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RESEARCH ARTICLE
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CUTANEOUS LEISHMANIASIS IN PESHAWAR REGION, KHYBER-PAKHTUNKHWA PAKISTAN.

Muhammad Fawad[1], Muhammad Shimran Khan Afridi[1], Basit Ali[2], Abdullah Khan[3], Siraj ud Din[4], Muazzam Ali Khan[5], Zahid Islam[6], Saad Rahman[1], Inayat Ullah[2,3]*, Farhat Sunny[1], Talha Khan[7], Muhammad Ibrahim[3].

[1]Department of Zoology Islamia College University Peshawar, Pakistan.
[2]Department of Chemical and Life Sciences Qurtuba University Peshawar, Pakistan.
[3]Department of Zoology Bacha Khan University Charsadda, Pakistan,
[4]Department of Botany Abdul Wali Khan University Mardan, Pakistan.
[5]Department of Botany Bacha Khan University Charsadda, Pakistan,
[6]Department Of Zoology University of Peshawar, Pakistan.
[7]Department of Zoology Quaid I Azam University Islamabad, Pakistan.

***Correspondence Author:** Inayat Ullah
*Department of Chemical and Life Sciences Qurtuba University Peshawar, Pakistan.
*Department of Zoology Bacha Khan University Charsadda, Pakistan.
Email address: inayatullahafridi2018@gmail.com

Abstract
Cutaneous Leishmaniasis is a zoonotic disease of tropical and subtropical regions and is caused by protozoan parasites of the genus Leishmania. For this study, samples were collected during the period of January to May 2022 in district Peshawar from Khyber Teaching Hospital and CMH Hospital.
The smear slides were prepared for each suspected patient, and these slides were then microscopically examined. A total of 239 samples were collected, of which 22.17% were positive and 77.82% were negative. In rural areas, the prevalence of cutaneous leishmaniasis was 23.72%, and in urban areas, the prevalence was 20.66%. The prevalence of cutaneous leishmaniasis is high in females, with a percentage of 88.67% and in males, 30.88%. The cutaneous leishmaniasis prevalence was high in the age group 1–15 years, with a percentage of 35.71%, while 9.8% were in the age group 45–60 years.
This study shows that in rural areas as a whole, prevalence was recorded as high as compared to urban areas due to a lack of awareness, an open housing system, and a low literacy rate. This study also shows that there has been a tremendous increase in cases of CL, and the disease has become endemic in many regions of Peshawar

1. INTRODUCTION:

Leishmaniasis is zoonotic disease caused by protozoan parasites of the genus leishmania, leishmaniasis is a protozoan infection of the tropical and subtropical regions of the world.it is transmitted and spread by the bite of the infected sand flies. Leishmaniasis is prevalent within and along the border of Afghanistan, India, and Islamic republic of Iran and Pakistan (1).
About 21 leishmania species have been reported to cause human infection. There is estimated 185 million people at risk for cutaneous leishmaniasis (OWCL) was not common in Pakistan and spreading rapidly in the refugee camps. Where the transmission is usually anthroponotic and human being are the reservoirs of the disease (2). Cutaneous leishmaniasis is disease of skin and not serious.

Visceral leishmaniasis (VL) damage your internal organ and serious leads death. Visceral leishmaniasis is also called kala-zar disease. Domestic animal such as dog, cat, can serve reservoir of the parasite. Transmission can occur from dog to sand fly the to human (3).
Human can transmit the parasite to each other through a blood transfusion or shared needle According to the world health organization (WHO) poverty is determining factor for the disease. Leishmaniasis often occurs in areas where the following condition are common;

- Poverty
- Malnutrition
- Famine
- Illiteracy
- Large migration caused by urbanization, emergency, situation or environmental changes (4).

1.1. History;

In 1756, Alexander Russell made an important advance in the discovery of Leishmaniasis after examining a Turkish patient. According to Russell, "after it is cicatrized, it leaves in ugly scare, which remains through life, and for many months has livid color. When they are not irritated, they seldom give much pain. "Russell called this disease, "Aleppo boils" (5).
The disease became known as Leishmaniasis after William Leishman, a Glaswegian doctor serving with the British Army in India, developed one of the earliest stains of Leishmania in 1901. In Dum Dum, a town near Calcutta, Leishman discovered ovoid bodies in the spleen of a British soldier who was experience bouts of fever, anemia, muscular atrophy and swelling of the spleen. Leishman discovered this illness as "dumdum fever" and published his findings in 1903. Charles Donovan also recognized these symptoms in other alcazar patients and published his discovery a few weeks after Leishman. After examining the parasite using Leishaman's stain, these amastigotes were known as Leishman – Donovan bodies and officially, this species become known as L Donovan. By linking this protozoan with kala zar, Leishman and Donovan discovered the genus, Leishmania (6). (Figure no; 1) shows sand fly and leishmaniasis.

Figure No 1: Sand fly on skin and its effect after infection, cutaneous leishmaniasis.

2. MATERIALS AND METHODS:

2.1. Study Area

Peshawar is the capital of the Khyber Pakhtunkhwa and Khyber Pakhtunkhwa is province of Pakistan. It is the largest city of Khyber Pakhtunkhwa, Peshawar is situated in a large valley near the eastern end of the Khyber Pass, close to the Pak-Afghan border. Peshawar is irrigated by various canals of the Kabul River and by its right tributary, the Bara River. Peshawar's recorded history dates back to at least 539 BC, so it show that it is oldest city in Pakistan as well as the oldest city in South Asia.

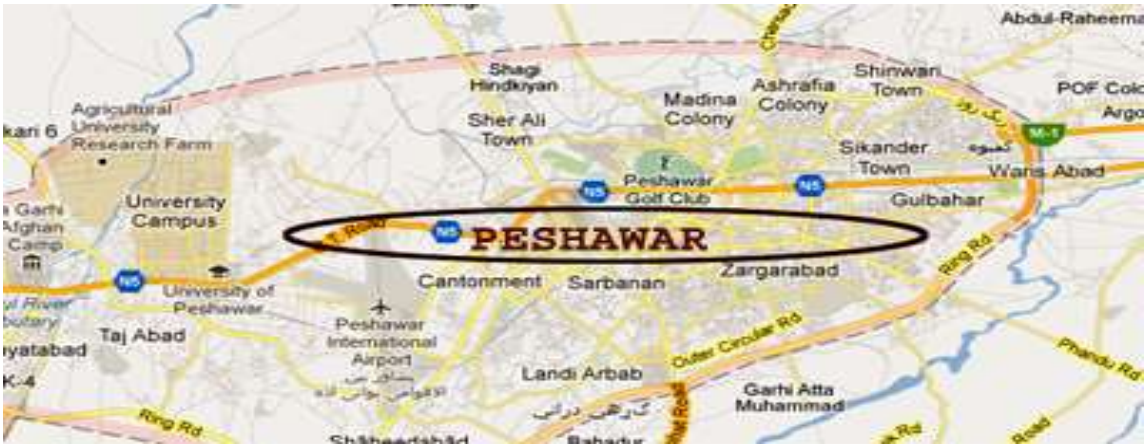

Figure no 2: Map of Peshawar selected area for study.

The climate of Peshawar is semi-arid, having very hot summer and winter is very cold. Winter in Peshawar starts in November and ends in late March, though it sometimes extends into mid-April, while the summer months are from mid-May to mid-September. The mean maximum summer temperature surpasses 40 °C (104 °F) during the hottest month, and the mean minimum temperature is 25 °C (77 °F). The mean minimum temperature during the coolest month is 4 °C (39 °F), while the maximum is 18.3 °C (64.9 °F). Figure No 2 shows map of Peshawar (study area).

2.2. Sample collection

A total of 339 archived slides were collected from smeared skin lesions of CL from suspected patients from Dermatology ward of different hospitals Peshawar CMH, Khyber Medical teaching hospital and random sampling from skin patient (during the period from January 2022 to May 2017) in the district Peshawar. Smeared slides were archived either Giemsa stained or unstained.

2.3. Microscopy

Slides and conforming patient's data were made available for this study. Received clean slides were stained with the help of Giemsa stain. The total slides were microscopically observed for the presence of Leishmania amastigotes under the study of light microscopy at 400 magnification.

2.4. Sample collection and smearing

Exudates of some from CL lesions were collected from patients visiting KTH Peshawar, an randomly from different villages in the study area During specimen collection, each patient was handed a printed questionnaire having information regarding age, sex, number of lesions, site and duration of lesion, type of lesion present and permanent address, occupation, type o house, number and species of domestic animals , 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.

Before the collection of exudates from 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 minutes.

3. RESULTS:

This study was carried out in district Peshawar to find out the prevalence of leishmaniasis. The total samples were 239 collected from the skin ward of Khyber teaching hospital (KTH). The patients were from different areas of rural and urban.

Table. 1: Overall monthly prevalence of leishmaniasis in district Peshawar

Months	**Total**	**Positive (%)**	**Negative (%)**
Jan-22	53	12(22.64)	41(77.35)
Feb-22	42	10(23.80)	32(76.19)
Mar-22	49	13(26.53)	36(73.46)
Apr-22	55	10(18.18)	45(81.81)
May-22	40	8(20)	32(80)
Total	239	53(22.17)	186(77.82)

The total samples 239 were collected in 5 months in which 53 were positive with 22.17 (%) and 186 were negative with77.82 (%).

- In January 2022, 53 samples were collected 12 were positive with 22.64(%) and were negative with 77.35 (%).
- In February2022, 42 samples were collected among them 10 were positive with 23.80(%) and 32 were negative with 76.19(%)
- In March 2022 the total samples were 49 in which 13were positive with 26.53 (%) and 36 were negative with 73.46 (%).
- In April 2022 the total samples were 55 in which 10 were positive with 18.18 (%) and 45 were negative with 81.81 (%).
- In May 2022 the total samples were 40 in which 08 were positive with 20 (%) and 32 were negative with 80 (%).

Table 2: over all Area wise prevalence of leishmaniasis in district Peshawar.

Area	Total	Positive (%)	Negative (%)
Rural	118	28(23.72)	90(76.27)
Urban	121	25(20.66)	96(79.33)
Total	239	53(22.17)	186(77.82)

The total samples 239 were collected on the base of area wise 118 were rural patients in which 28 were positive with 23.72 (%) and 90 were negative with 76.27 (%). In urban area the total sample were 121 having 25 were positive with 20.66 (%) and 186 were negative with 79.33(%).

Table 2.1: Gender wise prevalence of leishmaniasis in district Peshawar in urban area.

Gender	**Total**	**Positive (%)**	**Negative (%)**
Male	68	21(30.88)	47(69.11)
Female	53	47(88.67)	49(92.45)
Total	121	25(20.66)	96(79.33)

In urban area on the base of gender the total patients were 121 in which 25 were positive with 20.66(%) of and 96 were negative with 79.33 (%). 68 were the total male having 21 were positive with 30.88(%) and 47 were negative with 69.11(%). The total female was 53 in which 47 were positive with 88.67(%) and 49 were negative with 92.45(%).

Table 2.2: Gender wise prevalence of leishmaniasis in district Peshawar in rural area.

Gender	**Total**	**Positive (%)**	**Negative (%)**
Male	62	22(35.48)	40(64.51)
Female	56	6(10.71)	50(89.28)
Total	118	28(23.72)	90(76.27)

In rural area the gender wise prevalence was 118 were the total patient 28 were positive with 23.72(%) and 90 were negative with 76.27(%). Among them 62 were male in which 22 were positive with

35.48(%) and 40 were negative with 64.51(%). The total female was 56 in which 6 were positive with 10.71(%) and 50 were negative with 89.28(%).

Table 3: Over all Age wise prevalence of leishmaniasis in district Peshawar.

Age	Total	Positive (%)	Negative (%)
1-15	56	20(35.71)	36(64.28)
16-30	42	14(33.33)	28(66.66)
31-45	33	08(24.24)	25(75.75)
45-60	51	05(9.8)	46(90.19)
61-Above	57	06(10.52)	51(89.47)
Total	239	53(22.17)	186(77.82)

In the age wise prevalence there are five (5) different groups. In the first group 1-15 the total samples were 56 in which 20 were positive with 35.71 (%) and 36 were negative with 64.28 (%). In group second 16-30 the total samples were 42 in which 14 were positive with 33.33 (%) and 28 were negative with 66.66 (%). In group third 31-45 the total samples were 33 in which 08 were positive with 24.24 (%) and 25 were negative with 75.75 (%). In group forth 45-60 the total samples were 51 in which 05 were positive with 9.8 (%) and 46 were negative with 90.19 (%). In the last age group 61-Above the total samples were 57 in which 06 were positive with 10.52 (%) and 51 were negative with 89.47 (%).

Table 3.1: Age wise prevalence of leishmaniasis in male

Age	Total	Positive (%)	Negative (%)
1-15	34	15 (44.11)	19(55.88)
16-30	25	13(52)	12(48)
31-45	17	6(35.29)	11(64.70)
45-60	29	4(13.79)	25(86.20)
61-Above	29	5(17.24)	24(82.75)
Total	134	43(32.08)	91(64.91)

In the age wise prevalence, the total male was 134 in which 43 were positive with 32.08 (%) and 91 were negative with 64.91 (%). There are five (5) different groups. In the first group 1-15 the total samples were 34 in which 15 were positive with 44.11 (%) and 19 were negative with 55.88 (%). In group second 16-30 the total samples were 25 in which 13 were positive with 52 (%) and 12 were negative with 48 (%). In group third 31-45 the total samples were 17 in which 6 were positive with 35.29 (%) and 11 were negative with 64.70 (%). In group forth 45-60 the total samples were 29 in which 4 were positive with 13.79 (%) and 25 were negative with 86.20 (%). In the last age group 61-Above the total samples were 29 in which 5 were positive with 17.24 (%) and 24 were negative with 82.75 (%).

Table 3.2: Age wise prevalence of leishmaniasis in district Peshawar in female.

Age	Total	Positive (%)	Negative (%)
1-15	22	05(22.72)	17(77.27)
16-30	17	01(5.8)	16(94.11)
31-45	16	02(12.5)	14(87.5)
45-60	22	01(4.5)	21(95.45)
61-Above	28	01(3.5)	27(96.42)
Total	105	10(9.5)	95(90.47)

In the age wise prevalence, the total female was 105 in which 10 were positive with 9.5 (%) and 95 were negative with 90.47 (%). There are five (5) different groups. In the first group 1-15 the total samples were 22 in which 5 were positive with 22.72 (%) and 17 were negative with 77.27 (%). In

group second 16-30 the total samples were 17 in which 01 were positive with 5.8 (%) and 16 were negative with 94.11 (%). In group third 31-45 the total samples were 16 in which 02 were positive with 12.5 (%) and 14 were negative with 87.5 (%). In group forth 45-60 the total samples were 22 in which 01 were positive with 4.5 (%) and 21 were negative with 95.45 (%). In the last age group 61-Above the total samples were 28 in which 01 were positive with 3.5 (%) and 27 were negative with 96.42 (%). Figure No 2 shows amastigotes form in giemsa stained slide.

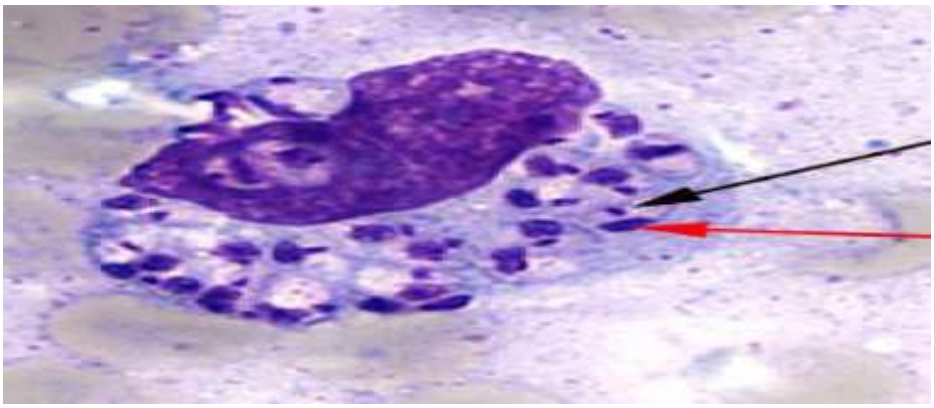

Figure No 2: Giemsa stained bone marrow aspirate smear from kala – azar patient showing amastigotes forms.

4. DISCUSSION:

Leishmaniasis is the protozoan disease infection of the tropical and sub-tropical regions of the world.it is transmitted and spread by bite of infected sand flies. leishmaniasis is prevalent within and along the borders of Afghanistan, India, Islamic republic of Iran and Pakistan (7). Cutaneous and visceral leishmaniasis is the common forms present in Asia. Cutaneous leishmaniasis (CL) is confined to the skin. CL is a preventable infection that is endemic in many regions of Pakistan (8).

About 21 leishmania species have been reported to cause human infection. Approximately it is estimated 185 million people at risk for cutaneous leishmaniasis (OWCL) was not common in Pakistan before the inflex of afghan refugees. Now it is endemic in many regions of Pakistan and spreading rapidly.

This study shows that the highest prevalence rate 26.22 % in the month of March and the lowest were 22.64 % in January. The result (9) show the highest result were 28% in September and the lowest prevalence were 9% in the month of December in Local population of Dir District.

The least number of patients were observed in April. The seasonal factors play important role in spread of Cl as Sand fly is more active during warmer months of the year as compared to February.

In gender wise the highest prevalence is 88.67 % in female and lower is 30.88 % in male. The reason of high prevalence in female is that Peshawar is urban city and female wear stylish cloths which have short sleeves so, that's why female is more infected than male, in age wise the highest prevalence were 35.71% in group 1-15 years and lower were 9.8% in group 45-60 years –above.

Similar findings were also shown by (10) in lower Dir District (KPK) it was highest (43.8%) in 1-15-year group and lowest (7.0%) in 46-60 years group.

The result of (10), *et al*., shows the high ratio (31.2%) aged 21–30 years old in Isfahan and Iran.

The result in Dargai Region of Pakistan also shows slightly higher prevalence among the ages of 11-20 years old that were 34.32% and among the age 60-70 were 2.94% which is same to the present study (10).

In our study the prevalence in young children was high because young children play outside of their houses so they were exposed to the sand fly bite. The prevalence in young children was also high because of the reason that their immune system is not fully developed.

5. CONCLUSION:

The cutaneous leishmaniasis in the district of Peshawar is not very high, but the prevalence of this disease in this area is due to insufficiency and inappropriate medicines. The prevalence rate is high in the month of March and low in April because of seasonal factors. The prevalence rate in females is higher than that in males due to the fact that females wear stylish and half sleeves on their shirts. Young people are more infected than old people because they play outside the houses and in contaminated areas. To reduce the incidence of CL, different stages are optional, i.e., the health professionals and the people should be aware of this problem, the movement of people across the Pak-Afghan border should be tested, personal protection from sandflies by using mosquito nets and hangings, the removal of diseased dogs and rodents, and spraying different mosquito repellent chemicals in houses and living areas, rodent holes, and dog environments to remove the vectors and decrease the prevalence of these chronic health problems.

6. RECOMMENDATION:

Cutaneous leishmaniasis is one of the major health problems. It is mainly spread by sand fly. Some are the following possible recommendations that can help in the restriction of cutaneous leishmaniasis.

- For minor protection from insects, use repellents and permethrin-impregnated mosquito nets.
- By using the appropriate dressing, use full sleeves as compared to half sleeves.
- Giving a lot of account for health zones.
- Primary diagnosis and prompt treatment at a public health center.
- Dropping scarcity and advancing access to health care services in those areas where cutaneous leishmaniasis is prevalent.
- Use of nets during sleep to escape the sand fly bite
- I ordered the spraying of the room with parathyroid insecticides.

7. AUTHOR CONTRIBUTIONS:

MF and IU presents the idea and also supervised the idea and wrote the manuscript, and the rest of the authors helped with sample collection and lab work.

8. CONFLICT OF INTEREST:

The authors declared that present study was performed in absence of any conflict of interest.

9. ACKNOWLEDGEMENT:

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