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Laboratory diagnosis, clinical manifestations, epidemiological situation and public health importance of cutaneous leishmaniasis in Shushtar County, Southwestern Iran

Hamid Kassiri^{1*}, Ali kassiri², Masoud Lotfi³, Parvaneh Farajifard⁴, Elnaz Kassiri⁵¹Department of Medical Entomology and Vector Control, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran²Department of Ophthalmology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran³Department of Public Health, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran⁴Shushtar Health Center, Ahvaz Jundishapur University of Medical Sciences, Shushtar, Iran⁵Mazandaran University of Medical Sciences, Sari, Iran

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ABSTRACT

Objective: To investigate as a descriptive and cross sectional study on epidemiology, the laboratory diagnosis and clinical manifestation of Cutaneous Leishmaniasis (CL) in Shushtar County. **Methods:** The investigation was carried out on referrals from different districts of Shushtar County and was diagnosed based on their ulcer clinical manifestations with CL using laboratory method. This study was actively developed over the period of 2008 to 2010. Surface of a clean slide was pressed on to the scraped part of the lesions and moved along to produce a smear on the slide. The slides were fixed with methanol, stained with standard Giemsa, and examined carefully under high magnification for an hour or more to look for amastigotes by a trained person. Two smears were prepared and examined from each patient and data were collected using different correlative patients characteristics to analyze information. The essential records including residential area, type and location of lesion, age, sex, the number of ulcers, season, and the history of traveling to endemic areas for this disease were carefully gathered. **Results:** Parasites presumed to be *Leishmania major* (*L. major*) based on the existence of a large vacuole in the cytoplasm. Totally 206 patients were referred to the central laboratory and their manifestation lesions were clinically examined. Of these, 64 cases (31.1%) were female and 142 cases (68.9%) were male. The findings documented that the rate of incidence of CL was in fall 61 cases (29/6%) and winter 80 cases (38/8%), which is one of the most important characteristics of zoonotic CL. Sixty-eight cases (33%) had ulcers on hands, 63 cases (30.6%) on feet and 30 cases (14.6%) on hands and feet. A total of 120 patients (58.3%) had only one ulcer. But 70 cases (34%) had 2 ulcers. The highest incidence was found among patients with two age groups of 20–29 years old (43.2%) and 10–19 years old (18%). The most cases resided in rural areas with 164 cases (79.6%). **Conclusions:** Analyzing of the composed results revealed that this area could be considered as one of the endemic foci of zoonotic cutaneous leishmaniasis caused by *L. major*. In view of the high prevalence rate and distribution of leishmaniasis in Iran, it is in necessity of data gathering about vectors and reservoirs of each district for prevention. Designing control programs play prominent role in this case. With respect to the increased prevalence of CL in Shushtar districts, health care observers should pay further attention on preventing.

1. Introduction

Leishmaniasis is the name of a group of protozoan

diseases that are transmitted by different species of female sandflies of subfamily phlebotominae that are considered as zoonosis. They are clinically included the cutaneous, visceral and, muco-cutaneous forms^[1].

In Iran, Cutaneous Leishmaniasis (CL) is seen both in urban and rural forms. In the urban form its reservoir host is human and is observed in different regions of Iran like Tehran, Kerman, Mashhad and Neyshabour. In the rural

*Corresponding author: Hamid kasseri, Department of Medical Entomology and Vector Control, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Tel: 0098-6113738269

Fax: 0098-6113738282

E-mail: hamid.kassiri@yahoo.com

form its reservoir hosts are rats and is seen in the counties and provinces like Natanz, Isfahan, Sarakhas, Lotfabad of Kashmar, Khuzestan and Ilam[2]. The trend of CL in Iran is rising and during recent years, new foci of the disease in the country have been identified[3].

There are currently 350 million people in 88 countries are at risk for the disease[4]. Iran is one of the countries in the Eastern Mediterranean where the prevalence of CL is high. Iran is among seven countries having most prevalence of the disease and about 30 000 cases of CL are annually reported[5]. Provinces such as Yazd, Bushehr, Khorasan, Fars, Ilam, Khuzestan and Isfahan with an incidence rate of 166 cases per 100 000 people have the highest incidence rate of the disease in Iran, and western and northwestern Provinces with an incidence rate less than 10 cases per 100 000 people have the lowest incidence rate[6–11]. Factors such as environmental changes, Immigrations and urban planning have a significant effect on the epidemiology of CL, and its urban type is strongly affected by natural disasters (such as earthquakes) or man-made disasters (such as war) [12].

Shushtar County is located in the Khuzestan Province, in southwest Iran, and has a subtropical climate and many plains. This county is one of the old endemic foci of rural CL. In controlling the CL, it seems that epidemiological and ecological studies have the most importance; because in these investigations the risk factors of distribution of the disease are evaluated and as a result preventive and control measures are improved. This study was designed to find out the status of the disease and become aware of its epidemiological process and factors affecting on it in the area.

2. Materials and methods

In this descriptive cross-sectional study, statistical population included all the patients who were under treatment and follow up with the diagnosis of CL through laboratory confirmation (amastigote observation in the lesion smear) and clinical signs in Shushtar County health centers from April 2008 by the end of March 2011. Demographic and epidemiologic information of each patient such as age, gender, living place (city or village), month, season, number of lesion and the place of lesions on the body were recorded in the questionnaire. Thus, data of 206 patients with CL in the County of Shushtar were collected. The data obtained were analyzed by SPSS version 19 through descriptive statistics indices (average and percentage).

3. Results

This study showed that a total of 206 patients with the CL during 2008–2010 referred to Shushtar County health centers.

Slides prepared from the lesion of these patients had a *Leishmania* parasite with large vacuole (the characteristics of *Leishmania major* (*L. major*) (Figure 1). During these years respectively 74 (36%), 93 (45.1%) and 39 cases (18.9%) were diagnosed. Also, out of the total number of patients, 64 (31.1%) and 146 cases (68.9%) were female and male, respectively (Table 1).

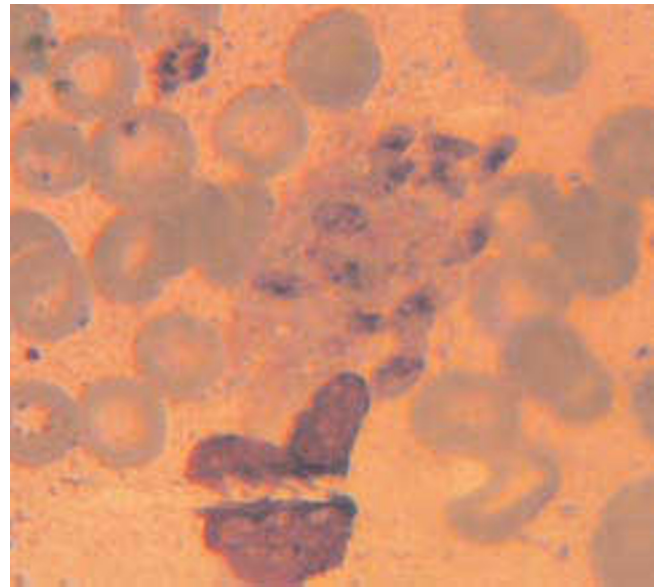


Figure 1. Amastigotes observed in smears taken from the lesions of patients, Shushtar County, Khuzestan province, Southwestern Iran (2008–2010).

Table 1

Frequency distribution of cutaneous leishmaniasis cases by sexes in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Years	Male [n (%)]	Female [n (%)]	Total [n (%)]
2008	42 (57.8)	32 (42.2)	74 (100)
2009	77 (82.8)	16 (17.2)	93 (100)
2010	23 (59.0)	16 (41.0)	39 (100)
Total	142 (68.9)	64 (31.1)	206 (100)

Based on the location, 164 (79.6%) and 42 cases (20.4%) were living in the village and the city, respectively (Table 2). Regarding the lesion place on the body, most patients have lesions on the hands (33%) and the feet (30.6%). As well as 14.6%, 9.7%, and 5.9% of patients respectively had lesions on the hands–feet, faces and hands–faces (Table 3). The disease cases distribution in the different age groups showed that the age groups of 20–29, 10–19, >5 and 30–39 years respectively with 89 (43.2%), 37 (18%), 28 (13.6%) and 19 cases (9.2%) showed the highest incidence of CL (Table 4).

Most cases of the disease were in early October (11.2%), December (10.2%) and May (9.2%) and lowest cases were in the months of August (2%) and July (2.4%), (Table 5). The CL prevalence in spring, summer, autumn and winter was determined respectively 44 (21.4%), 21 (10.2%), 61 (29.6%) and 80 cases (38.8%) (Table 6). Figure 2 shows a comparison

between the CL cases based on the season and sex during a three-year study period in Shushtar County. In terms of the number of lesions, 120 (58.3%), 70 (34%), 13 (6.3%) and 3 cases (1.4%) had one, two, three and four lesions on the body, respectively (Table 7).

Table 2

Frequency distribution of cutaneous leishmaniasis cases by residential places in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Years	Urban [n (%)]	Rural [n (%)]	Total [n (%)]
2008	16 (21.6)	58 (78.4)	74 (100)
2009	20 (21.5)	73 (78.5)	93 (100)
2010	6 (15.4)	33 (84.6)	39 (100)
Total	42 (20.4)	164 (79.6)	206 (100)

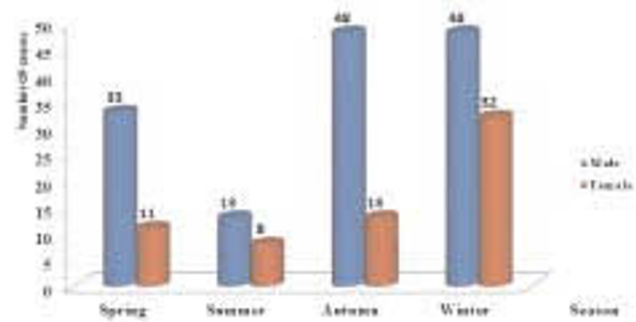


Figure 2. Frequency distribution of cutaneous leishmaniasis cases by seasons and sexes in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Table 3

Frequency distribution of cutaneous leishmaniasis cases by ulcer sites on the body in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Ulcer sites	2008 [n (%)]	2009 [n (%)]	2010 [n (%)]	Total [n (%)]
Hands	25 (33.8)	25 (26.9)	18 (46.1)	68 (33.0)
Feet	13 (17.6)	36 (38.8)	14 (35.9)	63 (30.6)
Faces	14 (19.0)	3 (3.2)	3 (7.7)	20 (9.7)
Trunks	1 (1.4)	2 (2.1)	1 (2.6)	4 (1.9)
Hands & feet	9 (12.0)	19 (20.5)	2 (5.1)	30 (14.6)
Necks	2 (2.7)	2 (2.1)	1 (2.6)	5 (2.4)
Faces and feet	3 (4.1)	1 (1.1)	0 (0.0)	4 (1.9)
Faces and hands	7 (9.4)	5 (5.3)	0 (0.0)	12 (5.9)
Total	74 (100)	93 (100)	39 (100)	206 (100)

Table 4

Frequency distribution of cutaneous leishmaniasis cases by age groups (Years) in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Age groups	2008 [n (%)]	2009 [n (%)]	2010 [n (%)]	Total [n (%)]
<5	18 (24.3)	7 (7.5)	3 (7.7)	28 (13.6)
5–9	8 (10.8)	9 (9.7)	2 (5.1)	19 (9.2)
10–19	13 (17.6)	11 (11.8)	13 (33.3)	37 (18.0)
20–29	19 (25.7)	58 (62.4)	12 (30.8)	89 (43.2)
30–39	13 (17.6)	3 (3.2)	3 (7.7)	19 (9.2)
40–49	2 (2.7)	2 (2.2)	3 (7.7)	7 (3.4)
>50	1 (1.3)	3 (3.2)	3 (7.7)	7 (3.4)
Total	74 (100)	93 (100)	39 (100)	206 (100)

Table 5

Frequency distribution of cutaneous leishmaniasis cases by months in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Months	2008 [n (%)]	2009 [n (%)]	2010 [n (%)]	Total [n (%)]
April	3 (4.1)	4 (4.3)	6 (15.4)	13 (6.3)
May	4 (5.4)	11 (11.8)	4 (10.2)	19 (9.2)
July	6 (8.1)	5 (5.4)	1 (2.6)	12 (5.8)
June	0 (0.0)	4 (4.3)	1 (2.6)	5 (2.4)
August	1 (1.4)	2 (2.2)	1 (2.6)	4 (2.0)
September	6 (8.1)	4 (4.3)	2 (5.1)	12 (5.8)
October	7 (9.5)	15 (16.1)	1 (2.6)	23 (11.2)
November	5 (6.7)	9 (9.7)	3 (7.7)	17 (8.3)
December	8 (10.8)	6 (6.4)	7 (17.9)	21 (10.2)
January	15 (20.3)	18 (19.4)	3 (7.7)	36 (17.5)
February	14 (18.9)	6 (6.4)	7 (17.9)	27 (13.1)
March	5 (6.7)	9 (9.7)	3 (7.7)	17 (8.2)
Total	74 (100)	93 (100)	39 (100)	206 (100)

Table 6

Frequency distribution of cutaneous leishmaniasis cases by seasons in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Years	Spring [n (%)]	Summer [n (%)]	Autumn [n (%)]	Winter [n (%)]	Total [n (%)]
2008	13 (17.6)	7 (9.5)	20 (27.0)	34 (45.9)	74 (100)
2009	20 (21.5)	10 (10.7)	30 (32.3)	33 (35.5)	93 (100)
2010	11 (28.2)	4 (10.3)	11 (28.2)	13 (33.3)	39 (100)
Total	44 (21.4)	21 (10.2)	61 (29.6)	80 (38.8)	206 (100)

Table 7

Frequency distribution of cutaneous leishmaniasis cases by number of ulcers on the body in Shushtar County, Khuzestan Province, Southwestern Iran (2008–2010).

Ulcer frequency

Years	1 [n (%)]	2 [n (%)]	3 [n (%)]	4 [n (%)]	Total [n (%)]
2008	41 (55.4)	25 (33.8)	7 (9.4)	1 (1.4)	74 (100)
2009	50 (53.8)	35 (37.6)	6 (6.5)	2 (2.1)	93 (100)
2010	29 (74.4)	10 (25.6)	0 (0.0)	0 (0.0)	39 (100)
Total	120 (58.3)	70 (34.0)	13 (6.3)	3 (1.4)	206 (100)

4. Discussion

About control of diseases that are transmitted by insects, first enough information should be collected about geographical distribution, seasonal abundance, and their relationship with the epidemiologic, demographic and ecologic factors. In this study a total of 206 cases of CL were found based on clinical symptoms and microscopic slides of patients' lesion, and an amastigote observation during the years 2008–2010.

One of the effective factors in catching various infectious diseases, including CL, is the age of the person. Generally the CL prevalence in the endemic areas increases up to age 15 and then it reduces probably because of acquired immunity. The high cases of the disease at early ages can confirm high endemicity of disease in the region. In the present study the highest incidence of the disease was observed in the age group of 20–29 years (43.2%). In Hamzavy et al study, more cases of CL in the Kermanshah Province was at the ages of 20–29, which was consistent with our results^[12]. In fact, this is the active age group of society, which is exposed to infection more for the reasons, including work and activity near the infected rodent colonies and sandflies. Furthermore, the movements of this age group to the disease foci are more than other age groups. On the one hand the population of this age group is more than other age groups; therefore, it causes to raise the probability catching disease in this age group. Due to less possibility of bites by sandflies, the frequency of the disease in the early age group is low. Due to the high level of immunity in the higher age group risk of the disease had been at a minimum level.

In the present study, out of the total cases of the disease, 68.9% were male and prevalence in this gender were 2.2 times the female, which it was consistent with Hamzavy's

study in the Province of Kermanshah^[12], Zahirmia's study in the province of Hamadan^[13] and Yaghoobi's study in the city of Ardestan (the center of Iran)^[14]. Cultural and behavioral factors, as well as the type of clothing possibly have caused men more than women are exposed to the disease. Due to type of job, men are obliged to stay outdoors more, therefore, are exposed more to be bitten by infected sandflies. Because of less movement in areas where the possibility of bites by sandflies is more (such as dilapidated places and desert areas), women are less bitten by sandflies at night. Therefore, the possibility of catching CL among them becomes less. In Khuzestan province, including Shushtar, most of the time, working continues until sunset and even the night, and from the sunset sandflies are active to bite. In the studies conducted by Talary in Kashan City^[15], Ebadi in Borkhar of Isfahan^[16], Karimi–Zarchi in Sarakhs city^[17], and Doroodgar in Kashan County^[1], CL cases were more on female gender that was not consistent with the present study. This difference in results has been mentioned in relation to the economic activities of the women and carpet weaving in low light rooms and basements. In such places, the sandflies also are active for days and continue to blood feeding^[14].

Location of the creation of lesions will comply with the numerous factors, including the sandfly species, social and cultural behavior of the people, climate and so on. The results of this study showed that most of the lesions were in the hands and feet that are uncovered parts of the body. Based on the study conducted by Hamzavy et al. in Kermanshah Province, most of the lesions were observed in the hands (49%), faces and neck (28%) and feet (22%)^[12].

In addition, Aflatonian and Sharifi's study in the city of Bam that is one of the most important urban foci of urban CL in Iran showed that more lesions were in the faces (46%) and hands (41.6%)^[18]. Rassi's study in Arsanjan County

reported most of the lesions in the hands (45.3%), feet (24.2%) and faces (20.5%)[19]. In Zahirnia's study in Hamedan Province, most of the lesions were reported in the hands and feet (71.6%) and faces (15.8%)[13]. Due to being short of mouth parts in the sandflies that cannot blood-feeding of the covered parts of the host, the possibility of a bite of the naked parts of the body by sandflies is higher and consequently the possibility of creation of the CL lesion in these areas become greater. Therefore, one of the effective factors in the distribution of the lesions in the body of patients is their coverage status. Also sandflies use chemical attractions such as the concentration of carbon dioxide, for selecting a suitable host and place for blood-feeding. These attractions in the hands and feet are more than other parts of the human body. Therefore to reduce the likelihood of contact with sandflies, by avoiding sleeping in non-roofed areas along with full body cover, it is necessary to attempt for the increase of awareness of individuals.

In terms of the disease occurrence season, in this study, most cases were found in the winter (38.8%) and fall (29.6%). In studies of CL in Counties of Khorramshahr (Khuzestan Province) and Bam (Kerman Province), most cases were reported in the winter that is similar to this study[18,20]. More cases of the disease in the Province of Kermanshah were observed in the fall (35%); however, 19% of disease cases were reported in the winter[12]. In another study carried out in Multan of Pakistan, all cases have been reported in the winter[21]. But in another study carried out in Saudi Arabia, a seasonal variety with An incidence maximum between June to November was observed[22]. Experimental studies indicate that the most appropriate temperature and relative humidity of the environment for the reproduction of sandflies are respectively 23–28 °C and 70%–100%. Therefore, according to meteorological data of Shushtar County, it can be inferred that in most months of the year (especially in spring and autumn) the environmental conditions are suitable for the sandflies to achieve the proper age for disease transmission. Principally in areas of the world where the growth and development of adult sandflies is seasonal, infection pattern of CL in human follows also a seasonal process. In areas where mature sandflies in the spring and early summer months are reproduced, the new cases of the CL disease usually appear in the late summer and autumn (according to the incubation period of the disease).

The results of this survey showed that most of the patients had one (58.2%) Or two sores (34%). In most studies, this pattern of lesions frequency is seen. In the county of Bam (southeastern Iran) out of a total of patients, 72.7% and 19.2% of cases respectively had one or two lesions[18]. As well as in Kermanshah Province (western Iran), 54.4% and 19.2% of patients respectively had one or two lesions[12]. In a study

of CL in Marvdasht County (southern Iran), 25.3%, 24.6%, 20.5%, 11.3%, and 18.08% of patients respectively had one, two, three, four and five or more ulcers on the body. The multiplicity of lesions in patients can be due to the blood-feeding behaviors of sandflies, which they are doing multiple bites for any time blood-feeding or due to the abundance of infected sandflies in place of the risk to the disease. According to the side effects of the disease in terms of health and beauty as well as the subsequent mental- psychological effects, the importance of control and prevention of the risk of the disease will be clearer.

In this study 79.6% of patients were resident of rural areas that are similar with Doroodgar's study in Kashan County[1]. In the Counties of Khorramshahr, Marvdasht, Gorgan, and Province of Hamedan respectively 65.75%, 57.5%, 53.1% and 54.8% of the patients with CL lived in urban areas.

The high cases of the disease in rural areas of the Shushtar County show CL infectivity in the rodent and sandflies. According to the abundance of rodents and sandflies, their infectivity, being close place of their life to a residence place of people and the possibility of spreading the disease in the city, the emergence of epidemics in the Shushtar city is not far from the minds. Therefore, according to this situation, the need for fundamental and serious measures to combat the reservoir hosts and vectors as well as other preventive measures have great importance.

Due to being exophile vectors and sleeping people during months of the disease transmission in outer places, controlling the rodent (which causes to reduce the abundance of sandflies) has an important role in reducing cases of the disease. Seemingly in the company of the rodent control, residual spraying has a low impact factor. The distribution of insecticide impregnated bed nets and advised to use it during the rest time, use of insect repellents, environmental health measures, proper disposal of garbage, the barn moved to the out of people's home, recommended to install lace to the doors and windows, to raise people's awareness of effective factors on the occurrence of the disease and how to transmit it to the man can greatly help in reducing CL cases.

Epidemiological factors such as age, gender, job, residence place and season have a serious impact on the CL prevalence. These findings not only specify the status of the disease in the County of Shushtar, but also they can be used to design and evaluate programs for controlling the disease.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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