

Outbreak and Clinical Features of Cutaneous leishmaniasis in 2019 at District Charsadda, KP, Pakistan

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Abstract

Cutaneous leishmaniasis (CL) is a vector-borne tropical disease caused by flagellated parasitic protozoans of the genus *Leishmania* which is one of the furthestmost predominant skin diseases around the globe. This study was aimed to find the epidemiological aspects of cutaneous leishmaniasis during the recent epidemic in district Charsadda in 2019. The suspected patients of leishmaniasis from different areas of District Charsadda were examined. We came across 150 cases during four months from September 2019 to Dec 2019. The statistical analyses were done to determine the association among different variables. The study revealed the highest frequency (59.3% n; 150) of infection in males as compared to the females (40.7% n; 150). The higher incidence of infection was noted in age-group-C 5-14 years (26.0%), followed by group-D (15-25) with 24.0% and group-E (25-34) with a 20.6% rate of infection. A higher infection was found in hand regions 34% Leg regions 16% and chest regions 14%. The finding of the Chi-square independence test between age groups of positive cases and between male and female affected body parts, there was no significant relationship between both populations. It is concluded that cutaneous leishmaniasis has emerged as the main health problem in district Charsadda. The present study reports that the cutaneous leishmaniasis outbreak in district Charsadda needs an instant response by the healthcare authorities. Moreover, vast awareness campaigns are required for the timely prevention of such outbreaks.

Introduction

Leishmaniasis contains a spectrum of diseases caused by a flagellate protozoan. *Leishmania* is an intracellular obligate protozoan parasite that can infect mammals and humans (1). The clinical

appearances of leishmania disease are cutaneous leishmania, visceral leishmaniasis (spleen or liver inflammation), mucocutaneous leishmaniasis: this parasite can transmit to humans by the bite of sandflies which belong to the family Psychodidae (2). leishmaniasis is frequently deadly and serious neglected tropical disease which is mostly affected. Leishmaniasis include a spectrum of diseases caused by the flagellate protozoan *Leishmania*, an obligate intracellular protozoan parasite that infects humans and other mammals¹. The clinical manifestations of the disease include cutaneous leishmaniasis (skin ulcers), mucocutaneous leishmaniasis, and visceral leishmaniasis (lethal spleen/liver inflammation); the parasite is transmitted to humans by the bite of infected sandflies (Psychodidae family) 2. Leishmaniasis is a serious and often fatal neglected tropical disease (NTD) that mainly affects the poorest of the poor and is associated with malnutrition, population displacement, poor housing, and a weak immune system (3, 4) Although human-biting sandflies occur in some other genera, the only proven vectors of human disease are the bite of phlebotomine female sandflies of the subspecies of *Phlebotomus* in the Old World and *Lutzomyia* in the New World [5,6, 7]. There are three clinical forms of leishmaniasis: visceral (also known as kala-azar), cutaneous, and mucocutaneous. VL is the most severe form of leishmaniasis, almost always fatal if untreated [8, 9]. Leishmaniasis are prevalent in 98 countries with an incidence of 1.3 million new cases each year, although only half is reported. The visceral form causes 300,000 cases (90% in Bangladesh, Brazil, Ethiopia, India, Nepal, South Sudan, and Sudan) and one million belong to the cutaneous (mostly in Afghanistan, Algeria, Brazil, Colombia, Iran, Pakistan, Peru, Saudi Arabia, Syria, and Tunisia) or mucocutaneous forms (especially in Brazil, Peru, and Bolivia)¹. The cutaneous cases can be divided into urban and rural. The most common type in Pakistan is called "urban" or "anthroponotic leishmaniasis (10, 11). The disease is transmitted from human to human, but rural or zoonotic leishmaniasis comes from the interaction of man with animals. Cutaneous lesions are usually single and often self-healing, but a presentation with multiple ulcers resulting from multiple bites from the sandfly is not rare in Pakistan. The disease has a very long history and lesions like leishmaniasis have been described dating back to the ninth century (Balkan sore). Cutaneous leishmaniasis has been given various names in different civilizations such as "Delhi boil" in India, "Baghdad boil" in Iraq, and "Saldana" in Afghanistan [12, 13]. The disease can present in various unusual clinical variants that can be difficult to diagnose, such as paronychia, chancriform, annular, palmoplantar, zosteriform, and erysipeloid forms [14, 15]. Several reports have been presented regarding the prevalence of visceral leishmaniasis (VL) and CL in northern areas of Pakistan. Were the pioneer and reported 30 cases of VL in the northern areas from 1957 to 1960 [16, 17].later, presented their study in detail. They surveyed various hospital records from 1957 to 1960 from nine villages of the northern areas and found 60 cases of VL. In 1974, they reported 20 cases of VL and CL. Visceral leishmaniasis is also reported from the Baluchistan province [18].And various other parts of the country [19]. The aim of the study has concentrated on exploring the epidemiological characteristics of the recent epidemic of cutaneous leishmaniasis in the population of district Charsadda KP Pakistan.

METHODOLOGY:

Study Area:

This study was conducted in District Charsadda. Where the total population of Charsadda is according to the 2017 census. District Charsadda is situated west of the capital city Peshawar east Mardan and north Nowshera District. Charsadda is about 25 kilometers away from Peshawar. Figure.No.1 Geographic location of District Charsadda Khyber Pakhtunkhwa.

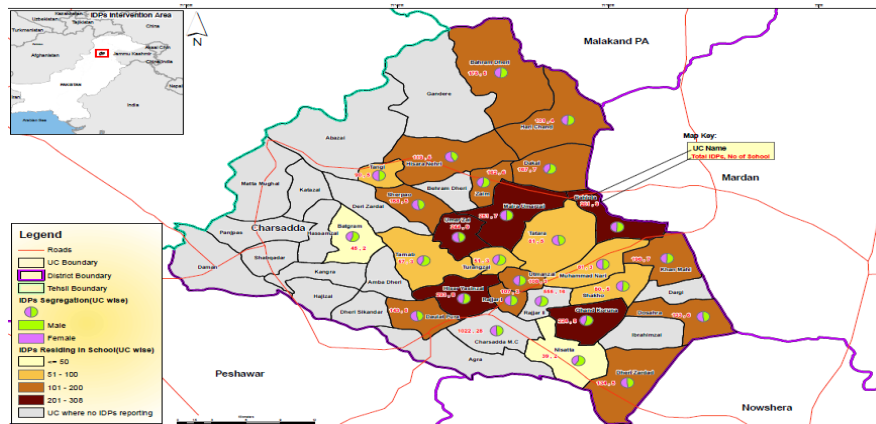


Figure.No.1: Map of District Charsadda

Data collection:

The current total of 150 CL samples was collected along with the clinical history from District Head Quarter Hospital Charsadda. The Data from September 2019 to Dec 2019 were obtained from different areas of Charsadda such as Charsadda Khas, Rajar, Sardaryab, Tangi, Shabqadar, and Dargai.

Clinical Examination:

Cutaneous leishmaniasis can easily be detected by direct observation of parasites in impression smears, skin biopsies stained with Giemsa stain (Sundar and Benjamin 2003). Based on Smear examination and clinical findings, all cases were evaluated.

Slide Preparation and Microscopy:

After the completion of the history and clinical checkup of the patients, a blood sample was collected from each patient by taking blood samples from the site of the wound with sterile lancets prick for making blood smears. Slides were kept for air drying; methyl alcohol was used for slide fixation followed by staining with Giemsa stain. Olympus microscope was used for the observation of slides with emersion oil. Whole smear slides were scanned, positive results were reported.

Data analysis:

Cutaneous leishmaniasis was diagnosed by direct observation of parasites in impression smears, skin biopsies stained with Giemsa stain [18]. Giemsa stain is one of the best histological stains used for the histopathological diagnostics of parasites. Based on smear examination and clinical

findings, the diagnosis of all the cases was performed. An Amastigotes form of the parasite was observed in the active lesion. The data obtained in the current study were analyzed based on; 1) month-wise (from Jan 2018 to July 2019) 2) age-wise (1-15, 16-30, 41-45, and >46), and socio-demographically (gender-wise and clinical manifestation and outcome basis).

Statistical analysis

Chi-square tests were applied to the data. The association between variables such as gender and age group and gender and lesions were tested. The level of significance (5%) was adopted in the test.

Results:

Gender-wise presentation

From July 2018 to March 2020 total of 152 cases of cutaneous leishmaniasis were reported. In total infected subjects, 89 patients were male (59.3%) and 61 were females (40.7 %.). While their calculated P=value was 0.229. As shown in Table.No.1.

Table.No.1 Body parts Affected by Cutaneous leishmaniasis: (The level of significance was taken 0.05)

Gender	Check	Face	Foot	Hand	Leg	Lip	Nose	Neck	Total	Frequency	P-value
Male	13	11	13	22	14	3	10	3	89	59.3%	0.230
Female	9	4	2	29	10	2	5	0	61	40.7%	
Total	22	15	15	51	24	5	15	3	150	100%	

Age-wise presentation of affected persons

Most affected persons were related with 5-14 years of age which was 26% followed by 15-24 years of age 24% and 25-34 years of age 20%. While the level of significant difference was 0.229. Depicted in Table.No.2.

Table. No. 2: Age-wise presentation of affected persons; (Level of significance was kept 0.05)

S. NO.	Groups	Age-Wise	Positive Case	Percentage %	P-value
1	A	>1	0	0.00	0.229
2	B	1 to 4	13	8.67	
3	C	5-14	39	26.0	
4	D	15-24	36	24.0	
5	E	25-34	31	20.6	

6	F	35-44	16	10.6
7	G	45-64	14	9.34
8	H	>65	1	0.67

Site wise CL distribution

The CL lesions on different body parts such as Cheeks, Face, Foot, Hand, Leg, Lip, Neck, and Nose. Hand region was the most affected 34% followed by leg 16% and Cheek 14% shown in Table.No.3 and Figure.No.2

Table. No. 3: Represents effected body parts & its frequencies.

Body Part	Frequency	Percentage
Cheek	22	14.67
Face	15	10.0
Foot	15	10.0
Hand	51	34.0
Leg	24	16.0
Lip	5	3.33
Neck	3	2.0
Nose	15	10.0
Total	150	100

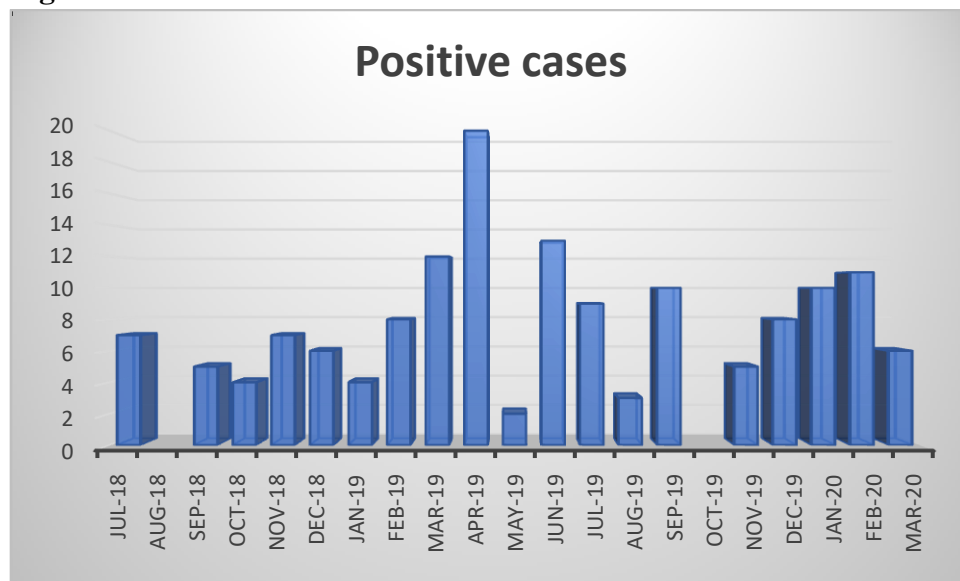


Figure.No.2: lesions presented in different regions of the body by the study participants.

Month-wise incidence of CL

In the month-wise infection rate, the highest prevalence was noted in April (2019) 13.34%, June (2019) 8.67%, and Feb (2020) 7.33%, while comparing a lower rate of infection was determined in other respective months of the 3 consecutive years. As shown in Figure.No.3.

Figure No.3. Month wise Prevalence of Cutaneous Leishmaniasis



Discussion

cutaneous leishmania infection can be attributed to overview of new global species arising from the cross-border movement, which is a well-documented danger factor. In 1997, the migrants from Kabul were the source of anthroponotic cutaneous leishmaniasis epidemic in an Afghan refugee settlement from the north-western part of Pakistan [20]. In the future, there is the possibility that people of this region participating NATO delivery may also become the source of establishing old world cutaneous leishmaniasis in NATO forces, which can be transmitted to their country. Highest prevalence of lesions was observed on an exposed surfaces of the body which were accessible to the mosquitoes biting that's why most of the infected people have lesion in their faces, foot, and hands. [21]. In our study, the higher infected population was male gender 59.3% as compared to the female gender 40.7% shown in the Table. No. 1, which is like the study (22). While with the different age-group context, the most affected age groups were noted as group-C (5-14) year of age 26.0%, group-D (15-25) year of age 24.0%, and group-E (25-34) year of age 20.6%, suggesting young agers were more effected. In other words, the lowest capacity of infection was noted in groups B, F, G, H, while group-A (>1) year of age with no infection rate, depicted in Table.No.2, shows similarity with findings identified by (23,24). Adding to that, Table.No. 1 also specifies infected parts of the body, where a higher infection was found on Hand regions 34% Leg regions 16% and check regions 14% which are like the findings recorded by (25,26). The higher rate of infection in April was 13.34%, June 8.67%, and Feb

7.33%. The finding of the Chi-square independence test for infected person's age groups and affected body parts, showing that there was a significant relationship between age groups of positive cases with a 5% significance level. The Chi-square (X^2) statistic was 56.000^a with 49 degrees of freedom (df) and the p-value (P) was 0.229. While for male and female affected body parts, the X^2 statistic was 36.000^a with 30 df and P was 0.208, which was greater than 0.05 (5% significance level). The result showed that there was no significant relationship between both populations. Leishmaniasis is spreading in the proportion of Charsadda district due to unawareness in the people. There is a dire need for a campaign in the public about the cure of the disease on time.

Conclusion:

From the present study, it can be concluded that several additional studies and raise awareness are needed to ascertain the reservoirs and vectors of the disease for the knowledge of parasites, related risk factors, and host immunological progressions which describe other infections like HCV, HBV, and TB. Further evidence is supplied by this study that leishmaniasis constitutes a serious skin disease problem in the District Charsadda KP. That there is a need to explore the conditions which encourage leishmaniasis in KPK and to raise awareness about leishmaniasis in Pakistan. Furthermore, the government of KP may have done several better arrangements for the control of such infections in other cities and districts of the province.

References:

1. Hotez PJ, Molyneux DH, Fenwick A, Ottesen E, Ehrlich Sachs S, Sachs JD. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med.* 2006;3:e102.
2. Hotez PJ, Remme JH, Buss P, Alleyne G, Morel C, Breman JG. Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. *Clin Infect Dis.* 2004;38:871–878.
3. Alvar J, Vélez I. D, Bern, C, Herrero M, Desjeux P, Cano (2012). & WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. *PloS one*, 7(5)
4. Chaudhary RG, Bilimoria FE, Katare S (2008) Diffuse cutaneous leishmaniasis: co-infection with human immunodeficiency virus (HIV). *Indian J Dermatol Venereol Leprol* 74:641
5. Kimutai R et al (2017) Safety and effectiveness of sodium stibogluconate and paromomycin combination for the treatment of visceral leishmaniasis in eastern Africa: results from a pharmacovigilance program. *Clin Drug Investig* 37:259–272
6. Clem A (2010) A current perspective on leishmaniasis. *J Glob Infect Dis* 2:124
7. Anwar, F., Shah, I. A., & Zeeshan, M. (2018). Prevalence of Syphilis in Blood Donors in District Mardan Khyber Pakhtunkhwa Pakistan. *International Journal of Contemporary Research and Review*, 9(07), 20257-20261.

8. Desjeux P (2001) The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 95:239–243
9. Durrani AZ, Durrani HZ, Kamal N (2012) Prevalence of Leishmania in sand fly in Pakistan. *Pak J Zool* 44(1):61–65
10. Qamar, Z., Anwar, F., Ahmad, R., Haq, I., Khan, A. M. K., Hussain, R., ... & Khan, J. (2021). Prevalence of Hepatitis C virus and determination of its genotypes in subjects of Tehsil Daggar District Buner, KP, Pakistan. *Clinical Epidemiology and Global Health*, 12, 100809.
11. Simon Brooker NM, Adil K, Agha S, Reithinger R, Rowland M, Ali I, Kolaczinski J (2004) Leishmaniasis in refugee and local Pakistani populations. *Emerg Infect Dis* 10:1681.
12. Ali A, Rehman TU, Qureshi NA, Rahman HU (2016) New endemic focus of cutaneous leishmaniasis in Pakistan and future epidemics threats. *Asian Pac J Trop Dis* 6:155–159
13. Kassi M, Kassi M, Afghan AK, Rehman R, Kasi PM (2008) Marring leishmaniasis: the stigmatization and the impact of cutaneous leishmaniasis in Pakistan and Afghanistan. *PLoS Negl Trop Dis* 2:e259
14. Ahmad, S., Anwar, F., Ullah, I., Alam, M., Khan, J., Abid-ur-Rehman, F. A., & Ullah, R. (2020). Epidemiological and clinical manifestation of dengue virus infection: A Recent Report of 2018 from District Battagram Khyber Pakhtunkhwa. *International Journal of Mosquito Research*, 7(6, Part A), 5-8.
15. Anwar, F., Khan, M., Salman, M., Ahmad, S., Ullah, F., Khan, J., ... & Abbas, M. (2021). Seroprevalence of hepatitis B virus in the human population of district Buner Khyber Pakhtunkhwa Pakistan. *Clinical Epidemiology and Global Health*, 10, 100688.
16. Anwar, F., Tayyab, M., Salman, M., Abdullah, Din, M., Khan, J., & Haq, I. (2020). Dengue outbreak 2018 in district Shangla KPK; clinical features and laboratory markers of dengue virus infection. *Future Virology*, 15(10), 693-699.
17. Anwar, F., Ahmad, S., Haroon, M., Haq, I. U., Khan, H. U., Khan, J., ... & Shah, I. A. (2019). Dengue virus epidemics: A recent report of 2017 from district Mardan, Khyber Pakhtunkhwa province, Pakistan. *International Journal of Mosquito Research*, 6(1), 46-49.
18. Anwar, F., Khan, M., Rehman, N., Akbar, F., Ahmad, S., Yousaf, M., ... & Zeeshan, M. (2021). Review and forecasting on COVID-19 outbreak; An insight to in silico candidate drug discovery. *Bioscience Research*, 1198-1210.
19. Anwar, F., Zubair, M., Shah, M., Ahmad, S., Mehmood, M., Bakht, S., ... & Shah, O. U. (2021). Molecular epidemiology of SARS-COV-2 in Mardan, Khyber Pakhtunkhwa Pakistan: A real world clinical experience. *Bioscience Research*, 1608-1613.
20. Rowland, M., Munir, A., Durrani, N., Noyes, H., & Reayburn, H. (1999). An outbreak of cutaneous leishmaniasis in an Afghan refugee settlement in north-west Pakistan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93(2), 133-136.

21. Kumar, R., Bumb, R. A., & Salotra, P. (2010). Evaluation of localized and systemic immune responses in cutaneous leishmaniasis caused by *Leishmania tropica*: interleukin-8, monocyte chemotactic protein-1 and nitric oxide are major regulatory factors. *Immunology*, *130*(2), 193-201.
22. Desjeux, P. (2001). The increase in risk factors for leishmaniasis worldwide. *Transactions of the royal society of tropical medicine and hygiene*, *95*(3), 239-243.
23. Edrissian, G. H., Mohammadi, M., Kanani, A., Afshar, A., Hafezi, R., Ghorbani, M., & Gharagozloo, A. R. (1990). Bacterial infections in suspected cutaneous leishmaniasis lesions. *Bulletin of the world Health organization*, *68*(4), 473.
24. Anwar, F., Tayyab, M., Khan, J., & Haq, I. (2020). COVID-19 and taking care and protection of patients with intellectual disabilities, need special care and equity.
25. Anwar, F., Tayyab, M., Haq, I., & Shah, O. U. (2021). Viral overload of COVID-19 pandemics: Overweight people a soft target to get an infection.
26. Afghan, A. K., Kassi, M., Kasi, P. M., Ayub, A., Kakar, N., & Marri, S. M. (2011). Clinical manifestations and distribution of cutaneous leishmaniasis in Pakistan. *Journal of tropical medicine*, 2011.