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RESEARCH TITLE

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AN OUTBREAK OF CUTANEOUS LEISHMANIASIS IN THE LOCAL POPULATION OF DIR UPPER, PAKISTAN

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Abstract

Leishmaniasis is an infectious disease caused by intracellular parasitic protozoans belonging to a genus of flagellate protozoa called *Leishmania*. More than 25 species of the genus *Leishmania* are present worldwide in which several species are pathogenic to humans. In many cases, cutaneous infection is formed but some species cause subcutaneous or deeper tissues and visceral infection. Cutaneous leishmaniasis is a skin disease caused by *Leishmania topica*. This disease is transmitted by sandflies. The disease is endemic along the entire Western border of Pakistan. During the present research, the samples were collected from infected people in different areas of Dir Upper. A total of 548 samples were collected among which 346 (63%) were positive cases. In the infected people, 297 (86%) were from the local population and 59 (14%) from Afghan refugees. A total of 235 (68%) males and 111(32%) females were affected. The high prevalence rate (48.5%) was noticed in the age of 11-20 years old people. The prevalence rate between the age of 0-10 was 29.1%. The low prevalence rate (13.6%) was noticed in the people over 30 years. Mostly one lesion was noticed in affected people (69%). The patients with two lesions were 22%. The numbers of affected persons with more than two active lesions were little in number i. e. 8.3%. Most of the lesions were reported on the face (36.1%). The second affected part of the body noticed in the study was hand (26.5%). Most of the lesions were dry (81%). It is concluded that a high prevalence rate was observed. People's awareness is very necessary.

Keywords: Cutaneous leishmaniasis, *Leishmania topica*, Dir Upper, Prevalence

1. Introduction

Leishmaniasis is an infectious disease caused by intracellular parasitic protozoans belonging to a genus of flagellate protozoa called *Leishmania* (order: Kinetoplastida, suborder: Trypanosomatidae). More than 25 species of the genus *Leishmania* are present worldwide in which several species are pathogenic to humans (Ullah *et al.*, 2014). In many cases, cutaneous infection is formed but some species cause subcutaneous or deeper tissues and visceral infection (Cox, 1993).

The classification of these parasites is still in a state of fluctuation following a change of status away from morphological and disease-associated characteristics towards biochemical and molecular conditions. Numerous species are reported from South America including *Leishmania lainsoni*, *L. naifli* and *L. shawi* that contaminate humans and *L. enriettii*, *L. hertigi*, *L. deanei* and *L. aristidesi* which are found only in wild animals. In the Old World, the main causative agents of cutaneous leishmaniasis were *L. tropica* and *L. maior* and visceral leishmaniasis was caused by *L. donovani*. Visceral leishmaniasis is caused by *L. chagasi* in the New World and *L. braziliensis*, *L. mexicana* and *L. peruviana* cause cutaneous and mucocutaneous leishmaniasis. Instead of humans from other organisms *Leishmania* species have been also isolated (Cox, 1993).

Cutaneous leishmaniasis is an infectious disease in which one or more lesions are present on the skin depending on the species of *Leishmania*, smooth ulcers, flat plaques,

nodules or hyperkeratotic wart-like ulcers may observed. The initial ulcers are generally papules present on a small area but in some cases, secondary lesions are also produced which may extend to mucosa or other part of the body (Ullah *et al.*, 2014). The most causative agent of cutaneous leishmaniasis in India and Pakistan is mainly *L. tropica* and man is the main common reservoir (Bari, 2006).

The sandflies are obligatory vectors and insect hosts of *Leishmania* species. *Leishmania tropica* is spread by *Phlebotomus sergenti*, *P. papatasi*, *P. caucasicus*, *P. longipes* and *P. pedifer* in the endemic areas (Cotran *et al.*, 1999). There are about 700 species of Phlebotomine sand flies of which about 70 are considered to transmit diseases to people. Of these, 37 species have been identified in Pakistan (Jones *et al.*, 1997).

Leishmaniasis are commonly present in 88 countries (mostly developing countries) and are endemic in all continents except Antarctica and Australia (Khan *et al.*, 2009). According to WHO, annually 0.4 million new cases of leishmaniasis occur approximately with almost 400 million people at threat of the disease. The overall prevalence of leishmaniasis is projected at 1.0-1.5 million new CL cases and 12 million cases with 0.5 million new visceral leishmaniasis cases per year (Khan *et al.*, 2009). In the New World Leishmaniasis is spread in 22 countries and in 66 nations in the Old World. In the 16 countries of Europe, human infections are found including Greece, France, Italy, Spain, Portugal and Malta. Leishmaniasis also originated in Central America, Mexico and South America from northern Argentina to southern Texas (excluding Uruguay, Canada and Chile), Asia (not Southeast Asia), the Middle East, Africa (particularly East and North Africa) and southern Europe (not common in travellers to southern Europe). It is also observed frequently in civilian and

military employees of Kuwait, Afghanistan and Iraq (Bari, 2006).

In Pakistan, the status of leishmaniasis has been changed. In parts of the country, both the cutaneous and the visceral types of the disease are being noticed in various parts of the country, including Khyber Pakhtoonkhwa (Khan *et al.*, 2009). The objective of the present research is to find out the infection rate of leishmaniasis in the local population as well as Afghan refugees of district Dir Upper.

2. Materials and methods

2.1. Study area

District Dir Upper is located in the Khyber Pakhtunkhwa. It lies in the Hidukush range between 35° 10-35° 16 N latitude and 71° 50 to 71° 83 E longitudes. In the North of Dir Upper are rugged mountainous peaks rising from 1100 to 3119 meters. District Swat is in the East while Afghanistan lies in the West (Fig. 1). The total area of Dir Upper is 3699 KM². The district is administratively divided into five Tehsils (Dir, Barawal, Chapar, Wari and Kalkot) (Wahab *et al.*, 2008).

2.2. Materials

Different types of materials were used during the present research work such as glass slide, coverslip, slide box, surgical blades, gloves, microscope, lancet, Giemsa stain, methyl alcohol, emersion oil, disposable syringe, digital camera, mobile, handbag and questionnaires (Fig. 2).

2.3. Collection of Samples

A total of 548 samples were collected from different areas of Dir Upper which included Akhagram, Karo Dara, Wari, Sahib Abad, Darora, Gandigar, Bibyaware, Khas Dir, Barawal, Sharingal, Nihag Dara, Ushari Dara, and Usorai Dara. The samples were taken from the local population as well as Afghan refugees from March to November 2022. To find out the disease ratio individual visit was made to every house (active survey). The study population included different groups of age. Special

questionnaires were filled out for people with active ulcers. The questionnaire incorporated information about inhabitants such as age, sites of lesion, sex, type of lesion, number of lesions, and date of occurrence of ulcer.

2.4. Procedure

The lesions were cleaned with methyl alcohol. Clean glass slides were used for making blood films, which were prepared as follows. Representative skin lesions were cleaned with 70% methyl alcohol. From the ulcerating skin lesion, exudate was collected through a puncture made at the raised margin of a lesion with the help of a sterile blood lancet. The exudates were placed at one end of a clean slide. These were smeared by a slide-to-slide method. Smears were then air dried and fixed in 100% methyl alcohol for 2-3 minutes. The prepared smears were stained by 10% Giemsa stain. Emersion oil was dropped on the stained smears. The smears were examined under the microscope using an oil emersion lens 100X for the finding of amastigotes of *Leishmania tropica* (Nawaz *et al.*, 2010).

3. Result

A total of 548 samples were collected from different Tehsils of Dir Upper which were Wari, Chapar, Dir, Barawal and Kalkot. A total of 346 (63%) numbers of patients were positive for CL and the amastigotes were seen in their body lesions, which shows high prevalence rate, and 202 (37%) were negative no amastigotes were seen (Fig. 2). In the total positive samples 297 (86%) were from local population and 59 (14%) were from Afghan refugees (Fig. 3). The data were also collected from both male and female sexes. There were 235 (68%) males and 111 (32%) females who were positive during the survey (Fig. 4). The mean age of males was 21.36 ± 23.82 and the mean age of females was 10.09 ± 10.81 . The difference between males and females was significant ($P < 0.05$). The prevalence rate was also found out month-wise in the present research. A high

prevalence rate was noticed in July, followed by August. In June, May and September the prevalence rate was moderate. A low prevalence rate was noticed in November, April and October (Fig. 5).

In positive cases, Tehsil-wise prevalence was also noticed. The prevalence rate was high in Tehsil Barawal followed by Tehsil Wari. An intermediate amount of prevalence was noticed. The prevalence of cutaneous leishmaniasis was observed at different ages of the people. Based on age the patients were classified into different groups. The majority of the cases (77.6%) were under the age of 20. The most affected group age was 11-15 in which 30.3% prevalence was observed (Table 1). The youngest affected patient was 10 months old child and the eldest was 71 years old. in Tehsil Dir and Chapar. A low prevalence rate was noticed in Tehsil Kalkot (Fig. 6).

In affected people, the active lesions were noticed. The total no. of lesions reported in the present survey was 509. These lesions were present on the different parts of the body. Some patients had one active lesion, some had two and some had three. In some patients, more than three lesions were also noticed. In 239 (69%) affected persons one active lesion was present. The no. of those patients who have two active lesions was 76 (22%). In some affected people three active lesions were present, which were calculated as 20 (5.8%). Four lesions were also noticed in some patients, no. of those patients was 4 (1.1%). In some patients there were more than four lesions were present and the no. of those people was 7 (2%) (Table 2).

The active lesions were observed on many parts of the body of the patients. Manly lesions were noticed on those parts which are almost naked, such as face, hand, nose etc. Among the positive samples, the active lesions present on face were 184 (36.1%). Some lesions were present on ear which was 11 (2.1%). In some patients, the lesions were

noticed on nose and the total no. of lesions which was present on nose was 35 (6.9%). The total no. of lesions present on neck regions in the different patients was 20 (4%). Some lesions were observed on the arm of the affected person that were 68 (13.4%) in number. The hand was also one of the affected regions in many cases the lesion was reported on hand. The total no. of lesions present on hand was 135 (26.5%). In some cases, the lesions were present on the legs. A total of 28 (5.5%) lesions were observed on a leg in different affected people. A total of 13 (2.5%) lesions were noticed on foot in different patients. 15 (2.9%) lesions were present on some other body parts in the affected persons (Table 3). Most of the lesions surveyed during the present research were dry (81%) and a small number of lesions were wet (11%) (Fig. 7).

4. Discussion

The survey "Prevalence of cutaneous leishmaniasis in Upper Dir district" was carried out from March to November 2012. During the present survey, the samples were collected from different infected people. The present survey shows that a high prevalence rate is present in the people of the district. Doroodgar *et al.* (2012) conducted research on CL in Iran. A total 6.4% infection rate was noticed out of 5098 inhabitants. Out of 326, 103 (2.0%) and 223 (4.4%) were noticed with active ulcers and scars correspondingly. In the age of 20-29, the highest incidence of active ulcer rate (23.3%) was reported, while in the age of 0-9, the lowest rate of infection (7.8%) was revealed. The age of most of the patients (49.5%) were under 30 years. In most cases (64.1%) one lesion was recorded and the rest of them with two or more lesions. The most affected parts of the body were hands (46.6%). During the present survey out of 548, 346 were positive, among these 235 were males and 111 were females. The prevalence was noticed in different ages of people. A high

prevalence rate was found in the age of 11-15 years old peoples which was 30.3%, which is different from the research of, Doroodgar *et al.* (2012) due to different places the surveys were conducted. In the present report, the active lesions were observed on different parts of the body of affected persons. Most of the active lesions were found on face 36.1%, which is also a different result from, Doroodgar *et al.* (2012) research due to the different climates both researches were conducted. A high prevalence rate was noticed in the under 30-year-old people in the present research, which shows similarities in both studies.

A survey was conducted by Ullah *et al.*, (2014) on Afghan refugees in Dir Upper. A total of 274 samples were collected among which 169 (62%) were positive cases. A total of 112 (66%) males and 57 (34%) females were affected. The high prevalence rate (49.7%) was noticed in the age of 11-20 years old people. Mostly one lesion was noticed in affected people (69%). The patients with two lesions were 22%. The numbers of affected persons with more than two active lesions were little in number i. e. 9%. Most of the lesions were reported on the face (36.4%). The second affected part of body noticed in the study was hand (26.5%). Most of the lesions were dry (81%) which shows a high correlation with present research.

Another study from Lower Dir by Khan *et al.* (2009) reported that out of 224 cases, 114 (51%) were positive for CL. Out of 224 participating individuals, the numbers of the local population were 129 and 95 were from Afghan refugees. The rate of infection in the local population was 55% and in Afghan refugees, 45% of individuals were infected. Mainly single lesions (56.7%) were present among the infected people. In 34.2% of patients, the lesions were present on face. 57.2% had several ulcers on different parts of the body such as face arms, face legs, arm

leg etc. In most cases (81.3%) wet lesions were reported and dry type of lesions were present in 18.7% of cases.

During the present survey, out of 548 cases positive were 346, among these 235 were males and 111 were females. Local as well as Afghan refugees were also affected by CL the rate of infection in local and Afghan refugees was 86% and 14% respectively. Most of the patients had single lesions (69%). During the present survey, most of the lesions in affected persons were dry (81%). The lesions were present on different parts of the body of affected persons. High numbers of lesions were present on the face (36.1%). The result of the present research and the study of Pakistan by Khan *et al.* (2009) show similarities in all aspects because of the same climatic conditions.

A survey conducted by Khan *et al.* (2009) further reported from Peshawar among 320 cases 223 were positive for CL. The local population (73.06%) was more highly affected than Afghan refugees (58.66%). A high amount of infection rate was noticed in the age of 0-9 and the lowest rate of infection was reported in adults. Males (76.47%) are more highly infected than females (57.75%). During the present survey out of 548, 346 were positive, among these 235 (68%) were males and 111 (32%) were females. Local as well as Afghan refugees were also affected by CL the rate of infection in local and Afghan refugees was 86% and 14% respectively. The prevalence was low in adult people as compared to young. The present showed quite similar results as reported by Khan *et al.* (2009) from Peshawar and Dir Upper.

Research was carried out on CL in Peshawar by Nawaz *et al.* (2010). In the survey out of 229 people 19 were Afghan refugees 276 were from the local population in 16 (5.79%) were positive for CL. High prevalence rate (10.9%) was noticed in the age of 0-9, followed by the age group 10-19 (6.66%). The face was highly infected. In the majority

of cases (9.5%) one lesion was noticed while 5.62% had 2 to 3 lesions, 5% had 4 to 5 lesions and 4% had more than 5 lesions.

During the present survey out of 548, 346 were positive, among these 235 (68%) were males and 111 (32%) were females. Local as well as Afghan refugees were also affected by CL the rate of infection in local and Afghan refugees was 86% and 14% respectively. Most of the patients had a single lesion (69%), and the percentage of those patients who had two lesions was 20%. Of those people who had three lesions were 20 (5.8%). The number of those patients who had more than three lesions was low (3.1%). Different ages of the people were affected by the infection. Between the ages 0-10, 29.1% of patients were reported as positive. Among the positive cases, 48.5% of patients were at the age of 11-20. The result of the present research and the result of Nawaz *et al.*, (2010) show similarities due to the same environmental conditions both the surveys were conducted.

A total of 305 cases of CL were reported by Mujtaba and Khalid (1998) in Nishtar Medical College Multan during 1995-97. Only dry types of lesions were noticed. All of the patients were infected by *L. tropica*. Later, Ayub *et al.* (2001) recorded 173 cases of CL from Multan. 30 confirmed cases were also reported from Nishtar Hospital, Multan by Ayub *et al.* (2003) from December 1999 to March 2000. Most of the patients (86%) were in the of 11-20. Males (77%) were more highly affected than females. During the present survey out of 548, 346 were positive, among these 235 (68%) were males and 111 (32%) were females. A high prevalence rate (48.5%) was noticed at the age of 11-20 years. Both the researches show similarities, most of the cases were infected by *L. tropica* in the present survey, which shows similarities with the research of Mujtaba and Khalid (1998).

478 cases were reported by Soomro *et al.* (2002) in Chandka Medical College Hospital, Larkana from February to July 2001. Children are highly affected (68%) as compared to adults. In most of the cases, open ulcers (77%) were found followed by nodular plaque and popular types of lesions. In the present survey, a total of 346 cases were positive out of 548, in which a high prevalence rate was reported in children (77.6%) which shows similarities with the research of Soomro *et al.* (2002).

A survey was conducted by Singer *et al.* (2008) on CL in Israel. Over all 161 cases of CL were recorded in the Jerusalem district in 2004 (n = 71) and in 2005 (n = 90) contrasted with 1 or 2 cases in each earlier year. In the noticed cases 74 (82%) were positive in 2005 under the microscopic examination. The rate of positive cultured cases was 53% (48); 41.6% (20) cases of these were serologically positive for *L. tropica* and not any were positive for *L. major*. In the present research out of 548, 346 (63%) were serologically positive for *L. tropica*, showing similarities with the research of Singer *et al.* (2008).

The review of Fazaelia *et al.* (2009) shows that CL is prevalent in an important rural district of Mirjaveh, Iran, presenting active scars and ulcers in 6.6% and 9.5% respectively. The maximum rates of both scars and active ulcers originated in the age group of 10 years contrasting to the older age groups. The upper limb was the most affected part with 41.7% of scars and 39.2% of ulcers. In the present research, different areas of patients were diagnosed with CL of District Dir Upper. From 548 affected patients the smears were collected, in which 346 were seen as positive for leishmaniasis. In these positive cases, most of them were children (77%), which shows similarities with the research of Fazaelia *et al.* (2009). In the present survey, it is observed that most

ulcers were present on the face, which shows differences between the two studies.

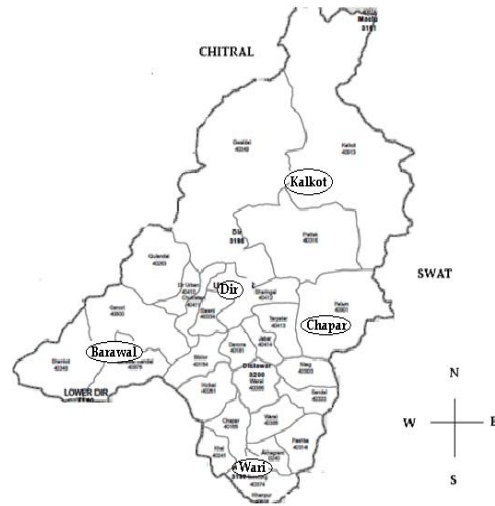


Fig. 1 Map of Dir Upper (Ullah *et al.*, 2014)

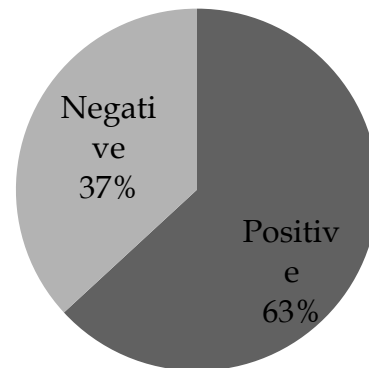


Fig. 2 Pie chart showing the percentage of positive and negative samples during present research

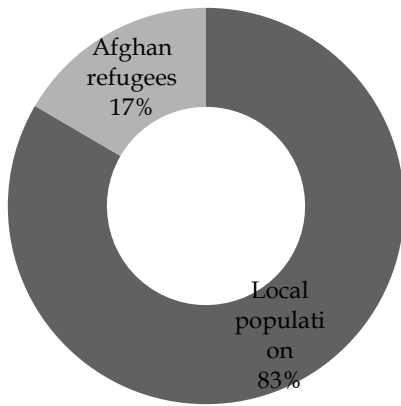


Fig: 3 Pie chart showing the percentage of infected cases of CL among the local population and Afghan refugees

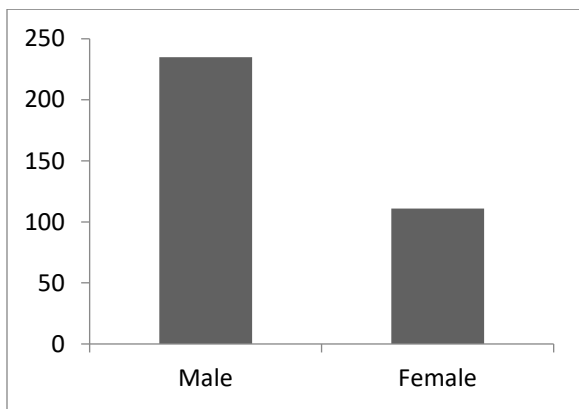


Fig: 4 Histogram showing positive samples of CL based on sex factor during the present research

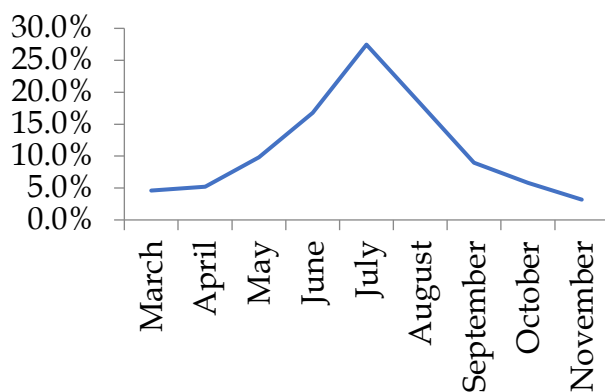


Fig: 5 Month-wise prevalence percentage during the present research

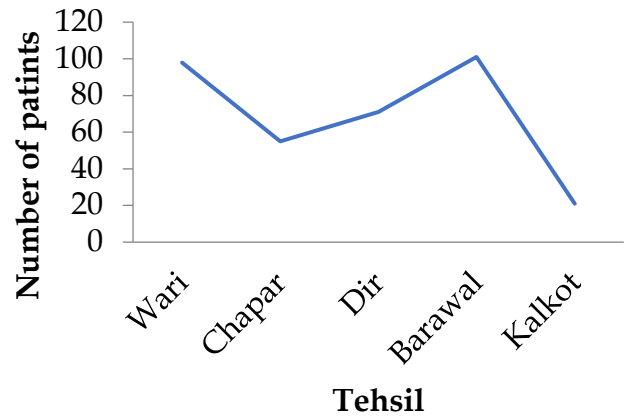


Fig: 6 Total Prevalence rate Tehsil wise during present research

Table: 1 CL positive samples based on age as well as sex factor reported during the present research

Age	Male	Female	Total	Percentage (%)
0-5	15	7	22	6.3%
6-10	58	21	79	22.8%
11-15	73	32	105	30.3%
16-20	37	26	63	18.2%
21-25	11	4	15	4.3%
26-30	9	6	15	4.3%
31-35	5	3	8	2.3%
36-40	7	3	10	2.9%
41-45	7	4	11	3.2%
46-50	6	3	9	2.6%
50<	7	2	9	2.6%
Total	235	111	346	100%

Table: 2 Positive samples of CL based on the number of lesions during the present research

Lesion	No of patient	total number of lesions
1	239	239

2	76	152
3	20	60
4	4	16
4<	7	42
Total	346	509

Table: 3 Site of lesions during present research

Site of lesion	No of patient
Face	184
Ear	11
Nose	35
Neck	20
Arm	68
Hand	135
Leg	28
Foot	13
Other	15
Total	509

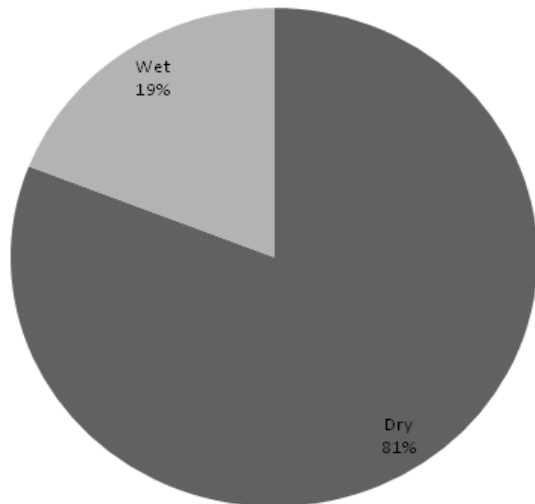


Fig: 7 Types of lesions found during the present study

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