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Prevalence and Comparative Analysis of Cutaneous Leishmaniasis in Dargai Region in Pakistan

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Abstract.- In the present study, 102 samples of exudates from cutaneous leishmanial lesions were collected from local Pakistanis and Afghan refugees residing in Dargai Tehsil of Malakand Agency Pakistan, and were analyzed. The lesions were more prevalent among the locals (60.79%) compared to refugees (49.21%), and 70.58% male compared to 30.42% female were infected with *Leishmania.* Infection was more prevalent among youth's ages of 11-20 year old (34.32%) compared to older adults. Exposed parts of the body (face, hands, legs, neck, ears and feet) were the main affected areas. Faces experienced more bites (36.28%), followed by legs (26.48%), and hands (21.57%). Lesions lasting duration of 3-4 months was more prevalent (42.16%).The present research also disclosed some key factors that seem to be responsible for the prevailing rate of incidence.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, *L. tropica*, phlebotomine sand flies, Afghan refugees, BHU, cutaneous lesion.

INTRODUCTION

Leishmania is an obligate intracellular protozoan parasite that is transmitted by female phlebotomine sand fly bite in promastigote form into the human skin. The injected parasites multiply repeatedly in the phagolysosomes of macrophages in form of amastigotes. The cutaneous form of the disease is caused mainly in Pakistan by *Leishmania tropica* and *L. major*. Cutaneous leishmaniasis being one of the most frequent vector-borne parasitic infections has the highest incidence in Kabul, Afghanistan with an estimated 67,500 to 200,000 cases annually (Khan and Muneeb, 2010; Faulde *et al*., 2008, 2009; Kolaczinski *et al.,* 2004).

Due to the migration of several million refugees to North-western Pakistan, cutaneous leishmaniasis has spread to previously non-endemic areas in Pakistan. Leishmaniasis is also a major problem faced by the soldiers and peacekeeping forces stationed in Afghanistan and tribal areas of Pakistan.

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After a most comprehensive report on outbreak of cutaneous leishmaniasis in Dir Afghan refugee's camp in Pakistan (Roland *et al.,* 1999) many epidemiological studies in different parts of Pakistan were carried out. Presence of both anthroponotic and zoonotic cutaneous leishmaniasis (CL) in all the four provinces of Pakistan were then detected. The cutaneous leishmanisis is becoming endemic in Larkana, Dadu and Jacobabad districts of Sindh. Recently 1210 cases of CL were found in lowland of Sindh (Bhutto *et al.,* 2009). High incidence of CL has been reported from various cities of Punjab and high altitude regions of Baluchistan. It is suggested that the invasion of infection in Sindh and Baluchistan has occurred from Iran, not from Afghanistan (Khan and Muneeb, 2010). Due to presence of one of the largest Afghan refugees' settlement adjacent to local Pakistani population in Dargai area, the present study was designed to evaluate the current threat and specific epidemiology of CL in the Dargai Tehsil area of Malakand Agency, Pakistan.

MATERIALS AND METHODS

Sample collection and smearing

Exudates (102) from CL lesions were collected from patients visiting THQ-hospital

Dargai, Basic Health units (BHU) of Afghan refugees camp Dargai, BHU of Kharkai, and randomly from different villages in the study area. During specimen collection, each patient was handed a printed questionnaire having information regarding name, father's name, age, sex, number of lesions, site and duration of lesion, type of lesion, present and permanent address, occupation, type of house, number and species of domestic animals in home, patient's previous history of the disease, family history of the disease, use of insecticide, impregnated bed nets and insect repellents, treatment, and follow up and any previous diagnosis through microscopy and/or biopsy.

Before exudates collection the skin lesion was sterilized with 100% ethanol. The active raised margin of the lesion was then pressed in between thumb and forefinger and punctured using a sterile blood lancet. The exudate from punctured margin was placed on both ends of the examination slide on the same side. Exudate on one end of the examination slide was smeared thick, while the other end was smeared thin, air dried and fixed in 100% methanol for 2-3 min (Garcia, 2001).

Stain preparation

Fresh Giemsa stain was prepared as used for staining blood smears (Kayser *et al*. 2003). Giemsa (Sorenson) buffer 0.67 M (100X) was prepared by dissolving 9.24 g of Na_2HPO_4 and 6.38 g of NaH_2PO_4 in 1000 ml of the deionized water and autoclaved. Working Giemsa buffer 0.006 M; pH 7.2 was prepared by diluting 10 ml of the stock Giemsa buffer with 990 ml of the deionized water. For preparation of stock Giemsa stain, 30 ml of the 3.5 mm glass beads, 270 ml absolute methanol, 3 g of Giemsa stain powder, and 140 ml of glycerol were mixed in a 500 ml airtight black bottle and placed on a shaker at an angle for 30-60 min daily for 14 days. Working Giemsa stain (2.5%) was prepared by mixing 40 ml working Giemsa buffer, 1 ml Giemsa's stain stock and 20 µL of 5% triton X-100 solution.

Staining and diagnosis by microscopy

The slides were dipped in working Giemsa stain for 45-60 min in a staining jar. After staining the slides were rinsed by dipping 3-4 times in the Giemsa (Sorenson's) buffer and air dried by placing upright in a rack. After staining, the slides were observed under oil immersion objective (10x100 magnifications) to see intra-macrophagic amastigotes of *Leishmania* spp. All of the 102 samples gave positive results showing both isolated as well as intra-macrophagic amastigotes (NCCLS, 2000; Ghosn *et al.*, 2008; Luz *et al*., 2009).

RESULTS

Age and sex-wise prevalence

Table I presents age and sex-wise distribution of leishmaniasis, the infection was more prevalent (34.32%) among age group of 11-20 years and was lower in the over 40 years old population. It may be due to their dynamic life style and activities that render them more susceptible to sand fly bites. A previous stud (Enami *et al*., 2009) found similar higher infection among age group of 10-14 years. The incidence was much higher in males (70.58%) than females (29.42%). One possible reason for higher incidence in males may be their tendency to put off shirts while sleeping at night during hot summers. This probably renders them more susceptible to sand fly bite. Females, on the other hand, do Parda (Hijab) and keep themselves covered in sheet that is thought to give them protection against the sand fly bite. However, previous studies conducted in areas where gender differences are not considered, equal or even higher prevalence has been encountered among females (unpublished data). A similar study (Enami *et al*., 2009) in Iran reported higher prevalence in males (6%) than females (4%).

Duration based prevalence of skin lesions

As shown in Table II, there were large numbers of patients with lesions of 3-4 month duration yielding a prevalence rate of 42.16%. The next category showing higher incidence (22.55%) were patients with 1-2 months old lesions. Patients with lesions as old as 5-6 months and greater than 6 months gave prevalence rates of 14.71% and 18.63%, respectively. Previous studies (Bhutto *et al*., 2009) reported similar results.

Table I.- Prevalence of cutaneous leishmaniasis in different age groups, and its sex-wise distribution in Daragi region of Pakistan.

Group	Age groups	Male	Female	%
1	1-10	2	11	12.75
2	11-20	24	11	34.32
3	21-30	14	4	17.65
4	31-40	11	4	14.70
5	41-50	7	0	6.86
6	51-60	9	0	8.82
7	61-70	3	0	2.94
8	71-80	1	0	0.99
Total		72 (70.6%)	30 (29.4%)	

*n=102; total percentage of infected subjects.

Table II.- Distribution of lesions of cutaneous leishmaniasis in all population with respect to duration of infected in Dargai region of Pakistan.

Duration (months)	Infected subjects	Percentage*
1-2	23	22.55
3-4	43	42.16
5-6	17	16.66
>6	19	18.63

*n, 102

Distribution of leishmanial lesions by number

Table III shows the categorization of patients by the number of lesions. The maximum number of patients (83.33%) carried 1-2 lesions. 10. 88% of the patient had 3-4 lesions and approximately 3% showed a greater number of lesions. However, single bites were more frequent, and almost all of the lesions were ulcerated and many of them were carrying super infection due to secondary bacterial infection. Lesions were pussy and painless.

Table III.- Categorization of infected subjects based on the number of lesion of cutaneous leishmaniasis in Dargai region of Pakistan.

No. of lesions	Infected subjects	Percentage
1-2	85	83.33
3-4	11	10.88
5-6	3	2.95
>6	3	2.95

Site-specific lesion distribution

Table IV shows the distribution of lesions with respect to site of occurrence. Lesions were mainly seen on exposed parts of the body, and occurred mainly on the facial areas (36.28%) followed by the legs (26.48%), hands (21.57%), and mixed location (9.81%) and other body parts (5.88%). However, a study (Enami *et al*., 2009) conducted in Iran showed that hands, feet, face, neck and other body parts were carrying 53%, 20%, 12%, 6% and 8% lesions respectively.

Table IV.- Distribution of lesions cutaneous leishmaniasis on different parts of body infected subjects in Dargai region of Pakistan.

Sites of body	Infected subjects	Percentage
Facial parts	37	36.28
Legs	27	26.48
Hands	22	21.57
Mixed	10	9.81
Other body parts	06	5.88

*n, 102

Nature of lesions recorded during the study

Figure 1 shows multiple leishmania lesions, and majority of the lesions (78.44%) were of dry type, characteristic to the anthroponotic cutaneous leishmaniasis, caused by *L. tropica.* Dry lesions were mainly observed in local Pakistanis. Wet types of lesions, characteristic to zoonotic cutaneous leishmaniasis, caused by *L. major*, were less frequent (21.57%) and were found mainly in Afghan refugees who had been infected in Afghanistan during their visit there.

House-hold characteristics that may influence the prevalence of leishmaniasis.

Table V shows the house-hold characteristics of the infected individuals, such as type of house and windows, species of domesticated animals and pets, sleeping habits and use of insect repellents and nets, which greatly affect the prevalence of infection. Majority of the patients (74.50%) were of low socio-economic level and dwelt in mud houses, which are probably more suitable places for sand flies. About 24.50% of the patients were living in brick houses. Higher percentage of patients (76.48%) were keeping domesticated animals and

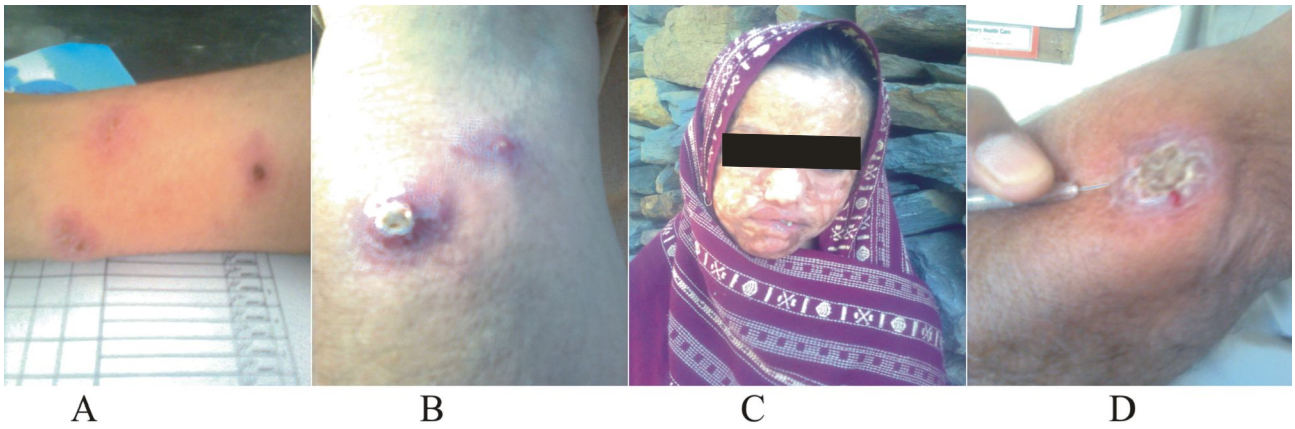

Fig. 1. Multiple leishmania lesions (A, B), extreme disfiguring due to leishmaniasis © and intra-lesion inoculation of drug for the treatment cutaneous leshmaniasis (D).

Table V.- Socio-economic conditions and household characteristics and the prevalence of lesions cutaneous leishmaniasis in Dargai region of Pakistan.

Household characteristics	Infected subjects	Percentage
House type		
Pakka house (brick made)	26	25.50
Kacha House (mud made)	76	74.50
Domestic animal/pets		
Present	24	25.53
Absent	78	76.48
Type of windos		
Meshed	40	39.22
Without meshed	62	60.79
Sleeping habit		
Covered	36	35.30
Uncovered	66	64.70
Use of nets and repellent		
Yes	03	2.95
No	99	97.05

pets like cows, buffalos, goats, sheep and dogs, while the rest (23.53%) were having no such animals in their homes. Most of the patients keeping animals were those who were living in the mud houses. Percentage of patients having unmeshed windows in homes was greater (60.79%) than those having meshed windows (39.22%). Greater number of the patients (64.70%) used to sleep outdoor without shirts. All of these were males. Some 35.30% of the individuals used to sleep covered, most of them were females. Only a small ratio (2.95%) of the patients used mosquito nets and repellents.

DISCUSSION

The CL is more prevalent in local population (61%) than refugees (39%). The reason of higher infection among the locals is not clearly known, however, it has been attributed to the novelty of the infection in this area. Therefore, immunologically local population is more susceptible to the infection than the refugees, who are thought to have developed protective immunity against CL, as this disease has long been existed in Afghanistan (Faulde *et al*., 2008). Another factor may be the large size of local population, thus more susceptible individuals in local population.

Only 36.37% patients were either under treatment or were treated partially, and 63.73% patients were not treated at all. In treated cases, response to intra-lesional treatment with meglumine antimoniate was good but show poor response in relapsed cases which may be due to the acquisition of partial treatment. The reason for leaving the treatment incomplete was that with two or three intra-lesion injections, there was a good response and patient thought that lesions were healed. Since, lesions are painless and have been neglected for long time giving more chances for spread of the infection. Also, the treatment is scarce and available only in refugees' camps (BHUs) which are beyond the reach of many patients.

Among other possible reasons are as follows; the pain caused by dermal injection of drug (Baily, 2011), and patients often do not cooperate during inter-dermal drug injection procedure, mostly by children, and if the injection is not properly administered, it becomes subcutaneous. Also Pakistani doctors who only practice medicine and surgery during their in-house training are unaware of *Leishmania* lesions, and usually keep on treating the patients with normal antibacterial creams and ointments. There are few medical practitioners who could inject the drug into dermis during intra-lesional treatment, which is a more effective treatment. Malnutrition may also be responsible for week immunological responses against this infection in different populations in different areas, thus lead to spread of infection.

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Disclosure

None of the authors have any conflict of interest.

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