

# FREQUENCY OF CUTANEOUS LEISHMANIASIS AMONG CLINICALLY SUSPECTED CASES, VISITING TERTIARY CARE HOSPITALS OF PESHAWAR

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## ABSTRACT

**Background:** Cutaneous leishmaniasis is a parasitic disease that is endemic in many countries in the world including Pakistan. **Objectives.** To determine the frequency and distribution of cutaneous leishmaniasis among suspected cases visiting various tertiary care hospitals in Peshawar. **Subjects & Methods.** This was a descriptive study in which a total of 320 clinically suspected people of cutaneous leishmaniasis visiting Lady Reading Hospital Peshawar and Kuwait Teaching Hospital were enrolled. This study was conducted from Sep. 2008 to Feb. 2009. **Results:** Out of 320 enrolled patients, 223 were found positive for cutaneous leishmaniasis during the study period. Prevalence of cutaneous leishmaniasis was higher in local population (73.06%) than in Afghan refugees (58.66%) visiting tertiary care hospitals. There was an increased burden of infection among children of 0-9 years while lowest prevalence was recorded in adults above 50 years of age. Prevalence was significantly higher amongst males (76.47%) as compared to females (57.75%). **Conclusion:** There is high prevalence of cutaneous leishmaniasis among suspected cases in Khyber Pakhtunkhwa, Pakistan and younger population is mainly affected. Prompt measures are recommended to control this disease.

**Key Words:** Cutaneous leishmaniasis, Frequency, Pakistan.

## INTRODUCTION

Leishmaniasis is transmitted to humans by the bite of an infected sand fly. The causative agent is *Leishmania*, a parasitic microorganism related to the trypanosomes.<sup>1</sup> The parasite has a digenetic life cycle which includes extracellular, flagellated promastigote forms and obligate intracellular amastigote forms.<sup>2</sup> At least 14 species of *Leishmania* commonly cause human leishmaniasis.<sup>3</sup> Cutaneous leishmaniasis appears to be an emerging disease in various parts of Pakistan. It is a chronic protozoan disease caused by various species of parasite leishmania.<sup>4</sup> *Leishmania tropica* and *Leishmania major* are 2 of the commonest causative species of this form which produce ulceration of the skin. The disease is endemic along entire Western border of Pakistan. Sindh (area associated with Balochistan), Punjab (Multan, Chakwal) and Northern Areas of Balochistan.<sup>4</sup> Two species of old World cutaneous leishmaniasis are endemic to Pakistan, Zoonotic cutaneous leishmaniasis (ZCL) or Rural or Wet type caused by *Leishmania major*, is associated with bites of *Phlebotomus papatasi* and occurs in rural areas of Pakistan.<sup>5</sup> Zoonotic cutaneous leishmaniasis is endemic to

the Southwest, mainly occurring in Balochistan and neighbouring Punjab and Sindh provinces.<sup>6,7</sup> Dry type leishmaniasis caused by *Leishmania tropica* and transmitted by the sand fly vector *Phlebotomus sergenti*, has a widest distribution, occurring in Urban areas of Southern Punjab (Multan) and Balochistan (Quetta), but also focally in the Northern Areas and Azad Kashmir.<sup>8</sup> Cutaneous leishmaniasis has been given various names in different civilization, such as “Delhi Boil” in India, “Baghdad Boil” in Iraq and “Saldana” in Afghanistan.<sup>8</sup> Reports of cutaneous leishmaniasis are received in hospitals of Khyber Pakhtunkhwa, raising concern about spread of the disease in Khyber Pakhtunkhwa. The objective of this study was to determine the frequency of cutaneous leishmaniasis among clinically suspected cases in local population (including afghan refugees) residing in Khyber Pakhtunkhwa through two main diagnostic and treatment centres in LRH (Lady Reading Hospital) and KTH (Kuwait Teaching Hospital) of Peshawar and investigate the distribution of this disease.

## SUBJECTS & METHODS

This descriptive study was conducted from 1<sup>st</sup> September, 2008 to 28<sup>th</sup> February, 2009 in two hospitals in Peshawar, Pakistan. A printed proforma was filled for each patient which had information regarding date of specimen collection, name, father's name, age, sex, area of residence, nationality, education, occupation, type of house, number of rooms, and number of persons in a house, species of domestic animals if any, types of windows (meshed or unmeshed), use of mosquito nets and use of

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mosquito repellent, family member visited any leishmaniasis endemic area, type of lesions, number, site, and duration of lesions. Samples were collected mainly from suspected people visiting hospitals for diagnostic confirmation of clinical cutaneous leishmaniasis. A diagnostic confirmation was undertaken by using three techniques, Slit-skin smear, biopsy and parasite culture.

Most of the patients were diagnosed by slit-skin smear. For this purpose, the nodular margin of the most active-looking lesion was sliced open and a smear taken with surgical blade / blood lancet. Smears were fixed in methanol, stained with Giemsa stain and examined under the microscope for identification of amastigotes of *Leishmania*.

For some patients, biopsies were also taken from nodular margins of the lesions using punch biopsy kit. Surgical exudates were obtained and kept in formalin. Exudates were used to prepare an impression smear.

The samples of some patients for culture were inoculated into biphasic NNN Media for examination of promastigotes of *Leishmania*. For this purpose Novey, McNeal and Nicole (NNN) medium, was prepared by using two parts of salt agar (Agar- 7g, NaCl-3g, and distilled water 450ml) and one part of rabbit blood i.e defibrinated.

Rabbit blood was collected aseptically into a sterile 50mL tube. Pieces of glass were added into the rabbit blood and shaken vigorously in order to defibrinate the blood. To reduce the risk of contamination, penicillin-streptomycin and antimycotic solutions were added to the defibrinated blood. Water was poured into a flask (one which will not crack in boiling water). Then 7g agar and 3g NaCl were added in this water and was mixed thoroughly. This flask was left in a container of boiling water until the agar was completely dissolved. This was then sterilized by autoclaving at 121°C for 15 minutes. This agar was then allowed to cool at 45-60°C. This medium was kept in screw capped bottle. About 0.6 mL of sterile defibrinated rabbit blood was then added to the liquid agar during cooling. The two were intimately mixed by rotating the tube between the palms of the hands, holding it in a vertical position. The tube was then allowed to set in a sloping position, so that the medium solidified in a slant. The *Leishmania* suspected exudates were

inoculated (0.1 ml) into the medium and incubated at 24-27°C. Special care was taken to avoid contamination with bacteria, which could lead to degeneration and death of *Leishmania* species. For this purpose a laminar flow hood was used. This was then examined every 4 days for promastigotes using a sterile wire loop to transfer a drop of the culture to a slide for examination. Statistical analysis was done using SPSS version 15, Chi square test was applied and P value  $\leq 0.05$  was taken as significant.

## RESULTS

A total of 320 suspected individuals, both from local Pakistani population and Afghan refugees were examined. Out of these, 223 were found harbouring intramacrophage amastigotes in their cutaneous lesions, showing 69.68% prevalence of cutaneous leishmaniasis among patients visiting tertiary care hospitals. Out of 223 positive cases, 44 (19.7%) were Afghan refugees while 179 (80.3%) were from the local population (Table I). Chi-Square analysis ( $\chi^2=5.632$ , d.f.=1, P=0.05) also indicated significant difference in prevalence among the two groups.

The highest prevalence rate (77.77%) was recorded in the age group between one and nine years of age and declined thereafter. The lowest rate was found in patients over 50 years of age (Table II). Chi-Square analysis ( $\chi^2=7.922$ , d.f.=5, P<0.05) indicated that there was significant difference among affected older and younger age groups.

Leishmaniasis was most prevalent in males (76.47%) compared to females 57.75% (Table III). Chi-Square ( $\chi^2$ ) test indicated a significant difference in the prevalence rate between males and females ( $\chi^2=12.226$ , d.f.=1, P=0.025).

More than seventy five percent of the seropositive cases came from the rural areas while 57.14% came from the urban areas. About 63.3% positive cases were found in illiterate individuals while 37.7% were literate.

**Table I. Prevalence of Leishmaniasis in local Pakistanis and Afghan refugees.**

Suspected population	Subjects examined	Positive cases	Prevalence percentage
Local population	245	179	80.2
Afghan population	75	44	19.7
Total	320	223	99.9

**Table II. Age distribution of diagnosed cutaneous leishmaniasis patients.**

Age (years)	Subjects examined	Positive cases	Prevalence percentage
0-9	90	70	77.77
10-19	97	69	71.13
20-29	70	48	68.57
30-39	25	16	64.00
40-49	21	12	57.17
50 and above	17	8	47.05
Total	320	223	69.68

**Table III. Sex-wise distribution**

Sex	Subjects examined	Positive cases	Percentage
Male	204	156	76.47
Female	116	67	57.75
Total	320	223	69.68

## DISCUSSION

This study revealed a higher number of local populations being affected by cutaneous leishmaniasis. This higher prevalence of cutaneous leishmaniasis in local population may be due to low immune status of this population who were previously not exposed to these pathogens while in Afghanistan cutaneous leishmaniasis has been endemic for centuries as described by Reithinger *et al.* from Afghanistan in 2003.<sup>9</sup> So circulating antibodies against this *Leishmania* species in Afghan refugees may have rendered them immune to pathogenicity of metacyclic promastigotes introduced by sand fly vector. The prevalence rates observed in our study are in the line with that of Hepburn *et al.*, who in 1993 recorded 61% parasitological confirmed cases in the Middle East.<sup>10</sup> Similar results were recorded in previous studies conducted on suspected subjects in Khyber Pakhtunkhwa Pakistan as reported by Shah *et al.* in 2007.<sup>11</sup>

All age groups were susceptible to *Leishmania* infection. The reason for higher prevalence in younger age groups is not clear but probably it may be due to poorly developed immune response among the growing children, as majority of the adults are immune to cutaneous leishmaniasis due to exposure. Older children seem to be more at risk than any other group or perhaps older children are

less disciplined in covering up during the hot summer nights as stated by Rowland *et al.* in 1999.<sup>12</sup> Males are at high risk of contracting this disease as observed in previous studies and the cause is occupational exposure and their habit of sleeping without shirts during summer.<sup>4,9,11,13</sup> Females wear clothes covering the whole body so it minimizes the chances of insect bites. Also the access of women to health care centres is limited in our country due to cultural barriers.

Sand flies seem more prevalent in rural areas due to perfect breeding sites. Present study showed that the high prevalence rate was not associated with over crowding in the house. However, the prevalence rate increased in those people who lived in houses where walls were made from mud and ceiling was of wood (thatched type), domestic animals were present and houses were single storey. The reason for higher prevalence in these situations may be that, the sand flies harbour in cracks and crevices of walls made from mud, the sand flies do not fly high or far, that is, they have a range of only 50 meters from their breeding sites. Another important contributory factor could be cattle breeding in the area. Cows are especially used in the fields which provide a ready warm blood meal for the sand flies and their dung may also form an ideal breeding site for the flies to lay eggs.<sup>9,11,14,15</sup>

## CONCLUSION

Our findings indicate that the prevalence of cutaneous leishmaniasis is increasing among suspected cases, visiting tertiary care hospitals in Khyber Pakhtunkhwa and is becoming a major health problem. The epidemiology of the disease is highly variable due to interplay of numerous factors in the parasites, vectors, hosts and environment. In view of the present study, it can be concluded that mostly young generations are the victims of the disease. Although cutaneous leishmaniasis does not cause death but it produces many structural deformities including disfiguring skin lesions.

Keeping in view the sudden resurgence of the disease, there is a need to make general public aware about the life cycle and control of the disease. Government should take interest in controlling the disease. Prompt and effective measures will be required to prevent cutaneous leishmaniasis.

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