Pakistan J. Zool., pp 1-10, 2025. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240128153014

A Retrospective Study on Human Cutaneous Leishmaniasis (CL) and First Report on Visceral Leishmaniasis (VL) from Islamabad, Pakistan During 2012-2023

Sundas Zahra[1], Wajiha Ali[1], Huma Khan[1], Ahmareen Khalid[2], Asma Khattak[2], Nida Khan[3], Minahil Attiq[1], Guo-Jing Yang[4,5], Rana Muhammad Kamran Shabbir[6] and Haroon Ahmed[1]*

[1]Department of Biosciences, COMSATS University Islamabad, Park Road, Chak Shahzad, Islamabad, Pakistan
[2]Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad, Pakistan
[3]Department of Medicine, Lady Reading Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan
[4]Key Laboratory of Tropical Diseases Control, National Health Commission of Peoples Republic of China, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University, Haikou, Hainan, P.R. China.
[5]Key Laboratory of Tropical Translational Medicine of Ministry of Education and School of Tropical Medicine and Laboratory Medicine. R.P. China
[6]Department of Zoology, Division of Science and Technology, University of Education Lahore, Punjab, Pakistan

Article Information
Received 28 January 2024
Revised 05 April 2024
Accepted 18 April 2024
Available online 08 July 2025
(early access)

Authors' Contribution
SZ, WA, HK performed the data collection, its documentation, analysis, and AHK and ASK assisted in the data collection and methodology section. HA and GJY supervised the study. NK, MA and RMKS critically review and revised the manuscript. All authors read and approved the final manuscript.

Key words
Leishmaniasis, Cutaneous leishmaniasis, Mucocutaneous leishmaniasis, Visceral leishmaniasis, Human, Pakistan

ABSTRACT

In Pakistan, leishmaniasis is the second most common disease after malaria and is a neglected tropical vector-borne illness that affects public health globally. No previous epidemiological investigation of human leishmaniasis has been reported in Islamabad, Pakistan. Therefore, the current study aimed to determine the prevalence of human leishmaniasis for the first time in the study area. The current retrospective study was conducted on suspected cases of human leishmaniasis reported in Pakistan Institute of Medical Sciences (PIMS) Hospital, Islamabad, during the years 2012-2023. All epidemiological and pathological information's were collected. For statistical analysis, the chi-square ($\chi 2$) test was applied, and a p value <0.05 was considered significant. A total of 290 suspected cases of human leishmaniasis were reported, and 87.2% (253/290) were confirmed positive, comprising 97.3% (247/253) cutaneous, 2% (5/253) mucocutaneous, and 0.4% (1/253) visceral leishmaniasis cases. The highest frequency of positive cases was observed in November 2012 (6.2%, 18/290), followed by March 2013 (5.2%, 15/290). The majority of leishmaniasis cases were recorded in the age group 21-30 (24.5%), followed by age groups 31-40 (14.5%) and 11-20 (13.8%), respectively. Gender-wise findings revealed that the number of males (48.9%) were higher as compared to females (38.3%). In addition, the majority (98.6%, 286/290) of the cases had only one lesion formation, and the most frequent lesion sites were skin (42.8%, 124/290) followed by the face and head areas (28.9%, 84/290). The current findings revealed an increase in the frequency of human cutaneous leishmaniasis in Islamabad during 2012 and 2023. However, further epidemiological, and serological studies are required to determine the prevalence of human leishmaniasis in Pakistan.

* Corresponding author: haroonahmed@comsats.edu.pk
0030-9923/2025/0001-0001 $ 9.00/0

INTRODUCTION

Leishmaniasis, a group of vector-borne diseases, is caused by a eukaryotic protozoan parasite of genus *Leishmania* that belongs to the family Trypanomastidae (Roatt *et al*., 2020) and around 20 species of *Leishmania* are known to cause leishmaniasis in humans (Cecílio *et al*., 2022; Seaman *et al*., 1996). This parasite is transmitted through the bite of infected female phlebotomine sandflies

that sucks blood of the host (Kayani *et al*., 2021). Based on the causative *Leishmania* species, its location and immune response of the host, there are three major types of leishmaniasis, cutaneous (CL), mucocutaneous (MCL) and visceral (VL) leishmaniasis (Burza *et al*., 2018). CL displays single to multiple skin ulcers, lesions, or nodular lesions, MCL affects mucosal membranes and the VL involves infection of internal organs, such as the liver, spleen, and bone marrow, and it is lethal if not treated appropriately (Burza *et al*., 2018; Chappuis *et al*., 2007; Pearson and Sousa, 1996).

Leishmaniasis is considered a global public health issue and is one of the seven neglected tropical diseases worldwide according to World Health Organization (WHO Reports, 2023; de Souza *et al*., 2018). It is one of the most unrecognized tropical diseases, endemic in 98 countries around the world and estimated 350 million people at risk globally (Mcgwire *et al*., 2014; Jorjani *et al*., 2019). Approximately 0.2 to 0.4 million cases of VL and 0.7 to 1.3 million cases of CL are reported annually, with an estimated 20,000 deaths per year (Alvar *et al*., 2012). The 90% of VL cases are mostly reported from Bangladesh, Brazil, India, Kenya, Somalia, South Sudan, and Ethiopia (Hailu *et al*., 2016; Alvar *et al*., 2012) and 95% of CL cases occur in the Mediterranean basin, the Middle East, South America, and Central Asia (Torres-Guerrero *et al*., 2017; WHO, 2023). In 2018, over 85% of new CL cases reported to the WHO were from Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran, Iraq, Pakistan, Syria, and Tunisia (WHO, 2020; Moein *et al*., 2018). In Pakistan, around 21,700–35,700 cases of cutaneous leishmaniasis are reported annually (Alvar *et al*., 2012). CL is endemic in regions of Pakistan including Baluchistan, Punjab, and both CL and VL in Khyber Pakhtunkhwa (KP) (Alvar *et al*., 2012; Khan *et al*., 2019, 2021; Hussain *et al*., 2018). Moreover, the number of cases reported in non-endemic areas of Pakistan are also increasing due to massive migration from endemic areas of country and abroad (Kassi *et al*., 2008). CL has two main forms; an anthroponotic form caused by *Leishmania tropica* mostly found in urban areas while zoonotic form caused by *Leishmania major* is found in rural areas of Pakistan (Afghan *et al*., 2011).

The formation of small erythema on site of bite is the first sign of leishmaniasis infection and due to inflammatory response, it develops into an open ulcer or visceral lesion (Thakur *et al*., 2020). CL causes formation of one or more ulcerated lesions on exposed body parts (Pearson and Sousa, 1996) including face, forearms, legs, and areas where sand-fly bites usually occur (Ahmed *et al*., 2019; Al-Dhalimi *et al*., 2023). The *Leishmania* parasite goes through morphological changes (amastigotes and the promastigotes) each time it transfers between the host and the vector (Mandell *et al*., 2022). In all diagnostic methods for leishmaniasis, parasitological methods are mostly preferred, and common parasitological test is histopathology, an *in vitro* examination of the tissue or skin of suspected lesion, in which skin and mucosal parts are stained to detect presence of parasite (de Vries *et al*., 2015). Moreover, microscopical diagnosis includes the staining of smears for confirmation of *Leishmania* amastigotes in the affected tissues aspirates, smears, or biopsies (de Vries *et al*., 2015; Boelaert *et al*., 2007). The most appropriate antileishmanial treatment has not been asserted since various species of *Leishmania* display different symptoms (Kotb and Agil, 2015). Although many treatments are available, only chemotherapy has been found to be satisfactory for treating leishmaniasis (Croft and Coombs, 2003). Pentavalent antimonial have been used as first-line treatments against leishmaniasis disease for almost seventy years (Frézard *et al*., 2009; Torres-Guerrero *et al*., 2017) but due to its adverse effects its use as a treatment is affected (Llanos-Cuentas *et al*., 2008; Freitas-Junior *et al*., 2012).

CL is a neglected disease because of its low mortality, so people do not take preventive and control measures seriously, resulting in an increase in its incidence worldwide (Reithinger *et al*., 2007). Considering the increasing prevalence of CL in various parts of Pakistan, it is crucial to provide immediate healthcare support and public awareness campaigns for proper prevention of its further spread around the country (Sajjad *et al*., 2022). Epidemiological studies and information regarding leishmaniasis are also insufficient in Pakistan (Khan *et al*., 2016).

There is paucity of data on leishmaniasis in both endemic and non-endemic regions of Pakistan. Previously, only few a studies have been conducted in Pakistan's endemic regions, such as Baluchistan (Khan *et al*., 2021), KP (Naz *et al*., 2022), Punjab (Khan *et al*., 2016), and other locations of Punjab (i.e. Waziristan) (Hussain *et al*., 2018). To the best of our knowledge, this is the first epidemiological study that was designed to investigate histopathological assessed suspected cases of human leishmaniasis in Islamabad, Pakistan, reported during the years 2012-2023.

MATERIALS AND METHODS

Study area

This study was conducted in Islamabad (33°49' N, 72°24' E), Pakistan. Islamabad city has a total area of 906.50 square kilometers, with both urban and rural regions; the urban area is 220.15 sq. km, and the rural area is 466.20 sq. km. It is located on northern region of Pakistan

featuring a subtropical humid climate, cold winters, and hot, humid summers along with monsoon season (CDA, 2022). Studies have shown that the northern and western regions of Pakistan have higher prevalence of CL while VL is endemic in regions with mountain and farming communities (Tazerji *et al*., 2022). The data required for this study was retrieved from Pakistan Institute of Medical Sciences (PIMS) Hospital in Islamabad.

Study design

The current retrospective study was carried out over a six-months period from July 2023 to December 2023. A total of 290 suspected cases of human leishmaniasis were included in the study, which were registered in the PIMS hospital during 2012-2023. The patient data were retrieved from the Pathology Department of the PIMS Hospital, Islamabad, with the department head's explicit consent

The data included the following variables for epidemiological investigation; gender, age, month, year of reported cases, diagnostic tests used, number of lesions, lesion localization and pathological findings of each patient.

Clinical features

The suspected cases were evaluated using histopathology test and Fine Needle Aspiration Cytology (FNAC) test. Pathological findings of these reports confirmed CL in most of the cases, but few reports lacked specification of type of leishmaniasis (CL, MCL, or VL). We found few cases suspected to have MCL and VL leishmaniasis since their pathological characteristics resembled the mucocutaneous and visceral type of human leishmaniasis according to World Health Organization report (WHO, 2020).

Statistical analysis

The data was documented into MS excel sheets and statistically analyzed using SPSS software (version 26). The chi-square test was applied for statistical analysis and a p value <0.05 was considered significant. Frequency of variables like year, month, age, gender, number of lesion and their localization, diagnostic tests, and pathological findings of all patients were calculated using formula; Reported cases/ Total cases *100. Prevalence was calculated for confirmed positive cases of CL and suspected cases of MCL and VL by year, age, and gender, using the formula: Positive cases/ Reported cases *100.

RESULTS

In the current study, 290 suspected cases of human leishmaniasis were reported between February 2012 and July 2023 in PIMS hospital, Islamabad, of which 87.2% were confirmed positive (97.6% CL, 2% MCL, and 0.4% VL) and 12.8% were negative. The frequency of positive cases was higher among males (48.9%) compared to females (38.3%). The overall prevalence rate was 87.2% (253/290), and the average percentage of positive cases reported in the past 12 years was 7.8% (Fig. 1).

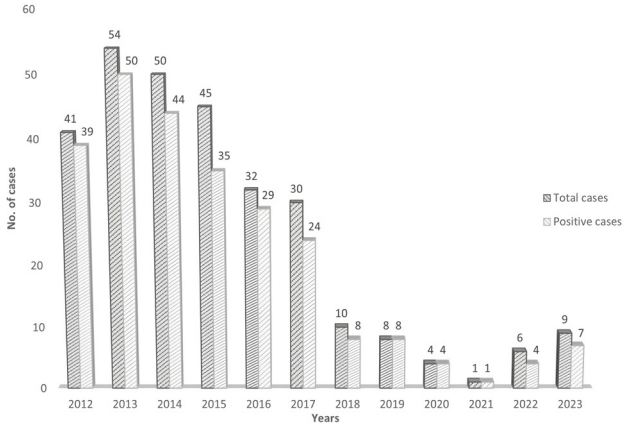

Fig. 1. Year wise prevalence among suspected cases of human leishmaniasis.

Year wise distribution

The highest number of positive cases was observed in the year 2013 (17.2%), and the percentage subsequently decreased significantly until 2021 (0.3%). The lowest frequency was observed during the years 2019-2021, and the reason might be the masking effect of the COVID-19 outbreak in these years. After the year 2021, a sudden increase has been observed in the percentage of positive cases from 1.4% in 2022 to 2.4% in 2023 (Fig. 1). This sudden rise in the number of positive cases suggests an increase in the prevalence rate of leishmaniasis in Islamabad, Pakistan. The statistical analysis showed a significant association among different years ($p < 0.05$) (Table I).

Month wise distribution

The highest frequency of positive cases was observed in the month of November 2012 (6.2%) (Fig. 2a), followed by March 2013 (5.2%) (Fig. 2b), and December 2012 (3.8%) (Fig. 2a). The study observed a varied distribution of positive cases in each month of each year (2012-2023). However, from 2012-2023, February had the highest frequency (14.1%), followed by March at 13.4% with the second highest positive cases. Moreover, the lowest frequency was observed in the month of October, with 2.4% positive cases.

Table I. Year wise distribution and prevalence of human leishmaniasis.

S. No	Year	Total cases	Positive (%)	Negative (%)	Prevalence (%)	Statistical analysis
1	2012	41	39 (13.4)	2 (0.7)	95.1	$\chi^2 = 179.407$
2	2013	54	50 (17.2)	4 (1.4)	92.6	d.f = 11
3	2014	50	44 (15.2)	6 (2.1)	88	p = 0.00
4	2015	45	35 (12.1)	10 (3.4)	77.8	
5	2016	32	29 (10.0)	3 (1.0)	90.6	
6	2017	30	24 (8.2)	6 (2.1)	80	
7	2018	10	8 (2.8)	2 (0.7)	80	
8	2019	8	8 (2.8)	0	100	
9	2020	4	4 (1.4)	0	100	
10	2021	1	1 (0.3)	0	100	
11	2022	6	4 (1.4)	2 (0.7)	66.7	
12	2023	9	7 (2.4)	2 (0.7)	77.8	
	Total	290	253 (87.2)	37 (12.8)	87.2	

Prevalence of CL, MCL, and VL leishmaniasis

In addition to CL, the pathological characteristics of a few cases resembled MCL and VL. Among 253 positive cases, 97.6% were confirmed CL while 2% positive cases were suspected for MCL and 0.4% for VL (Table II).

Age and gender wise distribution

A total of 70 CL positive and 1 MCL positive case were identified in the age group 21–30, which had the highest frequency of positive cases (24.5%). Followed by age group 31–40 (14.5%) had 41 CL, 1 MCL and age group 11–20 (13.8%) had 38 CL, 1 MCL, and 1 VL positive cases. A single MCL-positive case was identified in the age groups 61–70 and >80, while positive cases in other age groups were identified as CL-positive. The lowest percentage was observed in age groups aged >80 and 0–10 years old, with 2.1% and 2.4%, respectively (Table II, Fig. 3). The statistical analysis revealed a significant association ($p<0.05$) between positive cases in all age groups.

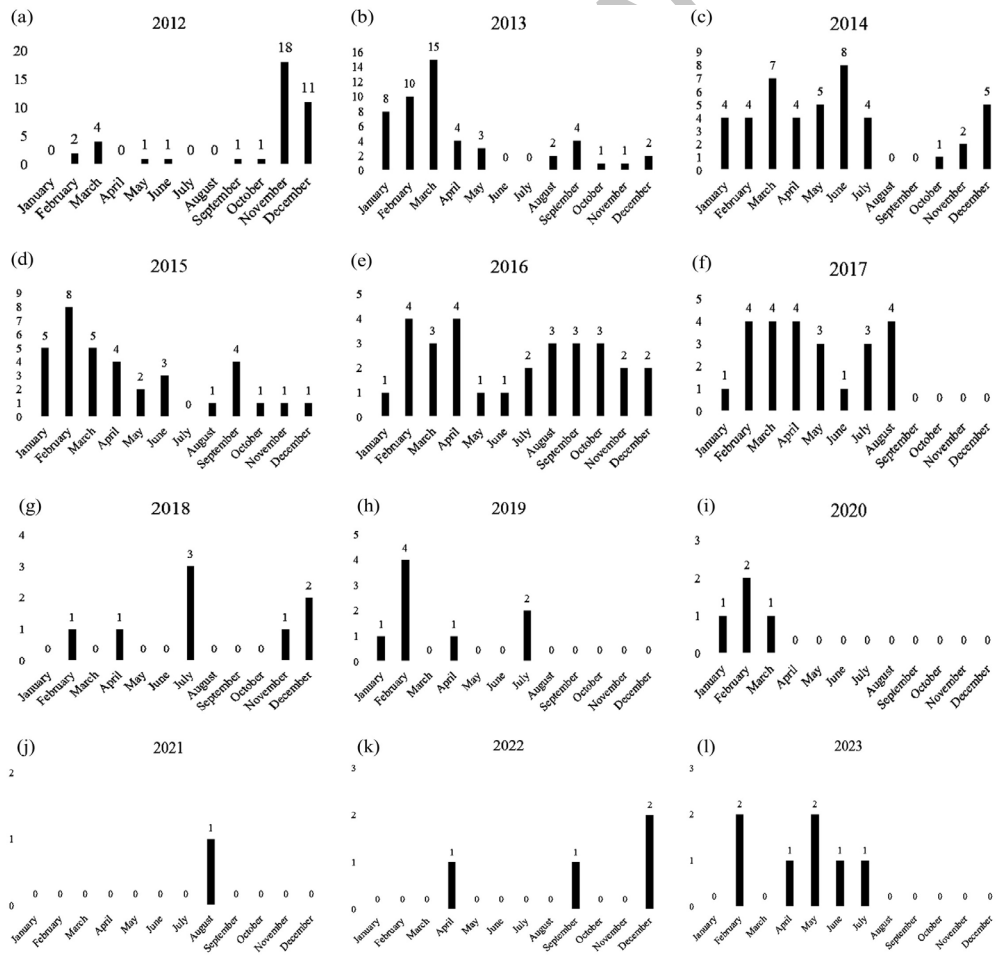

Fig. 2. Month wise distribution of positive cases of human leishmaniasis during 2012 to 2023.

Table II. Prevalence of cutaneous (CL), mucocutaneous (MCL) and visceral (VL) leishmaniasis by age and gender.

Variables	Total cases	Positive cases			Total number of positive cases (%)	Negative cases (%)	Prevalence (%)	Statistical analysis
		CL	MCL	VL				
Age (years)								
0-10	9	7	0	0	7 (2.4)	2 (0.7)	77.8	$\chi^2 = 133.062$ d.f = 71 p = 0.00
11-20	46	38	1	1	40 (13.8)	6 (2.1)	87	
21-30	79	70	1	0	71 (24.5)	8 (2.8)	89.9	
31-40	47	41	1	0	42 (14.5)	5 (1.7)	89.4	
41-50	31	25	0	0	25 (8.6)	6 (2.1)	80.6	
51-60	28	24	0	0	24 (8.3)	4 (1.4)	85.7	
61-70	30	26	1	0	27 (9.2)	3 (1.0)	90	
71-80	14	11	0	0	11 (3.8)	3 (1.0)	78.6	
>80	6	5	1	0	6 (2.1)	0	100	
Gender								
Male	160	137	4	1	142 (48.9)	18 (6.2)	88.8	$\chi^2 = 3.103$ d.f = 1 p = 0.78
Female	130	110	1	0	111 (38.3)	19 (6.6)	85.4	
Total	290	247	5	1	253 (87.2)	37 (12.8)	87.2	

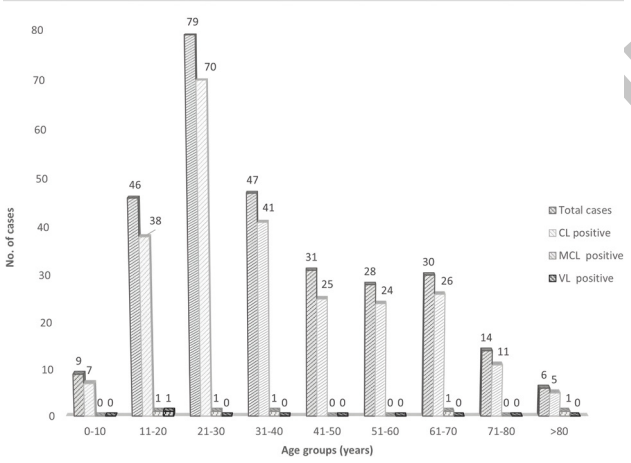

Fig. 3. Age wise frequency of cutaneous (CL), mucocutaneous (MCL) and visceral (VL) leishmaniasis cases among suspected cases

In gender-wise distribution, the number of positive cases was higher (48.9%) in males compared to females (38.3%). The results revealed that there were 137 CL, 4 MCL, and 1 VL positive cases in males (142/290), while 110 CL and only 1 MCL positive case in females (111/290) were reported (Table II, Fig. 4). The gender-wise distribution was statistically insignificant (p>0.05).

Diagnostic tests

The diagnosis of leishmaniasis cases was confirmed by histopathological testing, which was used in 71.73% cases and the FNAC test in 20.34% cases, and both tests were conducted on 7.93% cases. These tests were performed on reported cases that were suspected of having CL, however, a small number of patients also displayed clinical traits common to VL and MCL (Table III).

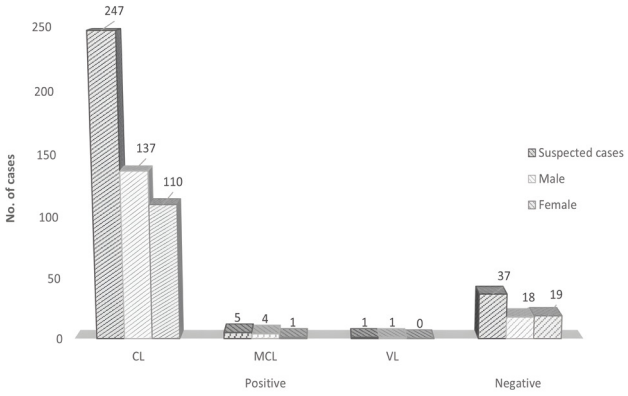

Fig. 4. Gender wise frequency of cutaneous (CL), mucocutaneous (MCL) and visceral (VL) leishmaniasis cases among suspected cases.

Lesion numbers and localization

The majority of the patients (286, 98.62%) had only one number of lesions formed, while 3 patients (1.03%) and one (0.34%) had two and three lesions, respectively (Table III).

Table III. Clinical characteristics of suspected cases of human leishmaniasis.

Variables	Categories	Number (n)	Percentage (%)
Lesion localization	Skin	124	42.75
	Face and head	84	28.97
	Hands	27	9.31
	Upper limbs	23	7.93
	Lower limbs	24	8.28
	Other/multiple areas	8	2.76
Number of lesions	One	286	98.62
	Two	3	1.03
	Three	1	0.34
Diagnostic tests	Histopathology	208	71.73
	FNAC*	59	20.34
	Both	23	7.93
Diagnosis/ Pathological findings	Leishmaniasis (unspecified)	122	42.07
	Cutaneous leishmaniasis	131	45.17
	Non-leishmaniasis/others	37	12.76

Based on the site of lesion formation, out of 290 suspected cases, the majority had lesions on the skin (42.75%), followed by the face and head areas (28.97%), hands (9.31%), upper limbs (7.93%), lower limbs (8.28%) and 8 (2.8%) lesions were found on other areas of the body (Table III). Mucous areas were also affected, and a few lesions were observed in mouth areas like the mandible, parotid area, salivary gland, and nasal area.

Diagnosis/ pathological findings

The histopathological and FNAC tests verified 253 (87.2%) positive cases. Out of which 122 (42.07%) patients had leishmaniasis (type not specified in clinical reports), 131 (45.17%) had leishmaniasis on the skin, and 37 (12.76%) were not diagnosed with leishmaniasis of any type (Table III). After analyzing unspecified leishmaniasis cases mentioned in clinical reports, we observed that 97.3% cases were CL, 2% were MCL, and 0.4% VL. The clinical features of cases that are not identified show similarities to several kinds of leishmaniasis, according to the World Health Organization report (WHO, 2020).

DISCUSSSION

CL is considered endemic in many regions of Pakistan, including Baluchistan, Punjab, and both CL and VL in Khyber Pakhtunkhwa (KP) (Alvar *et al*., 2012; Khan *et al*., 2019, 2021; Hussain *et al*., 2018). However, CL is the second most prevalent vector-borne disease after malaria in Pakistan (Ejaz *et al*., 2007). According to our knowledge, not a single epidemiological study has been conducted in Islamabad, Pakistan. Therefore, the current study was designed to investigate and analyze the epidemiology and histopathology of human leishmaniasis in Islamabad, Pakistan. The findings of the current study revealed that 290 suspected cases of human leishmaniasis were reported during the years 2012-2023 of which 87.2% (253/290) were positive and 12.8% were negative. These positive cases included 97.6% CL, 2% MCL, and 0.4% VL.

Preliminary research conducted in Pakistan has shown that CL is more common than other forms of the disease, such as in Baluchistan (Khan *et al*., 2021), Rawalpindi and Peshawar (Naz *et al*., 2022), Waziristan (Hussain *et al*., 2018) and KP (Khan *et al*., 2016). However, VL is also prevalent in a few areas of KP, Pakistan (Khan *et al*., 2019). In addition, the studies from Pakistan's neighboring countries also revealed that CL is prevalent there, including in Iran (Norouzinezhad *et al*., 2016; Razavi *et al*., 2021), Afghanistan (Faulde *et al*., 2008; Fakhar *et al*., 2017), and India (Sharma *et al*., 2005; Aara *et al*., 2013). However, CL is not endemic in China (Lun *et al*., 2015).

The highest frequency of positive cases was observed in 2013 (17.2%), which decrease gradually until 2021 (0.3%). The years 2019-2021 were periods of COVID-19, and peoples insecurity about visiting hospitals during this outbreak or false diagnosis and masking effect of the coronavirus might be the reasons for this lowest percentage observed (Mouliou *et al*., 2021). But after the year 2021, a sudden rise has been observed, with 1.4% in the year 2022 reaching 2.4% in the present year 2023. This is an alarming situation because this rise in the infection rate can be a sign of increasing prevalence of CL in Islamabad or overall Pakistan. Moreover, a higher prevalence rate was observed in the month of November of 2012 followed by March of 2013. Similar results had been observed in study from Iran showing infection rate higher in winter-spring season (September to March) (Razavi *et al*., 2021) but a few other studies had shown inconsistent findings (Khan *et al*., 2021; Nighat *et al*., 2022).

In the present study, the infection rate was higher among males than females, which correlates with the other studies from Pakistan (Khan *et al*., 2016; Sajjjad *et al*., 2022), and India (Aara *et al*., 2013). Our findings contradict a study from Muzaffargarh (Nighat *et al*., 2022), revealed that the infection rate was higher among females compared to males. In our study results, individuals in the age group 21-30 had the highest number of positive cases, followed by 11-20 and 31-40, while adults aged >80 and children 0-10 had the lowest frequency. Similar results

have been observed in studies from India (Sharma *et al*., 2005) and Pakistan including KP (Khattak *et al*., 2022) and Muzaffargarh (Nighat *et al*., 2022). But in contrast, a few studies revealed a higher prevalence in children aged 0-15 (Hussain *et al*., 2018; Sajjjad *et al*., 2022).

Lesion formation in our study was observed in all 290 cases, and 286 (98.6%) had only one lesion, while the other 4 (1.4%) cases had two or more lesion counts. Correspondingly, a few other studies (Norouzinezhad *et al*., 2016; Nighat *et al*., 2022) relate to our findings that the frequency of number the of lesions is mostly one in CL cases. Moreover, in the current study, the site of lesion formation was higher on exposed areas like the skin, face, and head. Similar results were observed in studies from Baluchistan (Khan *et al*., 2021) and KP (Sajjjad *et al*., 2022). In contrast, a study conducted in Iran (Razavi *et al*., 2021) showed the lowest frequency of lesion formation on the face but highest frequency on the hands.

The human leishmaniasis epidemiology is shifting with the emergence of the diseases in different areas of the globe. The most common reasons for changes in the epidemic scenario are migration of human beings and variations in environment due to human activity (Kolaczinski *et al*., 2004; de Vries and Schallig, 2022). More studies are needed to assess this situation's severity in Pakistan and take preventive and control measures accordingly to prevent further spread of this scarring disease.

CONCLUSION

The current findings revealed that CL is more prevalent and that the general prevalence of human leishmaniasis is increasing in Islamabad, Pakistan. Nevertheless, our study lacked serological or molecular level evidence of human leishmaniasis, so it is recommended to conduct these studies in Pakistan, particularly in metropolitan cities like Islamabad where travel and migration from endemic areas are anticipated. These studies are required to obtain definite evidence of the burden of human leishmaniasis in Pakistan. Furthermore, increasing public awareness regarding prevention, better diagnostic techniques, and effective treatments can aid in the complete eradication of these life-threatening diseases from Pakistan.

DECLARATIONS

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

Ethical statement and IRB approval

The ethical approval was received from the Ethics Review Board of the Department of Biosciences at COMSATS University Islamabad.

Statement of conflict of interest

The authors have declared no conflict of interests.

REFRENCES

Aara, N., Khandelwal, K., Bumb, R.A., Mehta, R.D., Ghiya, B.C., Jakhar, R., Dodd, C., Salotra, P. and Satoskar, A.R., 2013. Clinco-epidemiologic study of cutaneous leishmaniasis in Bikaner, Rajasthan, India. *Am. J. trop. Med. Hyg.*, **89**: 111–115. https://doi.org/10.4269/ajtmh.12-0558

Afghan, A.K., Kassi, M., Kasi, P.M., Ayub, A., Kakar, N. and Marri, S.M., 2011. Clinical manifestations and distribution of cutaneous leishmaniasis in Pakistan. *J. trop. Med.*, **2011**: 359145. https://doi.org/10.1155/2011/359145

Ahmed, H., Khan, M., Rehman, H.U., Noor, H., Khan, N.A., Sheikh, M.A.K.J.A. and Ahmad, W., 2019. Cutaneous leishmaniasis pattern: A Pakistani perspective. *J. Ent. Zool.*, **7**: 868-873.

Al-Dhalimi, M.A. and Jasim, S.H., 2023. Dermoscopic evaluation of cutaneous leishmaniasis. *Arch Dermatol. Res.*, **315**: 531–540. https://doi.org/10.1007/s00403-022-02387-3

Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J. and den Boer, M., 2012. WHO Leishmaniasis Control Team., 2012. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*, **7**: e35671. https://doi.org/10.1371/journal.pone.0035671

Boelaert, M., Bhattacharya, S., Chappuis, F., El-Safi, S.H., Hailu, A., Mondal, D., Rijal, S., Sundar, S., Wasunna, M. and Peeling, R.W., 2007. Evaluation of rapid diagnostic tests: Visceral leishmaniasis. *Nat. Rev. Microbiol.*, **5**(**Supp. 11**): S31-S39. https://doi.org/10.1038/nrmicro1766

Burza, S., Croft, S.L. and Boelaert, M., 2018. Leishmaniasis. *Lancet*, **392**: 951–970. https://doi.org/10.1016/S0140-6736(18)31204-2

Capital Developmental Authority, 2022. *Facts and statistics, Islamabad*. https://www.cda.gov.pk/about_islamabad/vitalstats.asp

Cecílio, P., Cordeiro-da-Silva, A. and Oliveira, F., 2022. Sand flies: Basic information on the vectors of leishmaniasis and their interactions with Leishmania parasites. *Communication*, **5**: 305. https://doi.org/10.1038/s42003-022-03240-z

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, R.W., Alvar, J. and Boelaert, M., 2007. Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? *Nat. Rev. Microbiol.,* **5**: 873–882. https://doi.org/10.1038/nrmicro1748

Croft, S.L. and Coombs, G.H., 2003. Leishmaniasis–current chemotherapy and recent advances in the search for novel drugs. *Trends. Parasitol.*, **19**: 502-508. https://doi.org/10.1016/j.pt.2003.09.008

De Souza, A., Marins, D.S.S., Mathias, S.L., Monteiro, L.M., Yukuyama, M.N., Scarim, C.B., Löbenberg, R., and Bou-Chacra, N.A., 2018. Promising nanotherapy in treating leishmaniasis. *Int. J. Pharm.*, **547**: 421–431. https://doi.org/10.1016/j.ijpharm.2018.06.018

de Vries, H.J. and Schallig, H.D., 2022. Cutaneous leishmaniasis: A 2022 updated narrative review into diagnosis and management developments. *Am. J. clin. Dermatol.*, **23**: 823-840. https://doi.org/10.1007/s40257-022-00726-8

de Vries, H.J., Reedijk, S.H. and Schallig, H.D., 2015. Cutaneous leishmaniasis: Recent developments in diagnosis and management. *Am. J. Clin. Dermatol.*, **16**: 99-109. https://doi.org/10.1007/s40257-015-0114-z

Ejaz, A., Raza, N. and Iftikhar, N., 2007. Recurrent cutaneous leishmaniasis presenting as sporotrichoid abscesses: A rare presentation near Afghanistan border. *Dermatol. Online J.*, **13**: 15. https://doi.org/10.5070/D34ZZ1K7JX

Fakhar, M., Karamian, M., Ghatee, M.A., Taylor, W.R., Ghohe, H.P. and Rasooli, S.A., 2017. Distribution pattern of anthroponotic cutaneous leishmaniasis caused by Leishmania tropica in Western Afghanistan during 2013-2014. *Acta Trop.*, **176**: 22-28. https://doi.org/10.1016/j.actatropica.2017.07.028

Faulde, M., Schrader, J., Heyl, G. and Amirih, M., 2008. Differences in transmission seasons as an epidemiological tool for characterization of anthroponotic and zoonotic cutaneous leishmaniasis in northern Afghanistan. *Acta Trop.*, **105**: 131-138. https://doi.org/10.1016/j.actatropica.2007.10.011

Freitas-Junior, L.H., Chatelain, E., Kim, H.A. and Siqueira-Neto, J.L., 2012. Visceral leishmaniasis treatment: What do we have, what do we need and how to deliver it? *Int. J. Parasitol. Drug*, **2**: 11-19. https://doi.org/10.1016/j.ijpddr.2012.01.003

Frézard, F., Demicheli, C. and Ribeiro, R.R., 2009. Pentavalent antimonials: New perspectives for old drugs. *Molecules*, **14**: 2317-2336. https://doi.org/10.3390/molecules14072317

Hailu, T., Yimer, M., Mulu, W. and Abera, B., 2016. Challenges in visceral leishmaniasis control and elimination in the developing countries: A review. *J. Vector Borne Dis.*, **53**: 193.

Hussain, M., Munir, S., Khan, T.A., Khan, A., Ayaz, S., Jamal, M.A., Ahmed, I., Aziz, S., Watany, N., and Kasbari, M., 2018. Epidemiology of cutaneous leishmaniasis outbreak, Waziristan, Pakistan. *Emerg. Infect.*, **24**: 159–161. https://doi.org/10.3201/eid2401.170358

Jorjani, O., Mirkarimi, K., Charkazi, A., Shahamat, Y.D., Mehrbakhsh, Z. and Bagheri, A., 2019. The epidemiology of cutaneous leishmaniasis in Golestan Province, Iran: A cross-sectional study of 8-years. *Parasite Epidemiol.*, **5**: 2405-6731. https://doi.org/10.1016/j.parepi.2019.e00099

Kassi, M., Kassi, M., Afghan, A.K., Rehman, R. and Kasi, P.M., 2008. Marring leishmaniasis: The stigmatization and the impact of cutaneous leishmaniasis in Pakistan and Afghanistan. *PLoS Negl. Trop. Dis.*, **2**: e259. https://doi.org/10.1371/journal.pntd.0000259

Kayani, B., Sadiq, S., Rashid, H.B., Ahmed, N., Mahmood, A., Khaliq, M.S., Maqsood, R., Rashid, H., Hasan, S., Mushtaq, M.H., Zia, U.U. and Chaudhry, M., 2021. Cutaneous Leishmaniasis in Pakistan: A neglected disease needing one health strategy. *BMC Infect.*, **21**: 622. https://doi.org/10.1186/s12879-021-06327-w

Khan, A., Sajid, R., Gul, S., Hussain, A., Zehri, M.T., Naz, S., Simsek, S., Waseem, S., Afzal, M. S., Naqvi, S.K. Qasim, M. and Ahmed, H., 2021. Epidemiological and pathological characteristics of cutaneous Leishmaniasis from Baluchistan Province of Pakistan. *Parasitology*, **148**: 591–597. https://doi.org/10.1017/S0031182020002413

Khan, N.H., Bari, A.U., Hashim, R., Khan, I., Muneer, A., Shah, A., Wahid, S., Yardley, V., O'Neil, B. and Sutherland, C.J., 2016. Cutaneous leishmaniasis in Khyber Pakhtunkhwa province of Pakistan: Clinical diversity and species-level diagnosis. *Am. J. Trop. Med. Hyg.*, **95**: 1106–1114. https://doi.org/10.4269/ajtmh.16-0343

Khan, S.S., Shahab, M.H. and Naz, T., 2019. Visceral leishmaniasis in a resident of Swat, Khyber Pakhtunkhwa, Pakistan, presenting to Civil Hospital Karachi: A case report. *Cureus*, **11**. https://doi.org/10.7759/cureus.6059

Khattak, F.A., Khan, T.A., Hussain, M., Khan, S.N., Ullah, N., Rehman, B., Qasim, M., Safi, A. U.R., Ishfaq, M., and Afaq, S., 2022. Analysis of

associated risk factors among recurrent cutaneous leishmaniasis patients: A cross-sectional study in Khyber Pakhtunkhwa, Pakistan. *J. Infect. Publ. Hlth.*, **15**: 1175–1179. https://doi.org/10.1016/j.jiph.2022.09.010

Kolaczinski, J., Brooker, S., Reyburn, H., and Rowland, M., 2004. Epidemiology of anthroponotic cutaneous leishmaniasis in Afghan refugee camps in northwest Pakistan. *Trans. R. Soc. Trop. Med. Hyg*, **98**: 373-378. https://doi.org/10.1016/j.trstmh.2003.11.003

Kotb Elmahallawy, E. and Agil, A., 2015. Treatment of leishmaniasis: A review and assessment of recent research. *Curr. Pharm. Des.*, **21**: 2259-2275. https://doi.org/10.2174/1381612821666141231163053

Llanos-Cuentas, A., Tulliano, G., Araujo-Castillo, R., Miranda-Verastegui, C., Santamaria-Castrellon, G., Ramirez, L., Lazo, M., De Doncker, S., Boelaert, M., Robays, J., Dujardin, J.C., Arevalo, J. and Chappuis, F., 2008. Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. *Clin. Infect. Dis.*, **46**: 223–231. https://doi.org/10.1086/524042

Lun, Z.R., Wu, M.S., Chen, Y.F., Wang, J.Y., Zhou, X.N., Liao, L.F., Chen, J.P., Chow, L.M. and Chang, K.P., 2015. Visceral leishmaniasis in China: An endemic disease under control. *CMR. Clin.*, **28**: 987–1004. https://doi.org/10.1128/CMR.00080-14

Mandell, M.A., Beatty, W.L. and Beverley, S.M., 2022. Quantitative single-cell analysis of Leishmania major amastigote differentiation demonstrates variably extended expression of the lipophosphoglycan (LPG) virulence factor in different host cell types. *PLoS Negl. Trop. Dis.*, **16**: e0010893. https://doi.org/10.1371/journal.pntd.0010893

Mcgwire, B.S. and Satoskar, A.R., 2014. Leishmaniasis: clinical syndromes and treatment. *QJM Int. J. Med.*, **107**: 7-14. https://doi.org/10.1093/qjmed/hct116

Moein, D., Masoud, D., Saeed, M. and Abbas, D., 2018. Epidemiological aspects of cutaneous leishmaniasis during 2009–2016 in Kashan city, central Iran. *Korean J. Parasitol.*, **56**: 21. https://doi.org/10.3347/kjp.2018.56.1.21

Mouliou, D.S. and Gourgoulianis, K.I., 2021. False-positive and false-negative COVID-19 cases: Respiratory prevention and management strategies, vaccination, and further perspectives. *Expert Rev. Respir.*, **15**: 993-1002. https://doi.org/10.1080/17476348.2021.1917389

Naz, S., Aroosh, A., Raza, N., Islam, A., Ozbel, Y., Toz, S., Ahmed, H. and Waseem, S., 2022. Multiparametric approach to assess the disease severity and progression of cutaneous leishmaniasis infection. *Acta Trop.*, **235**: 106659. https://doi.org/10.1016/j.actatropica.2022.106659

Nighat, S., Malik, A.Y., Mehmood, A., Hashmi, M., Irfan, E., Tahir, I., Salam, M.A., Irshad, I. and Soomro, S., 2022. Prevalence of cutaneous leishmaniasis in Muzaffargarh, Punjab, Pakistan. *J. Pharm. Negat.*, **13**: 1330-1335.

Norouzinezhad, F., Ghaffari, F., Norouzinejad, A., Kaveh, F. and Gouya, M.M., 2016. Cutaneous leishmaniasis in Iran: Results from an epidemiological study in urban and rural provinces. *Asian. Pac. J. Trop. Biomed.,* **6**: 614-619. https://doi.org/10.1016/j.apjtb.2016.05.005

Pearson, R.D. and Sousa, A.Q., 1996. Clinical spectrum of Leishmaniasis. *Clin. Infect. Dis.*, **22**: 1–13. https://doi.org/10.1093/clinids/22.1.1

Razavi, M.R., Shirzadi, M.R., Mohebali, M., Yaghoobi-Ershadi, M.R., Vatandoost, H., Shirzadi, M., Gouya, M.M., Gharachorloo, F., Arshi, S. and Amiri, B., 2021. Human cutaneous leishmaniosis in Iran, up to date-2019. *J. Arthropod. Borne Dis.*, **15**: 143–151. https://doi.org/10.18502/jad.v15i2.7483

Reithinger, R., Dujardin, J.C., Louzir, H., Pirmez, C., Alexander, B. and Brooker, S., 2007. Cutaneous leishmaniasis. *Lancet Infect.*, **7**: 581-596. https://doi.org/10.1016/S1473-3099(07)70209-8

Roatt, B.M., de Oliveira Cardoso, J.M., De Brito, R.C.F., Coura-Vital, W., de Oliveira Aguiar-Soares, R.D. and Reis, A.B., 2020. Recent advances and new strategies on leishmaniasis treatment. *Appl. Microbiol. Biotechnol.*, **104**: 8965–8977. https://doi.org/10.1007/s00253-020-10856-w

Sajjjad, W., Haq, M., Haq, I., Khan, H.A., Basir, N.U., Mazhar, R., Ullah, M.J., Rashid, F., Ullah, M.S., Nafees, J. and Ahmad, Z., 2022. Epidemiological features of cutaneous leishmaniasis in hilly and plot areas of Tribal Districts, Khyber-Pakhtunkhwa Province Pakistan. *Pak. J. med. Hlth. Sci.*, **16**: 1132-1132. https://doi.org/10.53350/pjmhs221621132

Seaman, J., Mercer, A.J., Sondorp, H.E. and Herwaldt, B.L., 1996. Epidemic visceral leishmaniasis in southern Sudan: Treatment of severely debilitated patients under wartime conditions and with limited resources. *Annls Intern. Med.*, **124**: 664-672. https://doi.org/10.7326/0003-4819-124-7-199604010-00007

Sharma, N.L., Mahajan, V.K., Kanga, A., Sood,

A., Katoch, V.M., Mauricio, I., Singh, C.D., Parwan, U.C., Sharma, V.K. and Sharma, R.C., 2005. Localized cutaneous leishmaniasis due to Leishmania donovani and *Leishmania tropica*: Preliminary findings of the study of 161 new cases from a new endemic focus in himachal pradesh, India. *Am. J. Trop. Med. Hyg.*, **72**: 819–824. https://doi.org/10.4269/ajtmh.2005.72.819

Tazerji, S.S., Nardini, R., Safdar, M., Shehata, A.A. and Duarte, P.M., 2022. An overview of anthropogenic actions as drivers for emerging and re-emerging zoonotic diseases. *Pathogens*, **11**: 1376. https://doi.org/10.3390/pathogens11111376

Thakur, S., Joshi, J. and Kaur, S., 2020. Leishmaniasis diagnosis: An update on the use of parasitological, immunological and molecular methods. *J. Parasit. Dis.*, **44**: 253–272. https://doi.org/10.1007/s12639-020-01212-w

Torres-Guerrero, E., Quintanilla-Cedillo, M.R., Ruiz-Esmenjaud, J. and Arenas, R., 2017. Leishmaniasis: A review. *F1000 Res.*, **6**: 750. https://doi.org/10.12688/f1000research.11120.1

World Health Organization, 2020. *Leishmaniasis* [Accessed 20 October 2020]. Available from: WHO, Geneva, https://www.who.int/mediacentre/factsheets/fs375/es/.

World Health Organization, 2023. *Leishmaniasis*, Accessed 25 Apr 2023. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis.