International Journal of Infectious Diseases 17 (2013) e1198–e1206

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

A newly emerged cutaneous leishmaniasis focus in central Iran

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ARTICLE INFO

Article history:
Received 18 May 2013
Received in revised form 3 July 2013
Accepted 3 July 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Cutaneous leishmaniasis
Epidemiology
Qom
Iran

SUMMARY

Objectives: This study was performed to evaluate the epidemiological status of cutaneous leishmaniasis (CL) in the most important endemic foci of Qom Province, central Iran. The city of Qom is the largest center for Shi'a scholarship in the world and is a significant pilgrimage destination.

Methods: During 2006–2011, all suspected CL patients with skin lesion(s) referred to regional health centers of Ghomrood and Ghanavat regions, and all actively detected cases, were examined clinically and parasitologically for CL. Patient information was recorded and patients were categorized based on the number and size of the lesions. Odds ratios (OR) of different risk factors were calculated.

Results: A total of 849 (59.2% male, 40.8% female) confirmed cases of CL were enrolled; the average incidence rate of the disease was 14.9 per 100 000 people. During the study period 2006–2011, the trend in CL incidence showed no sudden variations in the areas studied, except for an outbreak of CL in 2009. *Leishmania major* was identified as the causative agent based on internal transcribed spacer 1 (ITS1) ribosomal DNA PCR analysis. During the study period, the age distribution of CL cases was relatively stable, with the majority (50%) of patients aged 1–25 years. Most cases (n = 468; 55.1%) had a single lesion and 82 (9.6%) patients had four or more lesions (range 1–29). The risk of developing multiple lesions was significantly increased in patients with seasonal jobs (summer workers) (p = 0.023; OR 1.516) and significantly decreased in patients who were affected in winter (p = 0.010; OR 0.398). The risk of developing large-sized lesions (>1 cm) was significantly increased in patients in the age groups >25 years (p = 0.001–0.015; OR 2.5–3.5) and decreased in patients with seasonal jobs (summer workers) (p = 0.005; OR 0.570).

Conclusions: The present data show the importance of CL as a health problem in suburban areas of Qom Province. In order to identify other epidemiological aspects of leishmaniasis in this area, studies on vectors and reservoirs are recommended. Since leishmaniasis caused by *L. major* is typically zoonotic, control measures should focus on rodents as the main reservoirs and *Phlebotomus papatasi* as the main vector. Awareness should be raised in the high-risk populations comprising people with diabetes, young adults (<25 years old), and those who work outdoors during the summer.

1. Introduction

Leishmaniasis is a protozoan parasitic disease caused by *Leishmania* species. It is estimated that in about 100 countries, approximately 350 million people are at risk of acquiring leishmaniasis and 12 million are infected; an estimated two million new cases occur annually.[1] The two most common clinical forms of the disease, cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), are mainly seen in 14 of the 22 countries of the World Health Organization (WHO) Eastern Mediterranean Regional Office (EMRO) region, including Iran.[2] Self-healing zoonotic CL (ZCL) due to *Leishmania major* and anthroponotic CL (ACL) due to *Leishmania tropica* are two known types of CL that are spread across most parts of Iran. In total, approximately 17 out of the 31 provinces of Iran are endemic foci for ZCL.[3] According to the official reports of the Ministry of Health, the average incidence rate of CL is usually between 20 and 40 cases per 100 000 population.[4] The endemic regions in the central and south-western parts of the country (including Yazd, Semnan, Fars, Ilam, Khoozestan, and Isfahan), with an average incidence of more than 150/100 000 population, have the highest rates of CL.[4] The number of reported CL cases increased from 13 729 in 2002 to more than 24 000 in 2006 and thereafter,[4] and the disease prevalence is increasing and new foci of CL are emerging in Iran.[5–7]

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1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2013.07.003

The province of Qom is located in the center of Iran, 120 km southwest of the capital Tehran, and is the passageway to more than 17 provinces. Each year more than 14 million people travel to or pass through this province; about two million of them are pilgrims who may stay for a while in the city.[8] The city of Qom is currently the largest city for Shi'a scholarship in the world.[9] It is estimated that 10–20% of the total world Muslim population, and more than 38% of the local Muslim population of the Middle East, are Shi'a.[10] Shi'a Muslims consider Imam Ali as the rightful successor to Prophet Muhammad, and the first divinely appointed Imam.[10] There are an estimated 50 000 seminarians in Qom coming from more than 70 countries of Africa, Asia, the Middle East, and other parts of the world.[9] Thousands of immigrants from neighboring countries including Afghanistan and Iraq travel to Iran intermittently and also play a role in importing leishmaniasis to Qom. This huge number of travelers and immigrants make leishmaniasis a priority in health strategy planning for the province.

The most important foci of CL in Qom Province are the villages of Ghomrood and Ghanavat regions, and the first cases of CL were diagnosed in 1999 in an outbreak of leishmaniasis in Ghanavat region.[11] Based on observations, CL cases have constantly been referred from the province to regional health centers during recent years. However, there is no formal report on the endemicity of CL in suburban areas of Qom Province. Since ACL and ZCL have different features with respect to ecology, parasitology, entomology, and clinical characteristics,[12] epidemiological data are needed to establish effective control strategies in the region. This information could help in the integration of the surveillance of leishmaniasis into health system programs, monitoring of leishmaniasis trends during outbreaks, risk assessment for awareness of decision-makers and education of high-risk populations, investigation of fluctuations in vectors/reservoirs, and improving treatment strategies. This study reports the epidemic aspects of CL in Qom Province during recent years.

2. Materials and methods

2.1. The region

Qom is one of the 31 provinces of Iran and is situated between 50° 06′–51° 58′ E and 34° 09′–35° 11′ N, with an area of 11 237 km^2, covering 0.89% of the total land of the country. Qom Province is located in the central part of the country; it lies 120 km by road southwest of the capital Tehran and has one city, five counties, nine rural districts, and 256 villages[8] (Figure 1). Based on the most recent census of 2011, the province has a population of approximately 1 200 000, with 95.1% residing in urban areas and 4.9% in rural areas.[13] The climate of Qom Province varies from semi-desert to desert conditions; the annual rainfall in the last year was 86.9 mm and relative humidity ranged between 8.5% in June and 89.1% in December. Geographically, the province comprises mountainous areas, foothills, and plains, and in the last year, the minimum and maximum temperatures recorded were −14 °C in December and +47 °C in June.[8]

2.2. Population and sampling

This study was done during April 2006 to November 2011. All suspected CL patients with skin lesion(s) referred to regional health centers of the province were examined clinically and parasitologically for CL. In addition to this passive case detection, monthly routine house-to-house investigations in Markazi District (including Ghanavat and Ghomrood regions) for active detection of possible CL cases was done by trained staff during an outbreak in 2009. Furthermore, from 2006, in accordance with a Ministry of Health instruction, patients under treatment were actively followed up and visited in their homes to ensure treatment courses were fulfilled; possible side effects were recorded and other members of the family were checked for the onset of lesions. The population of the region is among the low income populations; patients would not be able to attend private clinics for CL, and even if they did, they would have to be referred to a health center for treatment services since Glucantime is only distributed in these health centers. Ghanavat and Ghomrood each has one health center. Leishmaniasis is included in the surveillance system, and the staff of these centers are informed of leishmaniasis, so we assume that every case of cutaneous leishmaniasis in the region should be diagnosed.

Patient information including demographic data and clinical history was recorded on specific forms. Using the average number of new cases that occurred each year during the study period as the numerator and the population at risk as the denominator, the average annual incidence rate of the disease was calculated. The size and the number of lesions were recorded at the time of diagnosis. For each lesion, two crossing diameters were measured using a metric caliper and the mean figure was recorded as the size of the lesion. If multiple lesions were present, all lesions were measured and a mean size was calculated and presented.

The skin was sterilized and exudates from the margins of the suspected lesions were taken, fixed with methanol, and stained with Giemsa, then examined under a microscope. Part of the lesion exudate was inoculated into Novy–MacNeal–Nicolle (NNN) medium overlaid with RPMI 1640 (Gibco Invitrogen, Carlsbad, CA, USA). This was incubated at 24 °C for 1 week and examined every day for parasite growth. Another sample was taken and transferred to 2-ml vials containing sterile phosphate buffered saline (PBS) and used for DNA extraction. The disease was diagnosed based on the clinical examination and microscopic observation of intracellular amastigotes in smear or promastigotes in NNN medium.

The most frequent type of treatment was local intra-lesional administration of meglumine antimoniate (Glucantime) followed by systemic Glucantime. The treatment responses and possible side effects in the patients who received standard therapy were recorded. Patients were categorized based on (1) the number of lesions: single lesion or multiple lesions, and (2) the size of lesions: small-sized lesion (<1 cm) and large-sized lesion (>1 cm). Odds ratios (OR) of different variables as risk factors in the outcome of disease were analyzed.

2.3. Molecular identification of parasites by PCR

2.3.1. DNA extraction

Parasite genomic DNA was extracted using the conventional phenol–chloroform procedure.[14] Briefly, 100 μl of each disrupted tissue sample was transferred to a 1.5-ml microtube containing 200 μl of lysis buffer (100 mM Tris–HCl, pH 8; 10 mM ethylene-diaminetetraacetic acid (EDTA), pH 8; 1% sodium dodecyl sulfate (SDS); 100 mM NaCl; 2% Triton X-100) (Sigma, St. Louis, MO, USA) with 20 μl proteinase K (100 mg/ml), vortexed, and incubated at 56 °C for 1 h. Three hundred microliters of phenol–chloroform (1:1) was added, vortexed, and centrifuged at 5000 rpm, 4 °C, for 5 min. The supernatant was transferred to a new microtube and chloroform extraction was performed again. An equal volume of isopropanol and 1/10 volume of 3 M sodium acetate (pH 5.2) was added to the supernatant, incubated at −70 °C for 15 min, and centrifuged at 12 000 rpm for 15 min; the precipitant was then washed with 70% ethanol by centrifugation at 12 000 rpm for 10 min. The pellet was air-dried and resuspended in 20 μl of distilled water and stored at −20 °C until use.

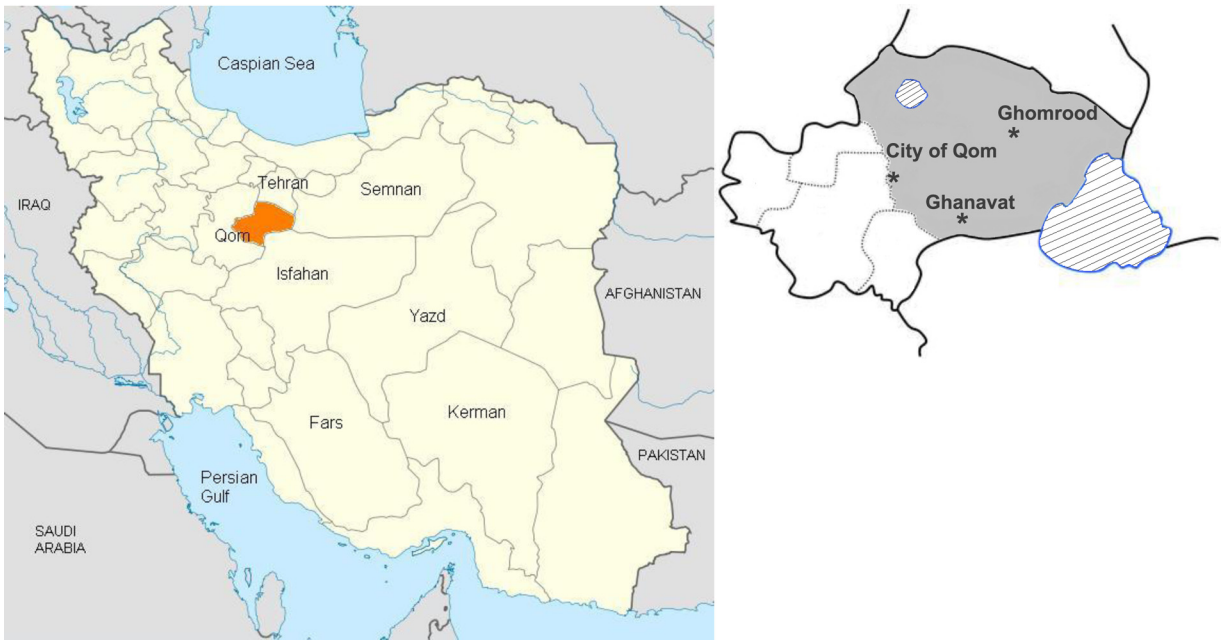

Figure 1. Geographic location of Qom Province and its districts, showing the regions of study. Hatched areas show lakes. Markazi District (including Ghomrood and Ghanavat) is highlighted.

2.3.2. PCR

Identification of the causative agent of the disease was performed using internal transcribed spacer 1 (ITS1) ribosomal DNA PCR. The Leishmania-specific target gene was amplified using primers Leish-F (5′-CCT CTC TTT TTT CNC TGT GC-3′) and Leish-R (5′-CAA CAC GCC GCC TCC TCT CT-3′), yielding a 600-bp fragment for *L. major* and an 800-bp fragment for *L. tropica*. Amplification of the target genes was carried out in a total volume of 25 μl containing 2 μl 10× PCR buffer, 1.5 mM $MgCl_2$, 0.5 μl of each primer (25 pmol/ml), 0.2 mM of each dNTPs, and 0.5 U Taq DNA polymerase (Fermentas Life Sciences, York, UK). The cycling program was initiated by early denaturation of 5 min at 95 °C, followed by 35 cycles of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 60 s, then by a final extension step at 72 °C for 5 min. PCR products were electrophoresed on 1% agarose gel and visualized under a UV transilluminator.

2.4. Data analysis

Data processing and the statistical analysis were performed using SPSS v. 18 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism v. 5.01 (GraphPad Software Inc., La Jolla, CA, USA) software. Pearson Chi-square, Fisher's exact, and univariate logistic regression analyses were used for the different comparisons. *p*-Values of ≤ 0.05 were considered significant.

3. Results

Clinically and parasitologically proven cases of CL were recorded from the villages of Ghomrood and Ghanavat regions. Patient data are given in Tables 1 and 2. A total 849 cases of CL (59.2% male, 40.8% female) were enrolled during the study period, from 2006 to 2011; 417 (49.1%) were residents of Ghanavat and 432 (50.9%) were recorded from Ghomrood region (Figure 2). During the study period, among the patients with skin lesions, only one case was diagnosed as having lupus erythematosus. In the areas under study, the maximum number of cases was recorded in 2009 with 312 cases (36.7% of total) and the least number of cases was recorded in 2008 with 34 cases (4% of total). With an average 138 new cases occurring each year during 2006–2011, and a population at risk estimated to be 954 000, the average annual incidence rate of the disease in Markazi District (including Ghomrood and Ghanavat) was calculated to be approximately 14.9 per 100 000 people.

Most of the CL patients were Iranian (88.2%), and 19.6% of the patients had traveled to a known endemic area during the last year. The mean ± standard deviation (SD) age of the patients was 29.5 ± 19.44 years, and most of the patients were assigned to the 1–15 and 16–25 years age groups, with approximately 25% of the total recorded CL cases for each group.

Based on PCR analysis, the parasites isolated from the lesions were characterized as *L. major*. The mean ± SD size of CL lesions was 1.41 ± 0.74 cm. While most of the patients (55.1%) had only one skin lesion, about 9.6% of the cases developed more than four lesions. The number of lesions per patient ranged from 1 to 29, with the hand and foot the most common sites of lesion onset (78.7% of total) (Figure 3). Seventeen percent of the patients were suffering from a chronic non-communicable disease such as diabetes, and housekeepers suffered more from CL than those in other occupation groups.

Eight hundred and thirty-one out of 849 patients responded to Glucantime (intra-lesional/systemic) alone or in combination with cryotherapy. In total, about 97.8% of the patients responded to Glucantime alone or in combination. Glucantime was well tolerated and only a few patients showed mild signs such as nausea, vomiting, malaise, and local inflammation. No serious side effects were recorded, but the treatment was interrupted in three patients because of impaired renal function.

Pearson Chi-square analysis showed a significant difference between the patients with single lesions and those with multiple lesions with regard to seasonal jobs (summer workers) ($p = 0.016$). The age group 1–15 years ($p = 0.018$) and the season of onset of winter ($p = 0.015$) were significantly different between patients with single lesions and those with multiple lesions. Univariate logistic regression analysis showed that the risk of developing multiple lesions was increased 1.5-fold in patients with seasonal jobs (summer workers) (Table 1) ($p = 0.023$; OR 1.516; 95%

Table 1
Results of regression analysis in cutaneous leishmaniasis (CL) groups categorized based on the presence of single or multiple lesions

Characteristics	CL groups: number of patients (%)			*p*-Value	OR	95% CI
	Single lesion	Multiple lesions	Total			
Gender						
Female[a]	204 (43.6%)	142 (37.3%)	346 (40.8%)	-	-	-
Male	264 (56.4%)	239 (62.7%)	503 (59.2%)	0.063	1.301	0.986–1.715
Nationality						
Iranian[a]	417 (89.1%)	332 (87.1%)	749 (88.2%)	-	-	-
Afghan	51 (10.9%)	49 (12.9%)	100 (11.8%)	0.378	0.829	0.546–1.258
Job						
Housekeeper[a]	141 (30.1%)	102 (26.8%)	243 (28.6%)	-	-	-
Farmer	61 (13.0%)	50 (13.1%)	111 (13.1%)	0.588	1.133	0.721–1.781
Rancher	75 (16.0%)	65 (17.1%)	140 (16.5%)	0.398	1.198	0.788–1.821
Student	77 (16.5%)	39 (10.2%)	116 (13.7%)	0.130	0.700	0.441–1.111
Seasonal worker	114 (24.4%)	125 (32.8%)	239 (28.2%)	0.023	1.516	1.058–2.172
Travel history						
No[a]	380 (81.2%)	303 (79.5%)	683 (80.4%)	-	-	-
Yes	88 (18.8%)	78 (20.5%)	166 (19.6%)	0.542	1.112	0.791–1.562
History of diabetes						
No[a]	394 (84.2%)	307 (80.6%)	701 (82.6%)	-	-	-
Yes	74 (15.8%)	74 (19.4%)	148 (17.4%)	0.168	1.283	0.900–1.830
Season of onset						
Spring[a]	32 (6.8%)	31 (8.1%)	63 (7.4%)	-	-	-
Summer	78 (16.7%)	66 (17.3%)	144 (17.0%)	0.655	0.873	0.483–1.580
Autumn	301 (64.3%)	262 (68.8%)	563 (66.3%)	0.687	0.899	0.534–1.513
Winter	57 (12.2%)	22 (5.8%)	79 (9.3%)	0.010	0.398	0.198–0.800
Area						
Ghanavat[a]	247 (52.8%)	185 (48.6%)	432 (50.9%)	-	-	-
Ghomrood	221 (47.2%)	196 (51.4%)	417 (49.1%)	0.221	0.845	0.644–1.107
Age, years						
1–15[a]	129 (27.6%)	83 (21.8%)	212 (25.0%)	-	-	-
16–25	107 (22.9%)	106 (27.8%)	213 (25.1%)	0.691	1.118	0.643–1.944
26–35	87 (18.6%)	70 (18.4%)	157 (18.5%)	0.744	0.908	0.510–1.617
36–45	63 (13.5%)	33 (8.7%)	96 (11.3%)	0.108	0.591	0.311–1.123
46–55	37 (7.9%)	37 (9.7%)	74 (8.7%)	0.720	1.129	0.581–2.194
≥56	45 (9.6%)	52 (13.6%)	97 (11.4%)	0.406	1.305	0.697–2.442
Type of treatment						
Local Glucantime	378 (80.8%)	172 (45.1%)	450 (53.0%)			
Systemic Glucantime	31 (6.6%)	160 (42.0%)	291 (34.3%)			
Local + cryotherapy	51 (10.9%)	46 (12.1%)	97 (11.4%)			
Cryotherapy	8 (1.7%)	3 (0.8%)	11 (1.3%)			
Lesion location						
Multiple locations on the body[a]	29 (6.2%)	60 (15.7%)	89 (10.5%)	-	-	-
Hand or foot	383 (81.8%)	285 (74.8%)	668 (78.7%)	0.071	1.360	0.925–1.575
Face	56 (12.0%)	36 (9.5%)	92 (10.8%)	0.060	1.311	0.969–1.572
Total	468 (55.1%)	381 (44.9%)	849 (100%)			

OR, odds ratio; CI, confidence interval.
[a] Reference group.

confidence interval (CI) 1.058–2.172). The risk of developing multiple lesions was decreased in patients who were affected in winter (p = 0.010; OR 0.398; 95% CI 0.198–0.800).

Regarding the lesion size, Pearson Chi-square analysis showed a significant difference between patients with small-sized lesions and those with large-sized lesions concerning seasonal jobs (summer workers) (p = 0.029), a history of diabetes (p = 0.021), and the age group 1–25 years (p = 0.014). Univariate logistic regression analysis showed that the risk of developing large-sized lesions was increased 1.56-fold in patients with a history of diabetes (Table 2) (p = 0.017; OR 1.562; 95% CI 1.084–2.251). Using backward-Wald analysis, chronic diseases remained an independent risk factor for the development of large-sized lesions (p = 0.018; OR 1.556; 95% CI 1.079–2.243). The risk of developing large-sized lesions was decreased in patients with seasonal jobs (summer workers) (p = 0.005; OR 0.570; 95% CI 0.385–0.843). The likelihood of developing large-sized lesions was increased 2.5–3.5-fold in patients aged ≥26 years.

4. Discussion

Qom Province is among the high risk regions for acquiring CL due to its proximity to other CL endemic foci such as Isfahan, Kashan, and Semnan at the center of Iran. The special religious status of Qom, with high numbers of pilgrims and immigrants, gives it a crucial role in the prevalence of leishmaniasis. Every year, many cases of CL are reported from Markazi District, including Ghomrood and Ghanavat. Detailed data of the geographical distribution of CL cases among the villages and possible reservoirs of disease are under review for publication: the same authors found that in Ghomrood region, among 104 villages, most of the cases were from Kooh-sefid with 27.8% of all confirmed cases, and in Ghanavat region, among 27 villages, most of the cases were from Ghanavat center with 23.5% of all confirmed cases during 2006–2011. Some rodents were identified as the possible vectors.

The average incidence rate of the disease in the region is close to the average incidence rate of 20–40 per 100 000 people estimated for the country.[5] Looking at neighboring provinces, a study of the prevalence of CL in 117 children in three areas in the southeast of Kashan, showed a prevalence of 7.2%.[15] The prevalence of CL in Orzoieh District, Kerman Province was found to be 4.7%,[16] and in 3024 individuals of three villages around Ardakan, Yazd Province, was found to be 30.4% and 24.6% for scars and ulcers, respectively.[6] In the city of Yazd, examination of 3176 primary school children showed a rate of 0.7% for scars and 0.2% for active lesions of ACL,

Table 2
Results of regression analysis in cutaneous leishmaniasis (CL) groups categorized based on lesion size

Characteristics	CL groups: number of patients (%)			*p*-Value	OR	95% CI
	Lesion size ≤1 cm	Lesion size >1 cm	Total			
Gender						
Female[a]	230 (39.9%)	116 (42.5%)	346 (40.8%)	-	-	-
Male	346 (60.1%)	157 (57.5%)	503 (59.3%)	0.478	0.9	0.672–1.205
Nationality						
Iranian[a]	516 (89.6%)	233 (85.3%)	749 (88.2%)	-	-	-
Afghan	60 (10.4%)	40 (14.7%)	100 (11.8%)	0.075	1.476	0.961–2.267
Job						
Housekeeper[a]	153 (26.6%)	90 (33.0%)	243 (28.6%)	-	-	-
Farmer	68 (11.8%)	43 (15.7%)	111 (13.1%)	0.759	1.075	0.677–1.707
Rancher	94 (16.3%)	46 (16.8%)	140 (16.5%)	0.411	0.832	0.537–1.290
Student	82 (14.2%)	34 (12.5%)	116 (13.7%)	0.151	0.705	0.437–1.136
Seasonal worker	179 (31.1%)	60 (22.0%)	239 (28.2%)	0.005	0.570	0.385–0.843
Travel history						
No[a]	465 (80.7%)	218 (79.8%)	683 (80.4%)	-	-	-
Yes	111 (19.3%)	55 (20.2%)	166 (19.6%)	0.764	1.057	0.737–1.516
History of diabetes						
No[a]	488 (84.7%)	213 (78.0%)	701 (82.6%)	-	-	-
Yes	88 (15.3%)	60 (22.0%)	148 (17.4%)	0.017	1.562	1.084–2.251
Season of onset						
Spring[a]	42 (7.3%)	21 (7.7%)	63 (7.4%)	-	-	-
Summer	97 (16.8%)	47 (17.2%)	144 (17.0%)	0.922	0.969	0.517–1.818
Autumn	381 (66.2%)	182 (66.7%)	563 (66.3%)	0.871	0.955	0.550–1.661
Winter	56 (9.7%)	23 (8.4%)	79 (9.3%)	0.589	0.821	0.402–1.678
Area						
Ghanavat[a]	286 (49.7%)	131 (48.0%)	417 (49.1%)	-	-	-
Ghomrood	290 (50.3%)	142 (52.0%)	432 (50.9%)	0.650	1.069	0.801–1.426
Age, years						
1–15[a]	155 (26.9%)	57 (20.9%)	212 (25.0%)	-	-	-
16–25	154 (26.7%)	59 (21.6%)	213 (25.1%)	0.074	1.916	0.938–3.910
26–35	105 (18.2%)	52 (19.0%)	157 (18.5%)	0.015	2.476	1.196–5.126
36–45	56 (9.7%)	40 (14.7%)	96 (11.3%)	0.001	3.571	1.664–7.667
46–55	45 (7.8%)	29 (10.6%)	74 (8.7%)	0.004	3.222	1.451–7.157
≥56	61 (10.6%)	36 (13.2%)	97 (11.4%)	0.006	2.951	1.370–6.356
Type of treatment						
Local Glucantime	320 (55.6%)	130 (47.6%)	450 (53.0%)			
Systemic Glucantime	190 (33.0%)	101 (37.0%)	291 (34.3%)			
Local + cryotherapy	61 (10.5%)	36 (13.2%)	97 (11.4%)			
Cryotherapy	5 (0.9%)	6 (2.2%)	11 (1.3%)			
Lesion location						
Multiple locations on the body[a]	62 (10.8%)	27 (9.9%)	89 (10.5%)	-	-	-
Hand or foot	451 (78.3%)	217 (79.5%)	668 (78.7%)	0.684	1.105	0.684–1.786
Face	63 (10.9%)	29 (10.6%)	92 (10.8%)	0.863	1.057	0.563–1.986
Total	576 (67.8%)	273 (32.2%)	849 (100%)			

OR, odds ratio; CI, confidence interval.
[a] Reference group.

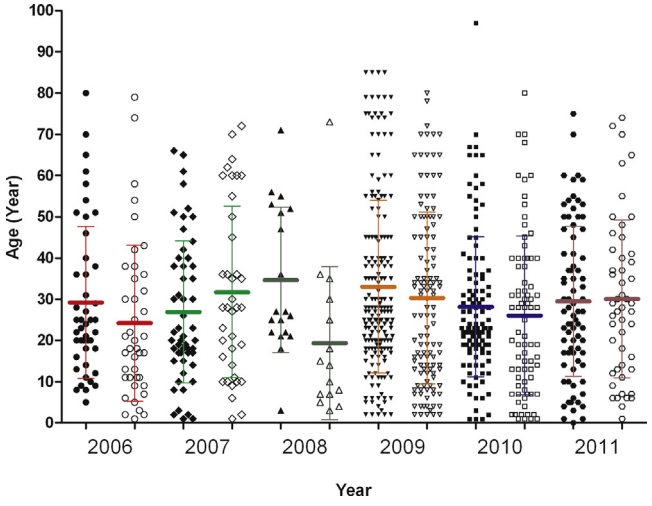

Figure 2. Age distribution of confirmed CL cases in Qom Province identified during 2006–2011. Filled symbols represent male patients; open symbols represent female patients. Mean (horizontal line) with ±SD (whiskers) for each group are shown.

and among 139 households the rate was 13.5% for scars and 0.5% for ulcers.[17]

Both ACL and ZCL are reported from countries neighboring Iran, including Afghanistan, Pakistan, Iraq, Saudi Arabia, and Turkey.[18–25] In the southeastern and Mediterranean regions of Turkey, hundreds of CL cases due to *L. tropica* are reported each year.[18] ZCL caused by *L. major* is a growing public health problem and is endemic in many parts of Saudi Arabia.[19] Both ACL and ZCL are major public health problems in all four provinces of Pakistan[20] and are widespread in Afghanistan.[21–24] In Afghanistan, the majority of leishmaniasis cases are caused by *L. tropica*.[21] WHO estimates more than 200 000 ACL cases in Kabul which is the most important endemic region of ACL in the world.[22] ZCL due to *L. major* is common in rural areas of north and north-west Afghanistan, as in Mazar-e Sharif and Balkh.[23,24] In the present population, a few cases of leishmaniasis were imported into the regions by people who had traveled to Afghanistan before the lesions appeared. Afghans might have a role in parasite maintenance and disease transmission in the area. However, a significant number of cases were Iranian with no history of travel to regions endemic for CL, so local transmission is occurring in these areas. Isfahan Province,

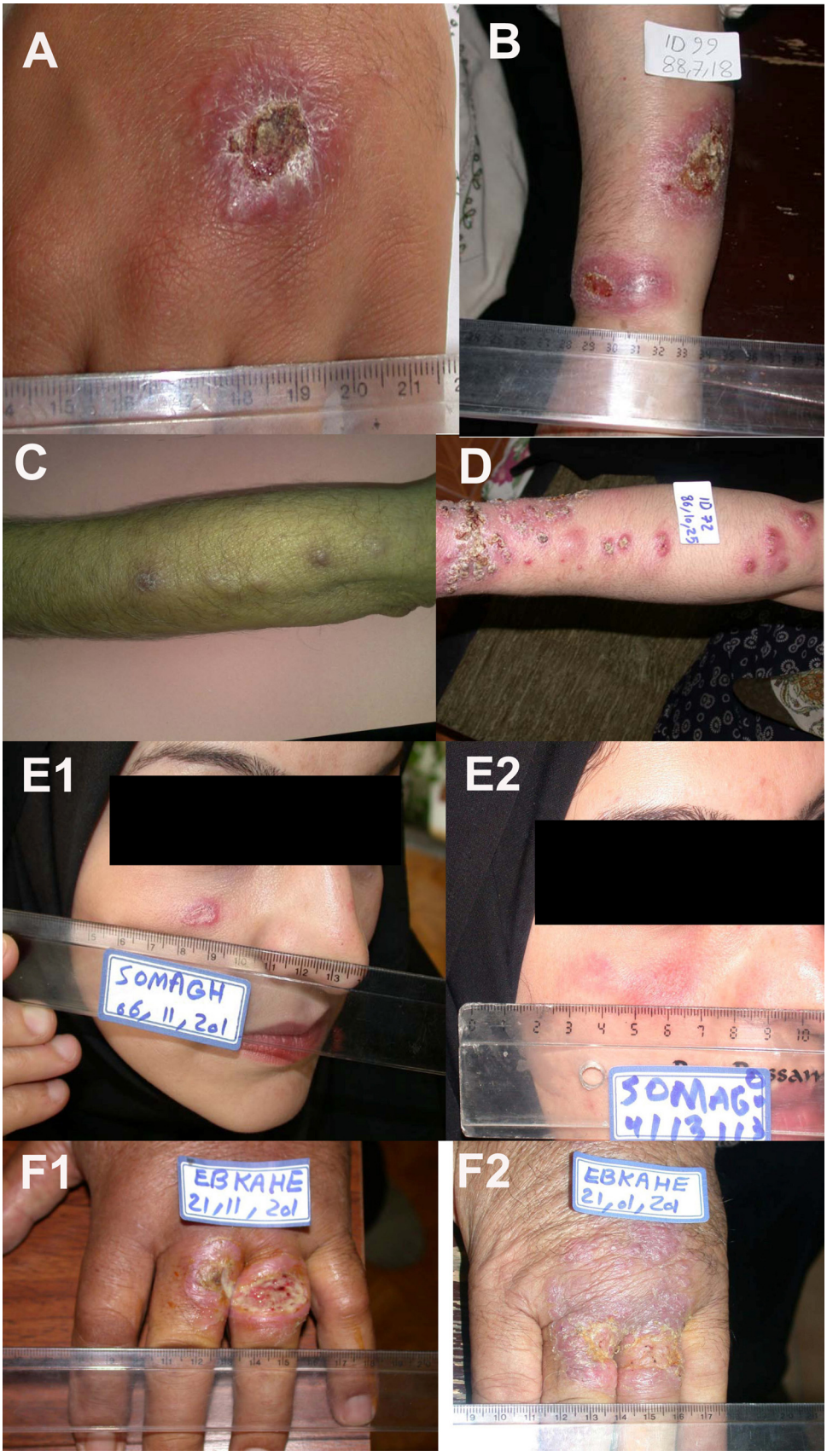

Figure 3. Representative pictures of skin lesions observed in cutaneous leishmaniasis patients. (A) single lesion; (B) multiple lesions; (C) and (D) multiple lesions with sporotrichoid extension along lymphatic vessels; (E) small-sized lesion (≤1 cm) before and after treatment; (F) large-sized lesions (>1 cm) before and after treatment.

which is the most important ZCL endemic focus in central Iran, is a neighboring province of Qom, and transmission of infection from Isfahan to Qom is possible. During the study period of 2006–2011, the trend in CL incidence showed no sudden variations in the areas under study, except for an outbreak in 2009. While changes in the incidence of CL hardly exceeded 20% of the mean rate during 2006–2008, in 2009 an outbreak of leishmaniasis occurred in the regions, in which the number of CL cases increased to more than 10 times and then decreased steadily to previous levels. Since surveillance strategies and diagnostic procedures were not changed or improved in that year, this increase in the report of confirmed CL cases was most likely due, not to better detection, but to a greater occurrence of disease. Possible explanations for this outbreak might be: the spread of disease from neighboring provinces like Isfahan, importing new cases through immigrants, changes in climate favoring an increase in vector/reservoir populations, etc.

Of the total 849 patients, the male/female ratio was >1 and the frequency of male patients with CL was higher than that of female patients. Other endemic regions of the country have the same pattern, such as Damghan[26] and Kashan.[15] Conversely, in Kerman Province, CL was found to be distributed more significantly in females (5.2%) than males (4.3%).[16] Some studies have shown an equal distribution of ZCL infection among the two sexes. In Baft[27] and in villages of Ardakan,[6] the sex distribution was found to be approximately equal. In the ACL-endemic city of Kabul, Afghanistan, a house-to-house investigation showed that females were at significantly higher risk of developing leishmaniasis lesions or scars than males.[28]

The transmission of leishmaniasis is highly dependent on climate conditions and on the ecology of vector/reservoir hosts.[12] Each species of sand fly has an annual cycle, and the highest transmission occurs at the end of this cycle.[29] The seasonal distribution of the disease shows that the highest rate of infection is in the autumn and the lowest in the spring and winter. The lower incidence of CL in winter was found to coincide with the lower risk of developing multiple lesions (OR 0.398). In this area, the period during which Phlebotomus sand flies are most active begins in the second month of spring (April) and lasts through the second month of autumn (November).[29] In ZCL, the average incubation period from mosquito bite to nodule development in the skin is around 6 months. Taking into consideration the peak period of sand fly activity and the incubation period for lesion development, an increase in CL cases is expected in the autumn. A similar seasonal pattern has been reported from other leishmaniasis endemic parts of the country. In Sabzevar (73.1%), Damghan (76.3%), and Kermanshah, the highest percentages of patients with ZCL were reported in autumn and the lowest in winter.[26,30–32] Faulde et al. described the seasonality of ZCL in Mazar-e Sharif, Afghanistan, where the maximum numbers of CL cases were recorded in September and October.[23] Conversely, in Iraq, in a survey among 107 CL cases presenting to the dermatology clinic of a general hospital in Alhaweja,[25] the highest number was recorded in February (32.1%) and the lowest number in April (3.37%). Weather conditions directly influence the fluctuation and breeding of sand flies[12] and the warm climate of Iraq should be considered when interpreting this discrepancy in seasonality of ZCL.

Most of the affected patients in the current study comprised housewives. It should be considered that normally all housekeepers are women, so the men were divided into other occupations. In reports from Damghan and Kalaleh, most of the CL patients were housewives.[31,33] In contrast, in Hamadan Province 85.7% of CL patients were workers who were job seekers migrating from other parts of the country.[34,35] People with certain occupations may be exposed more than others to sand fly bites because they are outdoors during the time of sand fly activity. It was observed that the risk of developing multiple lesions increased 1.5-fold in summer workers who work intermittently, especially as construction workers, in some months of the year (seasonal jobs); however, at the same time the risk of developing large-sized lesions was decreased in these patients.

During the period of this study, from 2006 to 2011, the age distribution of CL cases was relatively stable and about 50% of the patients were in the 1–25 years age range; however, all age groups were at risk of the disease. In known ZCL endemic regions of Iran, the highest risk group in the studied populations is often children aged less than 15 years. This was evidenced in Kerman Province (<10 years of age, with a rate of 6.3%, and >50 years, the lowest rate; study in 18 300 inhabitants aged 1–73 years),[16] Fars Province (≤10 years of age, with a rate of 70%; passive detection of any patient with skin lesion),[36] Shiraz (0–9 years of age, with a rate of 14.2%; age groups: <10 and ≥10 years),[7] Mirjaveh (≤10 years of age; in 3100 patients who were randomly selected and surveyed),[6] Hormozgan Province (10–14 years of age; in 1392 patients, age groups: 0–4, 5–9, 10–14, 15–19, 20–24, and >25 years),[37] Ardakan (10–14 years of age),[5] Ardestan (10–14 years of age; age range: 1–>55 years),[38] Damghan (10–19 years; in 465 patients, age range: 1–88 years),[31] and from part of Isfahan (most under 1 year of age).[39] Studies on CL associated with different age groups in Afghanistan have shown that ACL is more commonly seen in the younger age groups and that ZCL affects all age groups.[21,28,40] In Pakistan, in a study of 48 refugee camps (21 046 persons) and 19 neighboring villages (7305 persons) it was reported that younger age increased the risk of ACL lesion and ACL scar development.[41] In a case–control study in South Anatolia, Turkey, risk factors for outbreaks of CL due to *Leishmania infantum* were evaluated and it was concluded that people aged from 5 to 19 years were the highest risk group among the 282 persons studied.[42] Although the highest numbers of CL patients were aged under 25 years, the likelihood of developing large-sized lesions was increased 2.5–3.5-fold in patients aged ≥26 years. The effect of age might actually be the effect of immune reactions on the skin lesion size. In leishmaniasis it is known that the different outcomes of the disease are a function of both the parasite and immune response of the host.[43–46]

In this study, it was observed that most of the lesions appeared on the extremities, hands and feet, and this pattern of lesion development on the limbs is common in ZCL. It was reported that 45% of ZCL patients in Yazd,[47] 29.8% in Gonbad-e-Qabus,[48] and 35% in Orzoieh District[16] developed lesions mostly on the hands. There are studies that have indicated the development of CL lesions mainly on the feet (65.3% in Hormozgan Province)[37] and on the face (50.4% in Fars Province, 47.0% in Kashan).[15,36]

Over a half of the CL patients in the present study had one skin lesion. Likewise, most ZCL patients from other endemic regions of the country have presented a single lesion, as reported from Sabzevar (38.9%),[30] Damghan (42.7%),[26] Kermanshah (54.5%),[32] Khatam (55%),[47] and Bafgh (66.6%).[49] In contrast, in Gonbad-e-Qabus only 2.9% of the patients displayed one lesion and the majority showed more than three lesions,[48] similar to Orzoieh District, Kerman Province, where 39% of CL patients had multiple lesions.[16] In ZCL due to *L. major*, multiple lesions might be seen;[50] in the present study about 20% of CL patients had more than three lesions. Sand flies usually have a discontinuous blood-sucking habit and may sting several times at every attack and cause the development of several lesions on the skin.[12] In one report from Iraq, a total of 107 CL cases were diagnosed – 58% of them had multiple lesions, while 42% had a single lesion.[25] Patients with ACL due to *L. tropica* usually present with one lesion.[50]

Standard antimonial treatments are chosen for every confirmed CL patient in accordance with the guidelines of the Ministry of Health. Since most patients had a single lesion, usually on the hand

or foot, with mean size of 1.41 ± 0.74 cm, the local intra-lesional usage of Glucantime was the best choice of therapy, and resulted in a good response.

The present study shows that CL due to *L. major* is endemic in suburban areas of Qom Province. PCR revealed *L. major* to be the causative agent, but in order to identify all epidemiological aspects of ZCL, a study on the possible vectors and reservoirs of the disease and their role in the transmission cycle is recommended. Since leishmaniasis caused by *L. major* is typically zoonotic, control measures should be focused on the rodents as the primary reservoir hosts of ZCL, and on the *Phlebotomus papatasi* population as the main vector of ZCL in Iran. *P. papatasi* is probably active throughout the year and humans with active lesions may act as a secondary reservoir, so the detection and treatment of patients with active lesions should be considered. In the 2009 outbreak in the region, different control measures were implemented by the health system, including field control of rodents and active case finding, which seemed to reduce the increasing trend in CL dissemination. Based on these data, awareness should be raised in the high-risk populations comprising people with diabetes, young adults (<25 years old), and those who work outdoors during the summer. With regard to the treatment approach, the administration of Glucantime has been shown to be effective for most skin lesions, however a combination with other treatment methods is recommended for multiple/large CL lesions.

Acknowledgements

We would like to thank Dr Ali Khamesipour for kind help in providing the pictures. This study was supported by Qom University of Medical Sciences. The University had no role in the study design, collection/analysis/interpretation of data, writing of the manuscript, or the decision to submit the manuscript for publication.

Conflict of interest: The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2013.07.003.

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