Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/tf9r9q14

ASSESSING FREQUENCY AND RISK FACTORS RESPONSIBLE FOR CUTANEOUS LEISHMANIA AMONG PATIENTS REPORTED AT THE RURAL HEALTH CENTER, MANIKHWA DISTRICT SHERANI, PAKISTAN

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ABSTRACT

Background: Pakistan faces significant obstacles concerning cutaneous leishmaniasis (CL), as the disease's distribution is relatively uneven.

Aim: This study aims to determine the frequency of cutaneous leishmaniasis (CL) in the district of Sherani, in the Province of Baluchistan, Pakistan.

Methods: This study was conducted at the rural health center of District Sherani, Pakistan. About 300 patients were included in this study. Giemsa staining was used to examine tissue and blood specimens from the lesion locations under a microscope to confirm CL. SPSS (version 26) was used using a questionnaire to evaluate the demographic data and afflicted ulcer locations gathered from study participants.

Results: It was found that 73% of suspected patients were positive and 27% were negative for CL. The face (37.5%) was the most affected body part. Children under the age of 15 were highly affected. The areas with the most positive CL cases were the Union Councils of Manikhawa (29%) and Dana Sar (30%). Most of the patients (74.4%) lived in mud houses.

Conclusion: The results showed that those who live in substandard conditions are more vulnerable to infection. Thus, it is important to stress the need to modify their lifestyles, behaviors, and level of illness awareness.

Keywords: Cutaneous leishmaniasis, Sand fly, Frequency, Risk factors, Sherani District, Pakistan

1. Introduction

The neglected tropical illness leishmaniasis is brought on by a complex and varied group of obligate protozoan parasites (Genus: Leishmania). These are spread to mammalian hosts by female phlebotomine Sandflies of the genus Phlebotomus [1]. The parasite has two distinct stages, the promastigote and the amastigote stage. In the amastigote stage, parasites exist inside human cells in non-motile, intracellular form. While the promastigote is motile, a flagellated form of the parasite is found in the sandfly's gut [2]. Leishmaniasis is endemic in Pakistan and most commonly manifests

as cutaneous leishmaniasis (CL). In Pakistan, leishmaniasis is endemic and typically presents as cutaneous leishmaniasis (CL). Although visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL) remain less common but more severe [3]. An estimated 2 million new cases of leishmaniasis are reported annually. Leishmania is transmitted to around 350 million people, and many different cases from 98 nations in Asia, southern Europe, Africa, and South America have been reported [4]. The most prevalent type, CL, results in skin lesions on exposed body areas, primarily ulcers. These may result in severe disabilities or stigma, as well as permanent scarring. The Americas, the Mediterranean basin, the Middle East, and Central Asia account for about 95% of CL occurrences [5]. After malaria, human-CL is the most prevalent vector-borne infection in Pakistan and is endemic in several regions. In Pakistan, there are 37 out of 70 species of sandflies that can spread disease to healthy hosts [6]. CL has spread to formerly non-endemic regions of Pakistan as a result of the movement of millions of refugees to North-Western Pakistan. Another significant issue that soldiers and peacekeeping personnel stationed in Afghanistan and Pakistan's tribal regions must deal with is leishmaniasis. Recent large-scale population migration increased cutaneous leishmaniasis spread.

According to Yohannes and colleagues' (2019) article, in well-defined endemic locations, the incidence rate is relatively higher in children but declines in adults, most likely due to immunity development [7]. Gradoni (2018) reported that most cases are restricted to households.

This restriction suggests a limited flight range of sandflies, anthroponotic transmission, or genetic susceptibility [8]. Various factors add to the spread of the disease, these factors mainly include sex, which points to behavioral patterns that increase vector exposure, along with age, house design, construction materials, and the presence of domestic animals [9]. In cases of Leishmania major infection, lesions tend to heal on their own in 3–9 months, but those caused by Leishmania tropical infection take approximately 6–15 months to recover. [10].

Moreover, several reports have been published on the different therapeutic responses to conventional treatment modalities in comparison to adults [11]. Antimonials are the most commonly prescribed treatment, while a range of medications and topical therapies have been attempted, their efficacy has varied [12]. Alternative therapies include amphotericin B, miltefosine, pentamidine isethionate, amphotericin B, granulocyte-macrophage colony-stimulating factor, thermal therapy, cryotherapy, and antifungal medications like ketoconazole, fluconazole, itraconazole, and paromomycin [13].

This study was carried out in the tribal district of Sherani, which is situated close to the Pak-Afghan border and regular border-crossing is the primary factor of the infection's spread. The objective was to determine how often suspected patients with skin lesions tested positive for cutaneous leishmaniasis (CL). The health officials in the Sherani area are advised to take precautions to contain the CL outbreak.

2. Material and Methods

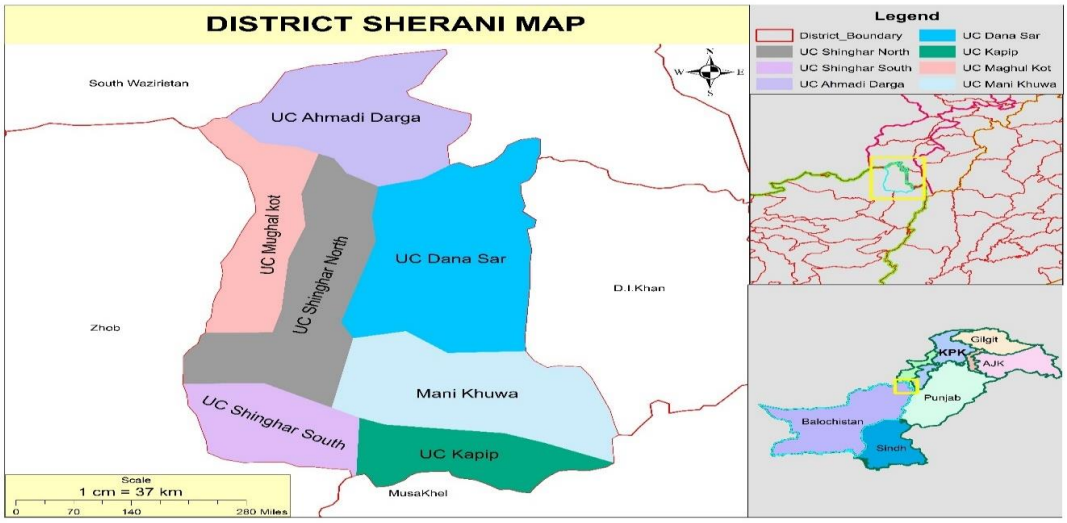
Study Design and Data Collection

This descriptive cross-sectional study was conducted for the duration of 1 year from January 2023 to December 2023 in the rural health center, Manikhwa district Sherani, Baluchistan Province (Figure.1), which comprises an area of 2800km2, having a population of 153,116 as of 2017 census. All the patients that reported one year of cutaneous leishmaniasis lesions on different areas of the body were included in this study upon consent, from six different union councils of district Sherani named (U/C Manikhawa, U/C Dana Sar, U/C Kapip, U/C Shinghar North, U/C Shinghar North, and U/C Maghul Kot) from the province Baluchistan of Pakistan. This study design was chosen to determine the various factors that may be the causes of Cutaneous Leishmania in the district of Sherani. A standard questionnaire was designed according to WHO/UNICEF protocol.

The study's objectives and procedures were explained to the patients, a written and informed consent was taken from the patients. Patients reported at the Rural Health Center were of age group between 1 to 50 years, including males and females. The medical records of the patients were used to collect the data concerning demographic characteristics, related age group, site, size and number of lesions,

gender of the patients, weight, travel history, and etiology. For younger children, questions were asked from their parents regarding the lesions on their body.

Figure 1: Study area map of District Sherani, Pakistan using ArcGIS software



All the patients who provided informed consent and lesions on different sites of the body were included in the study. Those who did not provide consent were excluded from the study.
After obtaining a complete clinical history, 300 samples were taken for isolation of *Leishmania* species.

Slide Preparation

Following the completion of the patient's clinical examination and history, sterile lancets were used to prick each patient's wound to obtain blood smears. Slides were allowed to air dry before being fixed with methyl alcohol and stained with Giemsa stain.
Giemsa stain-stained skin biopsies and direct examination of parasites in impression smears were used to diagnose cutaneous leishmaniasis (Anwar et al., 2021). One of the best histological stains for the histopathological diagnosis of parasites is Giemsa stain. The diagnosis of each patient was made using the results of the smear examination and the clinical observations. In the active lesion, the parasite was found in its Amastigotes form.

Data Analysis

This study included the data regarding, the frequency of males and females who were positively and negatively diagnosed with Cutaneous Leishmaniasis, the age of patients, patients reported with Cutaneous Leishmania from different areas of district Sherani, patients living in houses that are made of mud or sand and bricks or blocks, patients reported in different seasons and patients reported with Cutaneous Leishmania having lesions in different areas of the body.
The CL patients' data were entered into Microsoft Excel spreadsheets (Windows, 2016) and SPSS (v. 26) was used for analysis. After checking for a normal distribution, differences between diagnosed and undiagnosed groups for each risk factor were examined using chi-square tests, a nonparametric technique. If the p-value for the observed difference was less than 0.05, it was considered statistically significant.

3. Results

This study has focused on the frequency and risk factors that are responsible for the occurrence of Cutaneous Leishmania. In this study, 300 participants participated in a duration of 1 year from January 2023 to December 2023. They were the patients who visited the private clinic from all over the district

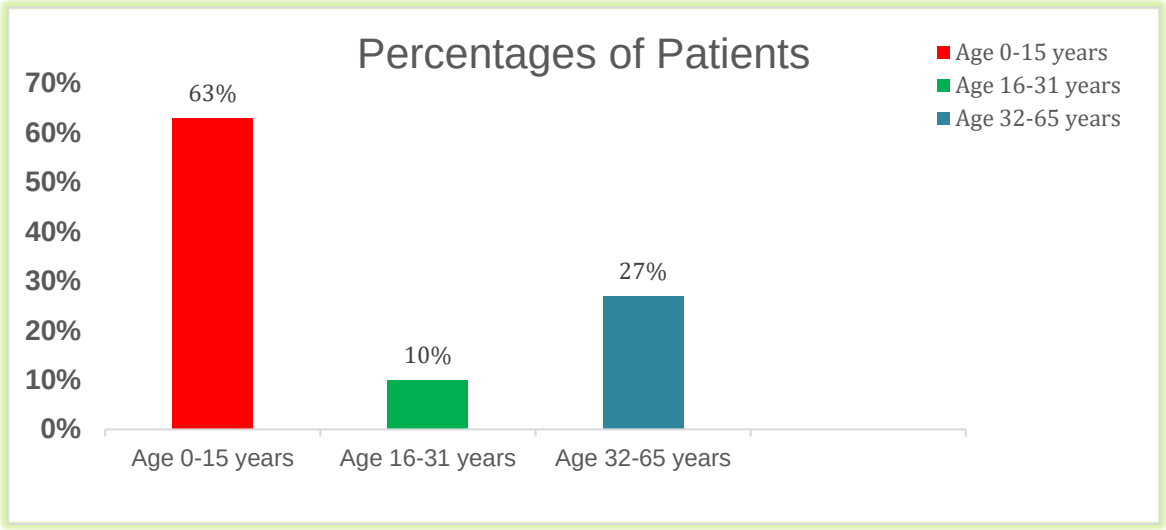
of Sherani. The participants, following informed consent, were asked about their living conditions, and the diagnosis was done using the staining technique. About 219 (73%) participants were detected as positive with CL and 81(27%) were detected as negative.
It was found that males were more positively detected with Cutaneous Leishmania (78.7%) than females (66.2%), as shown in Table 1.

Table 1: Frequency of Males and Females who were positively and negatively diagnosed with Cutaneous Leishmania

Variables	Patients with CL (%)	Patients without CL (%)	Total (%)
Percentage of Females	41.1	56.8	45.3
Percentage of Males	58.9	43.2	54.7

Patients 189 (63%) that reported in the period of 1 year were of age less than 15 years, 31 (10%) were from age 16-31 years and 80 (27%) were the age of greater than 31 years. (Figure 2)

Figure 2: Percentages of patients reported within age groups (0-15 years, 16-31 years, and 32-65 years



All 219 patients were confirmed cases of Cutaneous Leishmania, as the samples were tested with Gimme staining, as shown by the staining pictures under the microscope (figure 3).

Figure 3: Stained samples of Cutaneous Leishmania

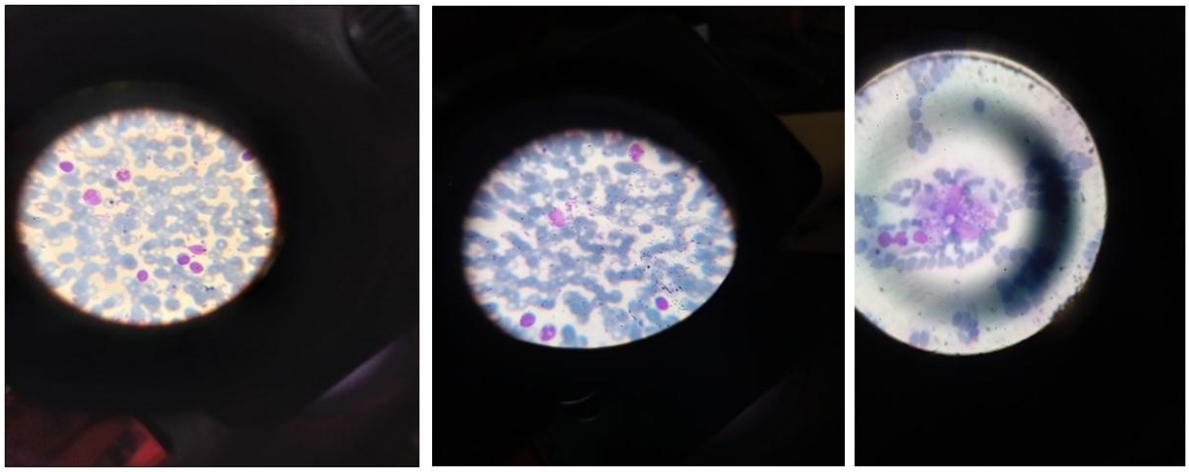
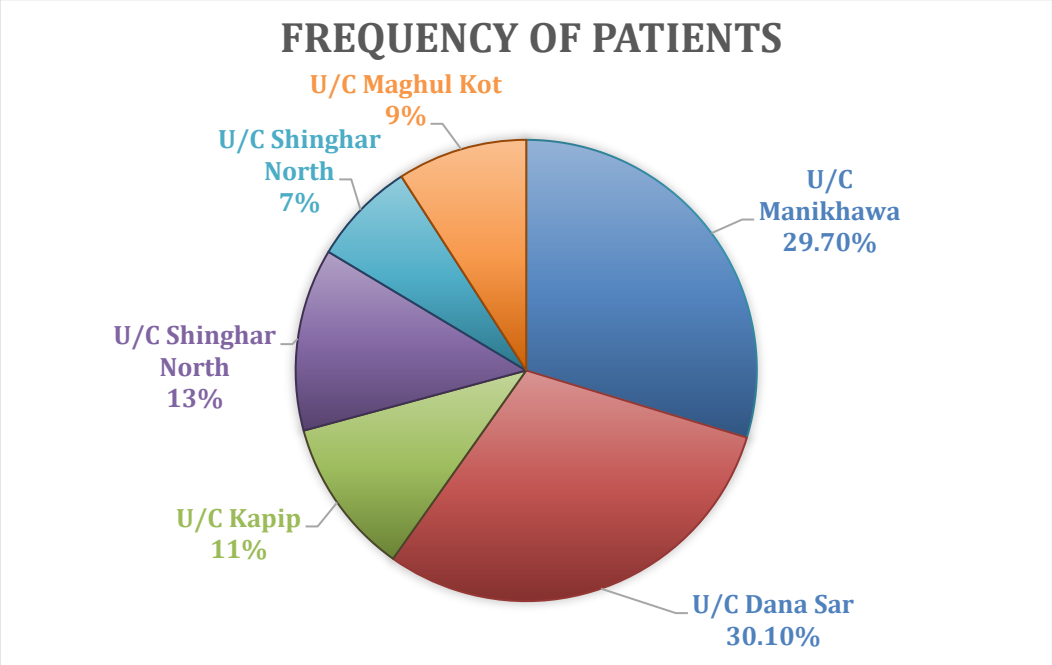


Figure 4 represents the frequency of patients with Cutaneous Leishmania concerning their area of residence, as the number of patients that visited was higher than the area of Dana Sar (30.1%). The lowest frequency of patients was from Shinghar North (7.3%).

Figure 4: Frequency of patients reported with Cutaneous Leishmania from different areas of district Sherani.



It has been seen that patients living in houses that are made of mud and sand were more (66.3%) prone to developing lesions, whether it is cutaneous leishmania or any other lesion-causing diseases. About 33.7% of patients lived in houses that were made of bricks and blocks. In the case of patients with Cutaneous Leishmania, about 67.6% of patients lived in houses made of mud or sand and 32.4% of patients lived in houses that are made of brick and blocks. (Table 2) (Figure 5).

Table 2: Frequency of patients living in houses that are made of mud or sand and bricks or blocks.

Variable	Patients with CL (%)	Patients without CL (%)
Patients living in Mud and Sand Houses	74.4	25.6
Patients living Brick and Block houses	70.3	29.7

Figure 5: Pictures of some mud houses in the district of Sherani

It has been seen that season affects the occurrence of Cutaneous Leishmania. In summers more patients were reported to the clinic who were diagnosed with C.L (41.6%), in winters 21.9% of patients, and in autumn 36.5% of C.L.

Figure 6: Frequency of patients reported in summer, winter, and autumn seasons

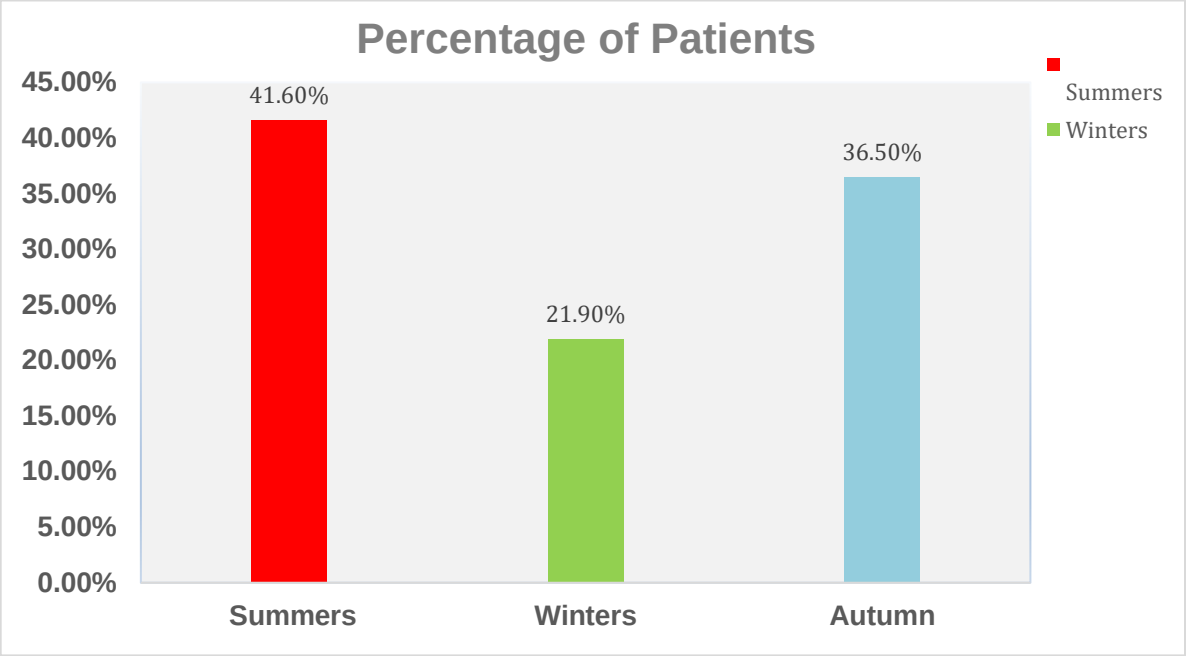


Table 3 represents the number of lesions that are present on the different parts of the body. On the cheek area, the number of lesions is more, 19 patients had single lesions, 18 had double lesions and 8 patients had three lesions. It was seen that females and males had more lesions on their cheeks compared to the other body areas (table 4). Figure 7 shows some of the patients with Cutaneous Leishmania.

Table 3: Frequency of patients reported with Cutaneous Leishmania having lesions in different areas of the body

Location of Lesion on the Body	Patients having Single Lesion	Patients having Double Lesions	Patients having three lesions
Under the eye	16	0	0
Ear helix	10	10	0
Chin	8	0	0
Lower Lip	8	0	0
Thigh	9	0	8
Foot	19	0	0
Cheek	47	18	8
Above Nose	20	0	0
Neck	8	0	0
Arm	10	0	0
Stomach	0	10	0
Hand	10	0	0

Table 4: Frequency of Females and Males with lesions on different parts of the body

Area of the Body	Female	Male
Under the eyes	18.4%	0.0%
Ear helix	0.0%	15.2%
Chin	8.8%	0.0%
Lower lip	8.8%	0.0%
Thigh	8.8%	7.3%
Foot	8.8%	7.9%
Cheek	37.5%	31.1%
Above Nose	0.0%	14.6%
Neck	8.8%	0.0%
Arm	0.0%	7.9%
Stomach	0.0%	7.9%
Hands	0.0%	7.9%

Chi-square value: 147.713, P-value: 0.000

Figure 7: Pictures of some patients diagnosed with Cutaneous Leishmania

Discussion

One of the biggest global public health issues, affecting both developed and developing countries, is Cutaneous Leishmania [14]. Many tropical countries across the world have endemic cases of leishmaniasis. The majority of people in Asian communities are from low-income neighborhoods with subpar lifestyles. Diseases transmitted by vectors are more prevalent in these people. Leishmaniasis is said to be prevalent not just in Pakistan but also in Afghanistan, China, India, and Iran [15]

A well-established risk factor for cutaneous leishmaniasis infection is the introduction of novel global species resulting from cross-border mobility. An outbreak of anthroponotic cutaneous leishmaniasis in a camp of Afghan refugees from northwest Pakistan in 1997 was caused by migrants from Kabul [16]. In 2021, nearly 20% of 3188 patients were reported from Bannu, Pakistan. The reason seems the high population density and high temperature. It has been seen that environments that are less hygienic and have high population density have higher Cutaneous Leishmania incidence [17]. The men have been reported to be more infected than females. The majority of men had work related to close contact with animals [18].

In our study, the patients who were diagnosed with CL were more males, aged less than 15 years, and were living in houses that are made of mud or sand. Similar results were reported previously [19]. Less female number of infected CL may be due to their confinement to their homes. A study from

2018, reported that in females 16.1% were infected as compared with males 24.5% [20]. According to a cross-sectional study conducted in 2021, CL data was retrieved from District Health Office D.I. Khan, which showed that men have been reported to have been diagnosed with CL than females. A higher number among them were in the age of up to 19 years [21]. The increase in the prevalence of CL in the younger population may be due to their exposure to infectious sand fly bites due to unhygienic living conditions. Cutaneous Leishmania has been observed in all ages but the high number has been reported to be of the age under 20. This may be due to the reason children play outside near the humid ground surfaces or in the shade of the trees, these are the places where sand fly breeds. High infection rate among children is also due to their immature immune systems [22] [23] [23][24]. Another study reported that 23% infection rate was of children <10 years old and 8% was of > 60 years old [25].

A current study has reported that about 74.4% of patients lived in houses that are made of mud or sand and 70.3% in blocks and brick houses. The high number of patients diagnosed with CL belonged to low economic status. A previous study concluded that the change in the pattern of the local and global climate, lifestyle, and agriculture is one of the main reasons contributing to the high vector population that leads to the increased CL spread [26]. According to the current study, 41.6% of cases were reported in summer, 21.90% in winter and 36.5% in autumn season. In another study, most cases were reported in moderate season [27]. Unlike some previous studies where cases were reported in the winter season [28]. Another study reported that the high prevalence was during the summer season. The change is due to the climate difference in different parts of the world so it will optimize the living conditions for the host reproduction. In our case, sand fly reproduction is more favorable in the summer seasons [28]. Similar to our study, a high incidence of CL was seen in the spring season [29] [30] [31].

Our study reported that a high number of patients (47) had a single lesion on their cheeks, 18 patients had double lesions on their cheeks, 19 reported lesion lesions on their feet, and 10 patients with single lesions on their hands. The frequency of females with lesions on their cheeks was higher at 37.5% than in males at 31.1%. Only males 7.9% were reported with lesions on their hands. About 8.8% of females were reported with lesions on their feet as compared to males whose frequency was 7.9%. Previous studies reported the same with most of the patients reported with single facial lesions [32] [33]. The face is the most exposed part to sand fly bites, preventive measures need to be taken such as using bed nets, wearing repellents, and avoiding outdoor activities [34]

According to Anwar et al, higher infection was reported in the hand regions, the legs, and then on the cheek regions [16]. According to Farahmand et al., patients' hands were the most affected site [35]. Another study reported that the patients with singular lesions were of high frequency 74.5%, patients with two lesions were 17.3% and 8.2% patients had three lesions [35]. Local and global climate pattern change has been the main contributor to changing the lifestyle of people which increases the vector population leading to increased Cutaneous Leishmania spread [25].

4. Conclusion

This study concludes that males are more infected with CL than females. The age group of 0-15 years was more exposed to the infection. Based on the findings, people living in poor conditions are more prone to the infection. Thus, improvement in living conditions, habits, and awareness about disease should be emphasized among people. Finally, the present data indicate that additional epidemiological research in this region and public health efforts to raise awareness of Cutaneous Leishmaniasis are needed. To avoid disease at both the individual and community levels, it is advised to assess the risk factors and develop control and management plans.

Acknowledgment

The authors would like to acknowledge the health department of Sherani and community members for providing invaluable assistance in collecting CL data.

Data Availability Statement
The corresponding author can provide the study's supporting information upon request.

Conflict of Interest:
The authors have no conflict of interest.

References

[1] T. Wijerathna, N. Gunathilaka, K. Gunawardena, Y. Fujii, and D. Gunasekara, "Detection of Leishmania donovani DNA within Field-Caught Phlebotomine Sand Flies (Diptera: Psychodidae) in Three Cutaneous Leishmaniasis Endemic Foci of Kurunegala District, Sri Lanka," *J Trop Med*, vol. 2021, 2021, doi: 10.1155/2021/6650388.

[2] H. M. Obaid and H. A. Shareef, "Epidemiological and clinical study of leishmaniasis in Kirkuk city, Iraq," *Iraqi Journal of Science*, vol. 59, no. 3, pp. 1195–1204, 2018, doi: 10.24996/IJS.2018.59.3A.7.

[3] I. Zeb, A. Ali, J. Nawab, M. Q. Khan, A. Kamil, and K. H. Tsai, "Cutaneous leishmaniasis in male schoolchildren in the upper and lower Dir districts of Khyber Pakhtunkhwa, and a review of previous record in Pakistan," *Acta Trop*, vol. 209, p. 105578, 2020, doi: 10.1016/j.actatropica.2020.105578.

[4] M. Saliba *et al.*, "Cutaneous leishmaniasis: an evolving disease with ancient roots," *Int J Dermatol*, vol. 58, no. 7, pp. 834–843, Jul. 2019, doi: 10.1111/ijd.14451.

[5] W. F. Refai, N. P. Madarasingha, B. Sumanasena, S. Weerasingha, R. Fernandopulle, and N. D. Karunaweera, "Cutaneous leishmaniasis in Sri Lanka: effect on quality of life," *Int J Dermatol*, vol. 57, no. 12, pp. 1442–1446, Dec. 2018, doi: 10.1111/ijd.14240.

[6] B. Kayani *et al.*, "Cutaneous Leishmaniasis in Pakistan: a neglected disease needing one health strategy," *BMC Infect Dis*, vol. 21, no. 1, Dec. 2021, doi: 10.1186/s12879-021-06327-w.

[7] M. Yohannes, Z. Abebe, and E. Boelee, "Prevalence and environmental determinants of cutaneous leishmaniasis in rural communities in Tigray, northern Ethiopia," *PLoS Negl Trop Dis*, vol. 13, no. 9, 2019, doi: 10.1371/journal.pntd.0007722.

[8] L. Gradoni, "A brief introduction to leishmaniasis epidemiology," in *The Leishmaniases: Old Neglected Tropical Diseases*, Springer International Publishing, 2018, pp. 1–13. doi: 10.1007/978-3-319-72386-0_1.

[9] D. Gebremichael, "Zoonotic impact and epidemiological changes of leishmaniasis in Ethiopia," *Open Vet J*, vol. 8, no. 4, pp. 432–440, 2018, doi: 10.4314/ovj.v8i4.13.

[10] H. Glans *et al.*, "Treatment outcome of imported cutaneous leishmaniasis among travelers and migrants infected with Leishmania major and Leishmania tropica: a retrospective study in European centers 2013 to 2019," *International Journal of Infectious Diseases*, vol. 122, pp. 375–381, 2022, doi: 10.1016/j.ijid.2022.06.025.

[11] Y. Hashiguchi, L. V. Nieto, N. C. Villegas, E. A. Gomez, and H. Kato, "Topical Treatment of Cutaneous Leishmaniasis: A Case Treated with A Glucantime-Based Lotion Experienced in Ecuador and A Mini Review," *Archives of Medical and Clinical Research*, vol. 01, no. 01, pp. 1–15, 2021, doi: 10.51941/amcr.2021.1103.

[12] H. Goto and J. A. L. Lindoso, "Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis," *Expert Review of Anti-Infective Therapy*, vol. 8, no. 4. Expert Reviews Ltd., pp. 419–433, 2010. doi: 10.1586/eri.10.19.

[13] H. J. C. de Vries and H. D. Schallig, "Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments," *American Journal of Clinical Dermatology*, vol. 23, no. 6. Adis, pp. 823–840, Nov. 01, 2022. doi: 10.1007/s40257-022-00726-8.

[14] D. B. H. FELLAH, M. RHAJAOUI, S. OUAHABI and M. LYAGOUBI, "Occurrence of Human Cutaneous Leishmaniasis in Zouagha My Yacoub Province (Morocco)," *Int J Agric Biol*, vol. 102, pp. 197–198, 2007.

[15] A. Z. Durrani, H. Z. Durrani, N. Kamal, and N. Mehmood, "Prevalence of cutaneous leishmaniasis in humans and dogs in Pakistan," *Pak J Zool*, vol. 43, no. 2, pp. 263–271, 2011.
[16] F. Anwar *et al.*, "Outbreak and Clinical Features of Cutaneous leishmaniasis in 2019 at District Charsadda, KP, Pakistan," *Ann Rom Soc Cell Biol*, vol. 25, no. 7, pp. 1583–6258, 2021.
[17] N. Tsirigotakis *et al.*, "Chapter 7 – Reduviid Predators," *Parasit Vectors*, vol. 3, no. 1, pp. 1–12, 2018.
[18] S. Mumtaz, A. H. Munir, M. Asghar, and Naheed, "Frequency and types of leishmaniasis in Khyber Pakhtunkhwa (KPK)," *Journal of Medical Sciences (Peshawar)*, vol. 24, no. 3, pp. 136–140, 2016.
[19] A. A. Abuzaid *et al.*, "Cutaneous Leishmaniasis in Saudi Arabia: A Comprehensive Overview," *Vector-Borne and Zoonotic Diseases*, vol. 17, no. 10, pp. 673–684, 2017, doi: 10.1089/vbz.2017.2119.
[20] M. Farahmand, H. Nahrevanian, H. A. Shirazi, S. Naeimi, and Z. Farzanehnejad, "An overview of a diagnostic and epidemiologic reappraisal of cutaneous leishmaniasis in Iran," *The Brazilian Journal of Infectious Diseases*, vol. 15, no. 1, pp. 17–21, 2011, doi: 10.1016/s1413-8670(11)70134-9.
[21] M. Rashid *et al.*, "Distribution of Cutaneous Leishmaniasis By Sex, Age Groups and Residence in Year 2020 in Cutaneous Leishmaniasis Population of District D.I.Khan, Pakistan," *Gomal Journal of Medical Sciences*, vol. 19, no. 1, pp. 28–34, 2021, doi: 10.46903/gjms/19.01.964.
[22] M. Yemisen, Y. Ulas, H. Celik, and N. Aksoy, "Epidemiological and clinical characteristics of 7172 patients with cutaneous leishmaniasis in Sanliurfa, between 2001 and 2008," *Int J Dermatol*, vol. 51, no. 3, pp. 300–304, 2012, doi: 10.1111/j.1365-4632.2011.05059.x.
[23] I. Bennis *et al.*, "Control of cutaneous leishmaniasis caused by Leishmania major in south-eastern Morocco," *Tropical Medicine and International Health*, vol. 20, no. 10, pp. 1297–1305, 2015, doi: 10.1111/tmi.12543.
[24] N. Haouas, O. Amer, A. Ishankyty, A. Alazmi, and I. Ishankyty, "Profile and geographical distribution of reported cutaneous leishmaniasis cases in Northwestern Saudi Arabia, from 2010 to 2013," *Asian Pac J Trop Med*, vol. 8, no. 4, pp. 287–291, 2015, doi: 10.1016/S1995-7645(14)60332-1.
[25] I. Sharifi *et al.*, "Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in preparation for an elimination plan," *Front Public Health*, vol. 11, no. April, pp. 1–22, 2023, doi: 10.3389/fpubh.2023.1091709.
[26] K. Holakouie-Naieni, E. Mostafavi, A. D. Boloorani, M. Mohebali, and R. Pakzad, "Spatial modeling of cutaneous leishmaniasis in Iran from 1983 to 2013," *Acta Trop*, vol. 166, pp. 67–73, 2017, doi: 10.1016/j.actatropica.2016.11.004.
[27] M. Khan, S. Ghayyur, and S. yasmin, "Distribution, clinical features, and epidemiology of cutaneous leishmaniasis: A case study of District Haripur, KPK, Pakistan," *Medical Reports*, vol. 2, no. September, p. 100025, 2023, doi: 10.1016/j.hmedic.2023.100025.
[28] T. T. Amin, H. I. Al-Mohammed, F. Kaliyadan, and B. S. Mohammed, "Cutaneous leishmaniasis in Al Hassa, Saudi Arabia: Epidemiological trends from 2000 to 2010," *Asian Pac J Trop Med*, vol. 6, no. 8, pp. 667–672, 2013, doi: 10.1016/S1995-7645(13)60116-9.
[29] F. Abedi-Astaneh *et al.*, "Risk mapping and situational analysis of cutaneous leishmaniasis in an endemic area of Central Iran: A GIS-based survey," *PLoS One*, vol. 11, no. 8, pp. 1–16, 2016, doi: 10.1371/journal.pone.0161317.
[30] M. A. and A. M. K. Hayat, U., Ayaz, S., "Cutaneous Leishmaniasis : Its Prevalence and Role of PCR in its Detection," *Journal of Islamabad Medical & Dental College: JIMDC*, vol. 4, no. 1, pp. 15–18, 2015.
[31] M. Khosravani, M. D. Moemenbellah-Fard, M. Sharafi, and A. Rafat-Panah, "Epidemiologic profile of oriental sore caused by Leishmania parasites in a new endemic focus of cutaneous leishmaniasis, southern Iran," *Journal of Parasitic Diseases*, vol. 40, no. 3, pp. 1077–1081, 2016, doi: 10.1007/s12639-014-0637-x.

[32] S. Ayaz *et al.*, "Cutaneous leishmaniasis in Karak, Pakistan: Report of an outbreak and comparison of diagnostic techniques," *Afr J Biotechnol*, vol. 10, no. 48, pp. 9908–9910, 2011, doi: 10.5897/ajb10.1987.

[33] M. Fakhar, M. Karamian, M. A. Ghatee, W. R. Taylor, H. Pazoki Ghohe, and S. A. Rasooli, "Distribution pattern of anthroponotic cutaneous leishmaniasis caused by Leishmania tropica in Western Afghanistan during 2013-2014," *Acta Trop*, vol. 176, no. July, pp. 22–28, 2017, doi: 10.1016/j.actatropica.2017.07.028.

[34] I. Zeb, A. Ali, J. Nawab, M. Q. Khan, A. Kamil, and K. H. Tsai, "Cutaneous leishmaniasis in male schoolchildren in the upper and lower Dir districts of Khyber Pakhtunkhwa, and a review of previous record in Pakistan," *Acta Trop*, vol. 209, no. February, p. 105578, 2020, doi: 10.1016/j.actatropica.2020.105578.

[35] M. Farahmand, H. Nahrevanian, H. A. Shirazi, S. Naeimi, and Z. Farzanehnejad, "An overview of a diagnostic and epidemiologic reappraisal of cutaneous leishmaniasis in Iran," *The Brazilian Journal of Infectious Diseases*, vol. 15, no. 1, pp. 17–21, 2011, doi: 10.1016/s1413-8670(11)70134-9.