Clinico-Epidemiologic Study of Cutaneous Leishmaniasis in Bikaner, Rajasthan, India

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Abstract. Cutaneous leishmaniasis is endemic to the Thar Desert of Rajasthan, Bikaner, India. The present study describes clinico-epidemiological data of all cases of cutaneous leishmaniasis CL in this region during 2001–2011. A total of 1,379 patients with 2,730 lesions were reported during the study period. Ages of patients ranged from 3 months to 86 years, and there was a predominance of infections in males. Most patients were from urban areas and lower middle socioeconomic groups. Lesions were dry, ulcerated nodules or plaques of different sizes commonly over face and upper limb. Skin smears were positive for parasites in 958 (69.5%) patients, and the remaining 45.8% (193 of 421) patients were positive by skin biopsy. Histopathologic analysis of the skin showed mixed granulomas consisting of macrophages, lymphocytes, epithelioid, and plasma cells. Species identification was conducted for 45 randomly selected patients by polymerase chain reaction, the infective species was *Leishmania tropica*. Most patients were treated with intra-lesional injections of sodium stibogluconate.

INTRODUCTION

Leishmaniasis is a vector-borne parasitic disease that is endemic to 88 countries. Global prevalence is approximately 12 million cases, with 1–2 million new cases each year throughout Africa, Asia, Europe, and North and South America. *Leishmania* causes different type of clinical manifestations ranging from cutaneous leishmaniasis and mucosal leishmaniasis to potentially fatal visceral leishmaniasis.^{1,2} According to the World Health Organization, the annual incidence of cutaneous and visceral leishmaniasis is 1–1.5 million and 500,000 cases, respectively.^{3,4}

Cutaneous leishmaniasis in the Old World is caused by *Leishmania tropica*, *L. major*, and *L. aethiopica*. New World leishmaniasis is caused by *L. Mexicana*, *L. amazonensis*, and *L. braziliensis*.³ Clinical manifestations range from spontaneously healing nodulo-ulcerative lesions to mutilating cutaneous or mucosal ulcers.¹ Rarely, diffuse cutaneous leishmaniasis occurs, particularly in immunocompromised patients.⁵

In India, cutaneous leishmaniasis is known to be endemic to the Thar Desert of Bikaner in Rajasthan State, which is located in northwestern India at an elevation of approximately 227 meters above mean sea level (Figure 1). Bikaner is situated in the middle of the Thar Desert. The area of the city is approximately 37.95 km²; the interior of the city is surrounded by a stone wall and is overcrowded. Most of the time climate of Bikaner is hot and dry with little rainfall (265 mm/year).⁶

The first evidence for cutaneous leishmaniasis in Bikaner was based on clinico-epidemiologic analysis of cases from a large-scale outbreak that occurred during 1971–1973.^{7–10} Agarwal and Chadda¹¹ in 1981 and Vyas and others¹² in 1994 reported the epidemiology of cutaneous leishmaniasis in Bikaner. In 2007, Kumar and others¹³ identified *L. tropica* as the causative species of cutaneous leishmaniasis in Bikaner in rural and urban areas. This disease, once restricted to the Thar Desert in India, is now showing a trend to spread to previously non-endemic areas, such as Himachal Pradesh. In 2005, Sharma

and others¹⁴ reported a new endemic zone of cutaneous leishmaniasis in the Subalpine Valley, which is located along the Satluj River in the Kinnaur District of Himachal Pradesh.

Cutaneous leishmaniasis is an emerging public health problem in certain regions of India, particularly in the Bikaner Region of the State of Rajasthan. The current study was conducted because no data are available on the clinico-epidemiological profile of this disease in this region after 1994. Epidemiologic information for cutaneous leishmaniasis in this region is of paramount importance for implementing appropriate control strategies to prevent the spread of this disease to other regions.

MATERIALS AND METHODS

The present study included persons with clinically diagnosed consecutive cases of cutaneous leishmaniasis who came to the Dermatology Department of Sandar Patel Medical College in Bikaner, Rajasthan, India, during January 2001–December 2011. Demographic data were recorded on printed proforma sheets and included age, sex, occupation, religion, habitat, area of residence, family history, history of keeping domestic animals, and socioeconomic status. Clinical details were also recorded and included number, size, site, and type of lesions; duration of disease onset; confirmative investigative modality; treatment given; and follow-up. Lesions were classified as early (duration less < 6 months) and late lesions (duration ≥ 6 months).

Lesions were also classified as papules, nodules, ulcerative nodules, plaques, and ulcerated plaques. Clinically diagnosed cases were confirmed by demonstration of parasites in Giemsa-stained skin slit smears from lesions. Skin biopsies were performed on patients who were smear negative. Parasite species was identified for some randomly selected patients and was determined by internal transcribed spacer 1 polymerase chain reaction–restriction fragment length polymorphism–(PCR) and kinetoplast DNA PCR at a molecular biology laboratory of the Indian Council of Medical Research in New Delhi. Relevant tests for individual patients were conducted before starting treatment and included routine hemograms, urine analysis, liver function tests, and renal function tests.

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FIGURE 1. Map of India showing Bikaner, Rajasthan (dark yellow). Small dark red dot shows Bikaner. **Inset:** map of India showing the State of Rajasthan (pale yellow) (http://en.wikipedia.org/wiki/File:Map_rajasthan_dist_Bikaner.png).

The following treatments were administered on case-by-case basis. Most patients were given intralesional injections of sodium stibogluconate (SSG, Pentostam; Albert David Ltd., Kolkata, India) at dose of 0.5 mL/cm² (100 mg/mL solution) of lesion once or twice a week for 5–7 injections. Some patients were treated with a single application of topical radiofrequency heat therapy (RFHT), which was introduced in 2009. The controlled heat was applied locally at 50°C for 30–60 seconds under local anesthesia, depending on the thickness of the lesion, with a localized current field radiofrequency generator (ThermoMed 1.8 device; Thermosurgery Technologies, Inc., Phoenix, AZ). Children and patients with multiple ulcers were treated with oral rifampicin at a dosage of 20 mg/kg of body weight/day for 4–6 weeks. Some patients who did not respond to intralesional SSG injections were treated with cryotherapy, oral dapsone, oral ketoconazole, topical paramomycin, or other antibiotics.

Complete cure was defined as total re-epithelization of the lesion with or without scarring and a negative skin smear. Data were tabulated and analyzed by using descriptive statistics in a Microsoft (Redmond, WA) Excel spreadsheet (2010). Records were analyzed for demographic profile, clinical data, method of diagnosis, and outcome of treatment given.

RESULTS

A total of 1,379 clinically diagnosed patients with cutaneous leishmaniasis with 2,730 skin lesions were reported during

the study period. The incidence of cutaneous leishmaniasis increased every year up to 2009, after which there was a decrease in 2010 and 2011. The highest number of patients was seen in 2009. Cases of cutaneous leishmaniasis during winter (November–April) were higher compared with those during summer (May–October). The lowest number of cases for a month was reported in October (58 cases), and the maximum number of cases was reported in January (181 cases).

Epidemiologic data showed that cutaneous leishmaniasis was prevalent in persons of all ages; the youngest patient was 3 months of age and the oldest was 86 years (mean age = 23 years) (Table 1). The highest proportion of patients ($n = 379$, 27%) were 11–20 years of age. The ratio of male to female patients was comparable. In persons < 20 years of age, male patients were affected more frequently. In persons 21–50 years of age, infection prevalence was higher in female patients. Most patients in our study were from old-walled localities, including Kotegate (552 patients, 40%) and Rani Bazar (441 patients, 32%). These regions are heavily populated areas and have poor sanitary conditions.

Students and housewives of the socioeconomically lower middle class population were most commonly affected. A positive family history was seen for 173 patients (13%). A total of 139 patients (10%) with a history of keeping domestic animals (including dogs, cows, and buffaloes) on their residential premises were positive for cutaneous leishmaniasis. Most patients who kept animals were from rural areas. The infection ratio between urban and rural populations was 4:1.

TABLE 1

Epidemiologic characteristics of patients with cutaneous leishmaniasis, Bikaner, Rajasthan, India

Characteristic	No. (%) patients
Age, years	
0–20	732 (53)
21–40	430 (31)
41–60	160 (12)
> 60	57 (4)
Sex	
M	764 (55)
F	615 (45)
Regional distribution	
Kotegate	552 (40)
Rani Bazar	441 (32)
Peripheral colonies	124 (9)
Rural area	262 (19)
Occupation	
Farmer	41 (3)
Labor	187 (14)
Serviceman	185 (13)
Housewife	323 (23)
Student	643 (47)
Economic status	
Higher	4 (0.3)
Middle	1,142 (83.8)
Poor	233 (16.9)
Habitat	
Rural	275 (20)
Urban	1,104 (80)

The time between the appearance of a cutaneous leishmaniasis lesion and the first diagnosis ranged from 2 days to 38 months. The average time when the patient first visited our clinic for treatment was 16.5 weeks after lesion onset,

and 1,168 patients came to our clinic between 4 and 24 weeks after lesion onset. Early and late lesions were observed in 1,191 (86%) and 188 (14%) patients, respectively.

A total of 2,730 lesions were present in 1,379 patients. Most patients had one lesion (805 patients, 58%), whereas two lesions were present in 275 patients (20%), three in 118 patients (9%), four in 71 patients (5%), five in 35 patients (3%), and only five patients had more than 10 lesions. Lesions varied in size from a few millimeters to 12 cm in diameter. A total of 1,938 (71%) of 2,730 lesions ranged in size between 0.5 cm and 3.0 cm, and only 48 lesions were > 5.0 cm. Most lesions were located on exposed parts of the body such as the face (33%), upper extremities (41%), and lower extremities (20%). The trunk was involved in only 2% of patients, and none of the patients had mucosal lesions. Of 2,730 lesions, nodular (26%) and ulcerated plaques (35%) were the most common (Figure 2). Papules (3%), ulcerated nodules (13%) and plaques (23%) comprised the remainder of the lesions.

Clinical diagnosis was confirmed in 958 (69%) patients by Giemsa-stained skin slit smears, which showed amastigotes of *Leishmania*. Skin biopsies were performed in 421 patients who were smear negative. Biopsy specimens were positive for *Leishmania* in 193 (45.8%) of 421 patients. Histopathologic analysis of lesions showed granulomatous inflammation comprised of parasitized macrophages, lymphocytes, epithelioid, and plasma cells. Internal transcribed spacer region 1 restriction fragment length polymorphism–PCR and kinetoplast DNA PCR were performed for 45 randomly selected patients and all patients were positive for *L. tropica*.

Many treatments, including intralesional SSG, oral rifampicin, thermotherapy, as well as cryotherapy were used on

FIGURE 2. Morphology of skin lesions in patients with cutaneous leishmaniasis from Bikaner, India. **A**, Nodule; **B**, ulcerated nodule; **C**, ulcerated plaque; **D**, crusted plaque.

TABLE 2

Treatment modalities and their complete cure rates in patients with cutaneous leishmaniasis, Bikaner, Rajasthan, India*

Treatment modality	Complete cure rate (%)						
	6 wk	8 wk	10 wk	12 wk	16 wk	20 wk	24 wk
Intralesional SSG twice a week (361 patients)	20	47	68	87	92	96	96
Intralesional SSG once a week (297 patients)	12	36	62	82	89	92	92
Radiofrequency heat therapy (185 patients)	21	39	46	82	92	96	98
Oral rifampicin (96 patients)	84	–	–	–	–	–	–
Cryotherapy (19 patients)	20	40	59	65	65	72	84

*SSG = sodium stibogluconate.

case-by-case basis, and complete cure rates in response to these treatments are shown in Table 2. Of 1,005 patients treated with intralesional SSG, 609 patients were given two SSG injections weekly and 396 patients were given injections once a week. Only 361 patients in the twice a week group and 297 patients in the once a week group completed the study; thus, 347 patients were lost to follow-up. Of 200 patients treated with RFHT, 15 patients were lost to follow-up. A total of 56% patients in RFHT group and 54% patients in SSG group came for follow-up at 18 months post-treatment and were found to be disease free. Forty three of 139 patients in the rifampicin group were lost to follow-up. Treatment with N₂O cryosurgery showed cure rates of 85% at 12 weeks. Ten of 10 patients who were treated with paramomycin topically twice a day for 10 days were cured completely after 12 weeks. Oral dapsone and ketoconazole were given only in refractory cases.

DISCUSSION

Cutaneous leishmaniasis is an important public health problem in developing countries, such as India. Bikaner is a known endemic area for this disease because of its dry and hot environment, which is favorable for sand fly breeding.⁶ The present study showed increasing infection trends from 2001 through 2009 and a sudden decrease in 2010 and 2011. The cause of this decrease is not well understood, although improved housing and/or treatment of some patients by private clinics in the city could be responsible for the decrease in cases of cutaneous leishmaniasis seen at our clinic.

A study by Sharma and others¹⁴ reported a higher incidence of cutaneous leishmaniasis during the summer in the northern state of Himachal Pradesh in India. However, in the present study, we found that an increased incidence of cutaneous leishmaniasis during winter. This finding could be caused by the fact many persons in this region sleep in the open during the summer months and thus are exposed to the bites of infected sand flies, which are most abundant during May–September. Lesions might develop in these persons after 2–3 months. These lesions might enlarge and ulcerate in approximately six months,¹⁵ when winter is at its peak. Sharma and others¹⁴ also identified *L. donovani* and *L. tropica* as causative agents of cutaneous leishmaniasis in patients from Himachal Pradesh. However, in the present study, all isolates tested were *L. tropica*. These data suggest that the clinic-epidemiologic profile of cutaneous leishmaniasis in Rajasthan and Himachal Pradesh could be different.

Comparison of clinico-epidemiological data from different studies is shown in Table 3. In the present study, the highest numbers of patients (27%) were 11–20 years of age, which is similar to that reported by Srivastava and others.⁶ In contrast, Sharma and others¹⁴ found a higher incidence of cutaneous leishmaniasis in persons 21–30 years of age. As age increased, the number of patients decreased; this finding may be caused by acquired immunity.

In the present study, the ratio of males to females was 1.24:1. This finding is similar to those in studies conducted in Bikaner.^{6,12} In contrast, Yemisen and others¹⁶ and Gurel and others¹⁷ in Turkey and Talari and others¹⁸ in Iran reported a higher incidence of females. Students and housewives were most commonly effected in this study, which is similar to that in the study by van der Meide and others.¹⁵ A positive family history was seen in 173 (12.5%) patients in the present study, and Yemisen and others¹⁶ and Gurel and others¹⁷ reported values of 9.6% and 18.4%, respectively. In the present study, the urban population was affected more than the rural population. This result is similar to those of earlier studies in the same location^{9,11} and may be caused by overcrowding and poor sanitary conditions.

The mean average time when patient first came to a clinic was 16.4 weeks in our study, and Sharma and others¹⁴ reported a value of 30 weeks in Himachal Pradesh in India. In the present study, early and late lesions were observed in 86% and 14% of patients, respectively; Sharma and others¹⁴

TABLE 3
Comparison of previous and present studies of patients with cutaneous leishmaniasis

Reference (study location)	Duration of study	Sex ratio, M/F	Most prevalent age group, years	Family history %	Mean time of admission	Early/late lesions	Mean no. lesions	Most involved site	Most common lesion type
Gurel and others ¹⁷ (Turkey)	1997–2000	49/51	5–9	18.4	14 weeks	90.8/9.2	1.6	Face	Ulcerated
Yemisen and others ¹⁶ (Turkey)	2001–2008	46/54	10–19	9.6	15.8 weeks	94.2/5.8	1.8	Face	Nodulo-ulcerative
Srivastava and others ⁶ (Bikaner, India)	1981–1982	59/40	10–19	–	8.0 weeks	–	1.4	Face	Ulcerative
Al Samarai and Abolaidi ¹⁹ (Iraq)	2004–2005	57/43	1–15	–	–	–	1.8	Upper limbs	Ulcerative
Sharma and others ¹⁴ (India)	2001–2003	49.6/50.3	21–30	–	7.8 months	75/25	1.58	Face and neck	Nodulo-ulcerative
Talari and others, ¹⁸ (Iran)	1995–2003	49.2/50.8	0–10	–	–	–	1.4	Hands	Ulcerative
Present study (Bikaner, India)	2001–2011	55.4/44.6	11–20	12.54	16.43 weeks	86.36/13.64	1.97	Upper limbs	Ulcerative

reported 75% and 25%, respectively. The average number of lesions was 1.98 in our study; Srivastava and others⁶ and Sharma and others¹⁴ reported averages of 1.4 and 1.58, respectively. Similar to earlier studies^{6,7} in the same location, our study found that exposed parts of the body are most affected, likely because sand flies can easily bite exposed parts during sleep. The most frequent type of lesion in the present study was the ulcerated plaque, and Sharma and others¹⁴ reported ulcerated nodules as the most frequent lesion.

Our study showed that 69.5% of skin smears were positive for *Leishmania* parasites, which is similar to findings in studies by Yemisen and others¹⁶ and Al Samarai and Alobaidi.¹⁹ Tissue smears from skin biopsies were positive in 45.8% of skin smear-negative patients in the present study, and Sharma and others¹⁴ reported only 37% positivity in tissue smears. On the basis of our PCR findings, *L. tropica* was identified as the causative organism of cutaneous leishmaniasis in Bikaner whereas *L. donovani* was found to be the causative species in most patients from Himachal Pradesh, India.¹⁴

Although the incidence of CL cutaneous leishmaniasis has decreased in the past two years, it is still a public health problem in this region of India. A closer look at sanitation programs to control vectors and improvement of living conditions for the population is needed for better control of the infection and preventing its spread to other regions.

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