	Few Limbs	Total	Systemic	Topical	Total
	No. (%)	No. (%)	No (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
2013	7 (53.8)	2 (15.4)	3 (23.1)	1 (7.7)	13 (100)	5(38.4)	8 (61.6)	13 (100)
2014	10 (38.5)	4 (15.4)	4 (15.4)	8 (30.7)	26 (100)	6 (23.1)	20 (76.9)	26 (100)
2015	7 (53.8)	1 (7.7)	1 (7.7)	4 (30.8)	13 (100)	1 (7.7)	12 (92.3)	13 (100)
2016	8 (50.0)	0 (0.0)	3 (18.8)	5 (31.2)	16 (100)	0 (0.0)	16 (100)	16(100)
2017	3 (23.1)	6 (46.1)	1 (7.7)	3 (23.1)	13 (100)	0 (43.2)	13 (100)	13 (100)
Total	35 (43.2)	13(16.0)	12 (14.8)	17 (20.1)	81 (100)	12 (14.8)	69 (85.2)	81 (100)
Year	Season					History		
	Spring	Summer	Autumn	Winter	Total	Relapse	New	Total
	No. (%)	No. (%)	No (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
2013	4 (30.7)	0 (0.0)	0 (0.0)	9 (62.3)	13 (100)	3 (23.1)	10 (76.9)	13 (100)
2014	0 (0.0)	3 (11.5)	7 (27.0)	16 (61.5)	26 (100)	1 (3.8)	25 (96.2)	26 (100)
2015	4 (30.7)	1 (7.7)	3 (23.1)	5 (38.5)	13 (100)	0 (0.4)	13 (100)	13 (100)
2016	2 (12.5)	1 (6.2)	7 (43.8)	6 (37.5)	16 (100)	0 (0.0)	16(100)	16 (100)
2017	7 (53.8)	1 (7.7)	5 (38.5)	0 (0.0)	13 (100)	0 (0.0)	13 (100)	13 (100)
Total	17 (21.0)	6 (8.4)	22(27.1)	36 (44.5)	81 (100)	4 (5.0)	77 (95.0)	81 (100)

Table 2: Distribution of cutaneous leishmaniasis cases based on lesion size, occupation and form of lesion in Karun County, southwestern Iran (2013-2017)

Years	2013 No. (%)	2014 No. (%)	2015 No. (%)	2016 No. (%)	2017 No. (%)	Total No. (%)
Lesion Size						
1cm	3(23.1)	1 (3.8)	2(15.3)	2 (12.5)	1 (7.7)	9 (11.1)
2cm	4 (30.8)	1 (3.8)	6 (46.2)	7 (43.7)	6 (46.2)	24 (29.7)
3cm	1 (7.7)	8 (30.8)	4 (30.8)	5 (31.3)	4 (30.8)	22 (27.1)
>3cm	5 (38.4)	16 (61.6)	1 (7.7)	2 (12.5)	2 (15.3)	26 (32.1)
Occupation						
Self-employment	2 (15.3)	3 (11.5)	0(0.0)	0 (0.0)	1 (7.7)	6 (7.4)
Housewife	4 (30.8)	5 (19.3)	4 (30.8)	3 (18.8)	5 (38.4)	21 (25.9)
Ranch	1 (7.7)	0 (0.0)	1(7.7)	0 (0.0)	0 (0.0)	2 (2.5)
Driver	0 (0.0)	3 (11.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.7)
Others Worker	0 (0.0) 2 (15.3)	0 (0.0) 3 (11.5)	1 (7.7) 0 (0.0)	5 (31.2) 1(6.2)	2 (25.3) 0 (0.0)	8 (9.9) 6 (7.4)
Employee	0 (0.0)	0 (0.0)	1 (7.7)	1 (6.2)	0 (0.0)	2 (2.4)
Student Children	3 (23.1) 1 (7.7)	6 (23.2) 3 (11.5)	3 (23.1) 2 (15.3)	3 (18.8) 3 (18.8)	4 (30.8) 1 (7.7)	19 (23.4) 10 (12.3)
Military	0 (0.0)	3 (11.5)	1(7.7)	0 (0.0)	0 (0.0)	4(4.9)
Form of Lesion						
With Secretion Without Secretion Lipoid Total	4(30.8) 9(69.2) 0(0.0) 13(100)	24 (92.4) 1(3.8) 1(3.8) 26(100)	11(84.6) 2(15.4) 0 (0.0) 13(100)	15(93.8) 1 (6.2) 0 (0.0) 16(100)	12 (92.3) 1(7.7) 0 (0.0) 13(100)	66(81.5) 14(17.3) 1(1.2) 81(100)

Table 3: Distribution of cutaneous leishmaniasis cases based on month in Karun County, southwestern Iran (2013-2017).

Years Months	2013 No (%)	2014 No (%)	2015 No (%)	2016 No (%)	2017 No (%)	Total No (%)
April	2(15.4)	0 (0.0)	2(15.4)	1(6.3)	1(7.6)	6(7.4)
May	2(15.4)	0(0.0)	2(15.4)	0(0.0)	0(0.0)	4(4.9)
June	0 (0.0)	0 (0.0)	0(0.0)	1(6.2)	6(46.1)	7(8.6)
July	0(0.0)	0(0.0)	1(7.6)	1(6.2)	0(0.0)	2(2.5)
August	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(7.6)	1(1.2)
September	0 (0.0)	3(11.5)	0(0.0)	0(0.0)	0(0.0)	3(3.7)
October	0 (0.0)	0(0.0)	0(0.0)	3(18.8)	0(0.0)	3(3.7)
November	0 (0.0)	3(11.5)	1(7.7)	1 (6.2)	4(30.1)	9(11.1)
December	0(0.0)	4(13.9)	2(15.4)	3(18.8)	1(7.6)	10(12.3)
January	2(15.4)	11(42.4)	1(7.7)	2(12.5)	0(0.0)	16(19.8)
February	4(30.7)	2(7.7)	3(23.1)	4(25.0)	0(0.0)	13(16.0)
March	3(23.1)	3(11.5)	1(7.7)	0(0.0)	0(0.0)	7(12.4)
Total	13(100)	26(100)	13(100)	16(100)	13(100)	81(100)

DISCUSSION:

There is a probable risk of CL infection to travelers visiting endemic regions. The statistical results showed that five patients had a visit to Mashhad, Chabahar, Abadan, Khorramshahr, and Fakkeh Counties in this five-year period. On the other hand, 76 patients had no history of visit, indicating that CL was endemic in Karun. The rural CL has been reported in rural areas of 17 out of 31 Iranian provinces (more than 50%) [19-21]. Provinces, such as Khorasan, Yazd, Bushehr, Fars, Khuzestan, Ilam and Isfahan showed the highest prevalence; whereas, provinces in the west and northwest of the country had the lowest prevalence [22]. This study showed that the mean incidence rate of the disease in this five years in the county, with a population of 180000 people, was one per 10000 people/population

per year. The highest incidence (34.6%) was observed in the age group of 20-29 years old. The highest incidence rate was observed in the age group of over 20 years old in Kashan [23], Hamadan [24], Damghan [25] and Isfahan [26]. In addition, the highest incidence rate was observed in the age group of over 10-30 years old in Brazil [27] and Pakistan [28]. Findings in Jajarm [29] and Esfarayen [30] Counties were inconsistent with this study. The higher incidence rate among younger age group could be because CL was endemic to these regions. This was because the incidence rate of CL increased in the age group below 15 years old and then reduced, probably due to acquired immunity. The highest incidence rate of CL in Karun was observed among the young age group.

In this study, the incidence rate of CL was higher among men than women with 58% of cases. In studies in Shush, Ganaveh and Ahvaz [31-33], the incidence rate was higher among males. In contrast, the incidence rate of CL was higher among females in studies of Ebadi in Isfahan [34] and Karimi-Zarchi in Sarakhs [35]. This finding suggests the higher risk of transmission at home in mentioned studies. As a result, enhancement of awareness, specifically among women, encouraging environmental sanitation inside and outside the living place, applying mosquito screen for doors and windows and using mosquito sleeping net at night are emphasized. The higher incidence rate of CL among men in other studies could be due to the greater presence of them in places with higher risk of vector biting, such as abandoned places, construction sites and desert areas and areas in the vicinity of rodent colonies at night [36].

The research findings showed that the rural residents accounted for the majority of patients (58%). Poor environmental health (the lack of a sewage disposal system, accumulation of domestic and construction wastes and animal feces, etc.), livestock farming near the living place, rodent colonies near the living place and abundance of vector phlebotomine sand flies were causes of higher incidence in rural areas [37].

Results showed that hands and legs were the most bitten sites. Studies in Kermanshah [38], Larestan [39], Mirjaveh [40] and Gorgan [41] Counties reported legs and hands with the most bite marks. Since legs and hands are less covered, they are more exposed to bites, and the chance of CL lesion is higher on them. As a result, it is recommended to cover these limbs in the investigated regions. It is also recommended to use mosquito sleeping nets treated with insecticide to prevent phlebotomine sand fly bites. The frequency of lesions in infected people, greater involvement of uncovered body parts and irreparable disfigurement complications of CL emphasize its control and prevention plans.

One of the most important factors affecting the CL is climate, which depends on seasons and months of the year. On the other hand, the seasonal distribution pattern of the disease in Karun clearly confirms the local transmission of the disease. The highest incidence rate of CL in Karun was observed in winter, indicating that phlebotomine sand fly is more active in the first half of the year and the second half accounts for the majority of CL cases. In other similar epidemiological studies in different parts of the country, the highest incidence of CL was observed in fall and summer, which is inconsistent with the current study [40]. Findings showed that although the number of lesions was different in the patients, the majority of them (38.3%) had only one lesion. Mohammadi - Azni et al. reported that 54% of the patients had one lesion, 23.7% had two lesions and the rest had three or more lesions [25]. This difference can be attributed to the difference in frequency of phlebotomine sand flies and their feeding habits [41]. Sharifi et al. also reported that the majority of patients (77.9%) had only one lesion [42].

CONCLUSIONS:

This was the first study on the prevalence of CL in Karun County, southwestern Iran, which showed high prevalence of infection. Some subjects should be applied in case of stable surveillance of CL infection situation in sand fly and rodent species including increase in health practice, promoting public awareness about CL, reducing the number of animal reservoirs, orderly monitoring immigrants and travelers

coming from endemic regions, demolition of rodent burrows, dermal protection from sand fly bites by bed nets, and spraying insecticides to interrupt the transmission of this parasitic infection.

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CONFLICT OF INTEREST:

The author declares that there is no conflict of interest in this study.

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