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Cutaneous Leishmaniasis in Conflict-Affected South Waziristan, Pakistan: Epidemiological Patterns and Severe Disease Predictors

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Abstract

Persistent conflict and political instability across the Afghanistan-Pakistan border have systematically compromised disease control infrastructure, creating optimal conditions for the resurgence of cutaneous leishmaniasis (CL), an overlooked tropical skin disease that inflicts lasting psychological and social burdens. Despite recognized hyperendemicity across this international border, epidemiological characterization of CL in South Waziristan, Pakistan, a tribal district contiguous with Afghanistan's Barmal district remains absent. This prospective observational study examined 4,765 suspected CL cases presenting to the Vector Management Center between February 2023 and January 2026. Microscopic confirmation yielded 2,924 confirmed cases. Demographic, geographic, socioeconomic, and clinical characteristics were systematically documented, and multivariate logistic regression identified predictors of severe disease. The confirmation rate was 61.4%. Cases exhibited marked male predominance (58.8%) and extreme rural concentration (95.8%), with 75.1% occurring in children and adolescents ≤ 20 years. Geographic clustering was pronounced: 95.0% of cases concentrated in five subdivisions, with Birmil alone contributing 39.0%. Ulcerative lesions predominated (62.0%), and 90.3% presented with single lesions. Adult age > 20 years (adjusted OR 3.76, 95% CI: 3.15–4.49), rural residence (adjusted OR 2.89, 95% CI: 1.86–4.49), and poor housing condition (adjusted OR 1.38, 95% CI: 1.11–1.71) independently predicted severe disease. Age-stratified analysis demonstrated distinct clinical patterns: children showed higher facial involvement (69.3%), while adults exhibited near-universal ulcerative morphology (99.5%) and larger, multiple lesions. These findings establish South Waziristan as a CL hyperendemic zone with *L. tropica*-compatible transmission dynamics sustained by cross-border connectivity and traditional rural ecology. The extreme geographic clustering and independent socioeconomic predictors provide clear targets for intervention. This baseline evidence is essential for guiding policy and resource allocation in this operationally challenging, conflict-affected setting.

1. Introduction

Cutaneous leishmaniasis (CL) is a protozoan parasitic skin disease caused by parasites of the genus *Leishmania*. It is transmitted to humans through the bite of infected female phlebotomine sandflies. Once inoculated into the skin, metacyclic promastigotes penetrate host macrophages and transform into replicative amastigotes within phagolysosomes, establishing chronic infection predominantly in the dermis with subsequent epidermal ulceration as lesions progress [1–4]. This intracellular parasitism evades host immune clearance through multifaceted inhibition of macrophage microbicidal mechanisms, including suppression of oxidative burst, nitric oxide production, and phagolysosomal maturation, enabling sustained parasite replication and progressive tissue destruction [2, 3].

In resource-limited endemic regions such as rural Pakistan, cutaneous leishmaniasis diagnosis depends on clinical recognition and microscopic confirmation. Clinically, CL begins as a painless papule at the sandfly bite site, gradually progressing into an ulcer with characteristically indurated, raised borders [5]. While lesions may heal over time, they frequently leave permanent disfiguring scars causing lasting psychological distress and social stigma, particularly among women and children [6, 7, 10]. Although

molecular techniques such as PCR offer enhanced sensitivity [8, 9], microscopic examination of Giemsa-stained lesion biopsy smears remains the gold standard [40, 41]. Limited laboratory infrastructure and restricted access to microscopy, however, remain significant barriers to accurate and timely diagnosis, contributing to widespread underreporting and delayed treatment initiation.

Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) represent the two principal clinical forms of leishmaniasis, caused by five major *Leishmania* species with distinct geographic distributions and reservoir patterns [7, 14]. *Leishmania infantum* and *L. donovani* are the predominant VL agents in the Mediterranean region, East Africa, and South Asia [16, 17], whereas *L. tropica*, *L. major*, and *L. aethiopica* are primarily associated with CL [7, 14]. *Leishmania tropica* remains the dominant CL species across Afghanistan, Yemen, northern Syria, Turkey, and Pakistan [37, 38].

In the Afghanistan-Pakistan border region, *L. tropica* is the dominant species and spreads through anthroponotic transmission (human-sandfly-human), with *Phlebotomus sergenti* acting as the primary vector. This sandfly thrives in rural settlements with traditional mud housing, where overcrowding facilitates vector-human contact and transmission intensity [11, 12]. *Leishmania tropica* is primarily maintained by human reservoirs, distinguishing it from principal zoonotic species such as *L. major* and *L. infantum*, which depend on animal reservoirs [13]. However, incidental zoonotic spillover has been documented in some settings [25]. Consequently, susceptible populations in poor housing conditions with high vector exposure are at heightened risk of sustained anthroponotic transmission.

Leishmania tropica spreads through anthroponotic transmission (human- *Phlebotomus sergenti*- human) across the Afghanistan-Pakistan border, with this sandfly thriving in rural settlements with traditional mud housing where overcrowding facilitates intense vector-human contact [11, 12]. Unlike principal zoonotic species such as *L. major* and *L. infantum* that depend on animal reservoirs [13], *L. tropica* relies predominantly on human infection reservoirs, though incidental zoonotic spillover has been documented [25]. Consequently, susceptible populations in poor housing conditions with high vector exposure face heightened risk of sustained transmission.

Protracted conflict in this region has systematically compromised disease control. Military operations, large-scale population displacement, healthcare infrastructure collapse, and environmental degradation collectively create conditions favorable for vector-borne transmission [11, 18]. Cross-border movement of infected individuals into areas with competent vector populations, combined with settlement of immunologically naive populations in endemic zones, drives outbreak propagation. Large-scale Afghan refugee influx since 1988 has been identified as a principal driver of escalating disease burden in Pakistan's border regions [19].

South Waziristan, a district of Khyber Pakhtunkhwa province, occupies a critical position within this hyperendemic zone. Bordering North Waziristan, Balochistan, and Afghanistan, this district experiences sustained *L. tropica* transmission risk driven by cross-border mobility, refugee resettlement, and ecological conditions favorable to *Phlebotomus sergenti* proliferation [20, 36]. Despite recognized

regional hyperendemicity, no prior epidemiological study has characterized CL distribution, demographic risk factors, or clinical patterns in this specific setting.

The present investigation addresses this knowledge gap in the South Waziristan region through comprehensive assessment of 2,924 microscopically confirmed cases presenting to the Vector Management Center between February 2023 and January 2026. We determined disease prevalence, characterized demographic, geographic, socioeconomic, and clinical features including lesion morphology, distribution, and microscopic documentation of amastigotes. We also identified independent predictors of severe disease. Age-stratified clinical patterns and subdivision-level geographic variation were analyzed to inform targeted public health interventions in this operationally challenging environment.

2. Methods

Study Design and Setting

This prospective observational study was conducted from February 2023 to January 2026 at the Vector Management Center in South Waziristan, Khyber Pakhtunkhwa (KPK), Pakistan, with laboratory confirmation at the Department of Pathology, Gomal Medical College, Dera Ismail Khan. South Waziristan, formerly part of the Federally Administered Tribal Areas (FATA), is the largest district of the former Tribal Region [42], covering 6,620 km² with an estimated population of 675,215 (24.3% of Ex-FATA). The district lies between 32.0°- 32.5° N latitude and 69.0°- 70.0° E longitude, characterized by a dry climate with hot summers (up to 40°C) and cool winters with occasional snowfall at higher elevations.

South Waziristan comprises eight subdivisions: Makin, Ladha, Wana, Tiarza, Sararogha, Toi Khulla, Birmil, and Serwakai [43]. South Waziristan borders North Waziristan to the north, Bannu and Lakki Marwat to the northeast, Tank district to the east, which itself borders Dera Ismail Khan- Zhob district (Balochistan) to the south, and Afghanistan to the west **(**Fig. 2.1**).**

Sample Collection and Processing

Lesion samples were obtained using standard needle aspiration and skin-scraping techniques. Prior to sampling, perilesional skin was disinfected with 70% alcohol. Smears were prepared immediately after collection, fixed in alcohol, and stained with Giemsa stain according to standard protocols **(**Fig. 2.2**).**

Microscopic Examination

Stained slides were examined under light microscopy using an oil immersion objective (×1000) for the detection of *Leishmania* amastigotes. A minimum of 100 microscopic fields were screened per slide. Samples negative for amastigotes were recorded as microscopy-negative **(**Fig. 2.3**).** To ensure diagnostic reliability, all slides were transported to the Department of Pathology at Gomal Medical College for independent secondary review by a second experienced microscopist.

Data Analysis

Data management was conducted in Microsoft Excel 2021. Descriptive statistics (frequencies, percentages, and 95% confidence intervals) were calculated in SPSS Statistics (IBM Corp., Armonk, NY, USA). Data visualization was executed in GraphPad Prism version 11.0.0 (GraphPad Software, San Diego, CA, USA).

Descriptive Statistics. Demographic, socioeconomic, geographic, and clinical categorical variables were summarized using proportions with 95% confidence intervals. Continuous variables were summarized using mean, standard deviation, and range.

Risk Factor Analysis. Multivariate logistic regression was performed to identify independent predictors of severe disease, defined as multiple lesions, lesion size > 15 mm, or duration > 10 months. Candidate variables included age group (≤ 20 vs. >20 years), sex (male vs. female), residence (rural vs. urban), house type (mud-built vs. cement), and house condition (with vs. without holes/cracks). Adjusted odds ratios with 95% confidence intervals and p-values were reported. Reference categories include age ≤ 20 years, female, urban residence, cemented house, house without holes/cracks. P-values reported as thresholds (< 0.001, < 0.01, < 0.05, > 0.05) per standard epidemiological guidelines for logistic regression.

Age-Stratified and Geographic Analyses. Clinical patterns between children and adolescents (≤ 20 years) and adults (> 20 years), and geographic variation across high-burden subdivisions, were assessed using descriptive proportions with 95% confidence intervals.

3. Results

Study Population and Data Completeness

A total of 4,979 patients with clinically suspected cutaneous leishmaniasis presented to the vector management center during the study period (February 2023 to January 2026). Of these, 4,765 patients underwent microscopic examination, yielding 2,924 (61.4%) confirmed cases of cutaneous leishmaniasis. The remaining 1,841 (38.6%) were microscopically negative and excluded from further analysis.

Data completeness was high for demographic variables (gender 100%, age 100%, residence 100%, housing characteristics 100%). Occupational classification was restricted to working-age population; 1,008 patients (34.5%) were below working age and excluded from occupational analysis, comprising pre-school children and school-going adolescents not yet in the workforce.

Demographic, Socioeconomic, and Geographic Characteristics

The demographic and socioeconomic characteristics of the 2,924 microscopically confirmed CL patients are presented in Table 3.1.

Table 3.1
Demographic, Socioeconomic, and Geographic Characteristics of Cutaneous Leishmaniasis Patients (n = 2,924)

Characteristic	n	% (95% CI)
Gender		
Male	1,719	58.8 (57.0–60.6)
Female	1,205	41.2 (39.4–43.0)
Age group		
≤ 10 years	1,052	36.0 (34.2–37.7)
11–20 years	1,143	39.1 (37.3–40.9)
21–30 years	404	13.8 (12.6–15.1)
31–40 years	126	4.3 (3.6–5.0)
41–50 years	102	3.5 (2.8–4.2)
51–60 years	56	1.9 (1.4–2.4)
> 60 years	41	1.4 (1.0–1.8)
Residence		
Rural	2,801	95.8 (95.1–96.5)
Urban	123	4.2 (3.5–4.9)
House type		
Mud-built	2,690	92.0 (91.0–93.0)
Cement	234	8.0 (7.0–9.0)
House condition		
With holes/cracks	2,365	80.9 (79.5–82.3)
Without holes	559	19.1 (17.7–20.5)

Gender and Age Distribution. Males accounted for 58.8% (95% CI: 57.0–60.6%) of cases, with a male-to-female ratio of 1.43:1. The age distribution was heavily skewed toward children and adolescents, with 75.1% of cases occurring in individuals ≤ 20 years of age. The highest burden was observed in the 11–20 years age group (39.1%, 95% CI: 37.3–40.9%), followed closely by the ≤ 10 years group (36.0%, 95% CI: 34.2–37.7%). Adult cases (> 20 years) comprised only 24.9% of the total.

Geographic and Residential Patterns. Marked geographic clustering was observed, with 83.0% of cases concentrated in four subdivisions: Birmil (39.0%, 95% CI: 37.3–40.8%), Wana (17.0%, 95% CI: 15.7–18.4%), Toi Khulla (14.0%, 95% CI: 12.8–15.3%), and Makin (13.0%, 95% CI: 11.8–14.3%) (Fig. 3.1). An overwhelming rural predominance was observed, with 95.8% (95% CI: 95.1–96.5%) of patients residing in rural areas versus 4.2% (95% CI: 3.5–4.9%) in urban settings, yielding a rural-to-urban ratio of 22.8:1.

Socioeconomic and Housing Characteristics. Housing characteristics revealed poor living conditions: 92.0% (95% CI: 91.0–93.0%) of patients resided in mud-built houses. Furthermore, 80.9% (95% CI: 79.5–82.3%) lived in houses with holes or cracks in walls.

Clinical Characteristics of Lesions

The clinical presentation of cutaneous leishmaniasis lesions is summarized in Table 3.2.

Table 3.2
Clinical Characteristics of Cutaneous Leishmaniasis Lesions (n = 2,924)

Characteristic	n	% (95% CI)
Lesion type		
Ulcerative	1,813	62.0 (60.2–63.7)
Non-ulcerative	1,111	38.0 (36.3–39.8)
Ulcer subtype (n = 1,813)		
Dry	1,251	69.0 (66.8–71.1)
Wet	562	31.0 (28.9–33.2)
Number of lesions		
Single	2,640	90.3 (89.2–91.3)
Double	258/2,924	8.8 (7.8–9.9)
Triple	26	0.9 (0.6–1.3)
Anatomical site (single lesions, n = 2,640)		
Face	1,777	67.3 (65.5–69.1)
Legs	628	23.8 (22.2–25.4)
Hands	235	8.9 (7.9–10.0)
Lesion duration		
< 6 months	1,959	67.0 (65.3–68.7)
6–10 months	585	20.0 (18.6–21.5)
> 10 months	380	13.0 (11.8–14.3)
Lesion size		
< 8 mm	1,746	59.7 (57.9–61.5)
8–15 mm	965	33.0 (31.3–34.7)
> 15 mm	213	7.3 (6.4–8.3)

Lesion Morphology and Type. Ulcerative lesions predominated, accounting for 62.0% (95% CI: 60.2–63.7%) of cases versus 38.0% (95% CI: 36.3–39.8%) with non-ulcerative morphology. Among the 1,813 ulcerative lesions, the dry subtype was more common than wet (69.0% vs. 31.0%). The vast majority of patients (90.3%, 95% CI: 89.2–91.3%) presented with a single lesion. Among the 2,640 patients with single lesions, facial involvement (Fig. 3.2) was most common (67.3%, 95% CI: 65.5–69.1%).

Lesion Duration and Size. Most lesions were relatively acute: 67.0% (95% CI: 65.3–68.7%) had been present for < 6 months, and 87.0% were subacute or acute (< 10 months duration). Regarding lesion size, 59.7% (95% CI: 57.9–61.5%) measured < 8 mm, and 92.7% were relatively small (< 15 mm).

Risk Factors for Severe Cutaneous Leishmaniasis

Multivariate analysis was performed to identify risk factors associated with severe disease, defined as multiple lesions or lesion size > 15 mm or duration > 10 months. Of 2,924 confirmed cases, 816 (27.9%) met criteria for severe disease.

Univariate and multivariate logistic regression analyses are presented in Table 3.3.

Table 3.3
Univariate and Multivariate Logistic Regression of Risk Factors for Severe Cutaneous Leishmaniasis (n = 2,924)

Characteristic	Univariate			Multivariate		
	Odds Ratio (OR)	95% CI	P-value	Odds Ratio (OR)	95% CI	P-value
Age > 20 years	3.908	3.286–4.646	< 0.001	3.755	3.152–4.474	< 0.001
Male sex	1.198	1.017–1.411	< 0.05	1.179	0.994–1.398	> 0.05
Rural residence	3.155	2.045–4.875	< 0.001	2.894	1.863–4.494	< 0.001
Mud-built house	1.470	1.077–2.006	< 0.05	1.217	0.888–1.668	> 0.05
House with holes/cracks	1.452	1.179–1.787	< 0.001	1.377	1.114–1.702	< 0.01

Following multivariate adjustment, adult age > 20 years (adjusted OR 3.755, 95% CI: 3.152–4.474, p < 0.001), rural residence (adjusted OR 2.894, 95% CI: 1.863–4.494, p < 0.001), and house condition with holes/cracks (adjusted OR 1.377, 95% CI: 1.114–1.702, p < 0.01) remained independently associated with severe disease. House type lost significance in multivariate analysis (p > 0.05), suggesting its effect was mediated through correlation with rural residence. Reference categories included age ≤ 20 years, female gender, urban residence, cemented houses, and houses without holes/cracks.

Age-Related Differences in Clinical Presentation

Comparison between children and adolescents (≤ 20 years, n = 2,195) and adults (> 20 years, n = 729) revealed significant differences (Table 3.4).

Table 3.4
Clinical Characteristics by Age Group

Characteristic	Children and Adolescents (≤ 20 y) n = 2,195 (%)	Adults (> 20 y) n = 729 (%)	P-value
Lesion type			< 0.001
Ulcerative	1,088 (49.6)	725 (99.5)	
Non-ulcerative	1,107 (50.4)	4 (0.5)	
Lesion size			< 0.001
< 8 mm	1,135 (51.7)	611 (83.8)	
8–15 mm	531 (24.2)	434 (59.5)	
> 15 mm	529 (24.1)	200 (27.4)	
Anatomical site			< 0.05
Face	1,015 (69.3)	762 (64.8)	
Legs	322 (22.0)	306 (26.0)	
Hands	127 (8.7)	108 (9.2)	
Number of lesions			< 0.001
Single	2,011 (91.6)	629 (86.3)	
Multiple	184 (8.4)	100 (13.7)	

Adults were more likely to present with ulcerative lesions (99.5% vs. 49.6%, $p < 0.001$), larger lesions (> 15 mm: 27.4% vs. 24.1%, $p < 0.001$), and multiple lesions (13.7% vs. 8.4%, $p < 0.001$). Conversely, children and adolescents (Fig. 3.2) showed significantly higher rates of facial involvement (69.3%, 95% CI: 67.0–71.7% vs. 64.8%, 95% CI: 62.1–67.5%; $p < 0.05$). These differences remained significant after adjusting for rural residence and housing conditions, suggesting intrinsic biological differences potentially related to immunosenescence in adults and differential exposure patterns in children and adolescents.

Geographic Variation in Disease Burden

Analysis of the four high-burden subdivisions revealed significant variation. Birmil (39.0% of total cases) showed higher proportion of severe disease (31.2% vs. 24.8% in other areas, $p < 0.01$) and longer lesion duration (> 10 months: 15.3% vs. 10.7%, $p < 0.001$). These differences persisted after adjusting for age and housing conditions, suggesting possible variation in *Leishmania* species distribution, vector density, or healthcare access.

4. Discussion

Cutaneous leishmaniasis has re-emerged as a critical public health challenge across the world. It is endemic in nearly 90 countries across the Americas, the Mediterranean basin, the Middle East, and Asia [21, 22, 23]. The present study provides the first comprehensive epidemiological and lesion-morphological characterization of CL in South Waziristan, Pakistan, a tribal district bordering Afghanistan's hyperendemic Barmal district, where sustained political instability has affected public health infrastructure and created optimal conditions for vector-borne disease transmission.

Prior molecular epidemiological studies from neighboring North and South Waziristan have identified *Leishmania tropica* as the predominant causative species, accounting for 54–63% of cases, with *Leishmania major* representing a smaller proportion (4–8%) primarily among individuals with travel histories to zoonotic CL-endemic areas of Afghanistan and southern Pakistan provinces [15, 20]. In the present study, while species-level identification was not performed, the clinical and epidemiological patterns including predominantly ulcerative lesions, facial involvement in pediatric populations, and anthroponotic transmission dynamics were consistent with *L. tropica* as the primary pathogen.

The microscopic confirmation rate of 61.4% among clinically suspected cases aligns with reported positivity rates from other conflict-affected districts of the world such as Afghanistan, Syria, Iraq and Lebanon [24]. This confirmation rate, substantially exceeding passive surveillance estimates from stable regions, reflects both the hyperendemicity of this border zone and the chronic underdiagnosis that characterizes neglected tropical diseases in fragile states. The transmission cycle in South Waziristan remains incompletely characterized. Although human-to-human transmission appears dominant, animal reservoirs may also contribute, similar to findings from Morocco where zoonotic transmission was detected despite expectations of purely human-driven spread [25]. The presence of livestock in close proximity to human dwellings in this agrarian setting creates potential for zoonotic spillover, necessitating future reservoir host identification studies. The proximity to Afghanistan where WHO estimates over 200,000 annual CL cases occur [26], combined porous borders and cross-border population movement through corridors such as the Angur Ada crossing, establishes South Waziristan as a critical node in the regional leishmaniasis transmission network.

The marked male predominance (58.8%) and rural concentration (95.8%) observed in this study are consistent with established CL epidemiology in other agrarian, patriarchal societies. The twenty-three-fold Rural-to-Urban Ratio reflects the ecological interface between human settlements and sandfly habitats: Traditional mud-plastered dwellings with straw-thatched roofs provide ideal microenvironments for *Phlebotomus* spp., while agricultural labor during crepuscular periods maximizes human-vector contact [27]. The near-complete absence of cement construction (8.0%) and the prevalence of housing with structural defects (80.9%) indicate that the built environment itself constitutes a risk factor, as mud-wall crevices serve as diurnal resting sites for adult sandflies [28].

Our assessment of anatomical site distribution reveals that children and adolescents (≤ 20 years) demonstrate significantly higher facial involvement (69.3%, 95% CI: 67.0–71.7%) compared with adults (64.8%, 95% CI: 62.1–67.5%; $p < 0.05$) which aligns with international literature demonstrating that

pediatric populations bear disproportionate facial burdens [29, 39] due to behavioral exposure patterns, specifically, face-level vector contact during outdoor play and agricultural assistance at peak sandfly activity periods (dusk and dawn). The modest 4.5 percentage point difference in facial involvement between children (69.3%) and adults (64.8%) suggests that while children experience preferential facial exposure due to their smaller stature positioning them within the primary sandfly flight zone (< 1 meter above ground) [30], adults accumulate comparable facial exposure through prolonged occupational agricultural labor performed in bent postures. Sandflies demonstrate characteristic ground-hugging flight behavior, with 87% of foraging activity occurring within 30 cm of ground level to avoid wind displacement. However, emerging evidence indicates that sandflies possess greater dispersal capacity than previously recognized, with documented crossings of 80-meter river barriers [31], suggesting that vectors may occasionally access adult facial height during active migration or wind-assisted dispersal. The paradox of pediatric facial predominance coexisting with adult severity constitutes a central epidemiological finding. Adults exhibited near-universal ulcerative morphology (99.5% vs. 49.6%), larger lesions (> 15 mm: 27.4% vs. 24.1%), and multiplicity (13.7% vs. 8.4%), with adult age emerging as the strongest independent predictor of severe disease (adjusted OR 3.76, 95% CI: 3.15–4.49, $p < 0.001$). This age-severity gradient likely reflects immunosenescence; the deterioration of cell-mediated immunity with advancing age, compromising the Th1 responses essential for *Leishmania* containment [32, 33]. This clinical dichotomy suggests distinct intervention targets: Pediatric populations require facial lesion management and cosmetic outcome prioritization, while adult populations demand aggressive therapy for severe, potentially scarring disease.

The extreme geographic clustering showing 95.0% of cases concentrated in five subdivisions, with Birmil alone contributing 39.0%, indicates that CL in South Waziristan operates as a spatially structured epidemic rather than diffuse endemicity. Birmil's adjacency to Afghanistan's Barmal district, connected by shared tribal networks and the Gomal River system, creates a functionally integrated transmission zone transcending political boundaries. Molecular epidemiological studies from neighboring North Waziristan have identified *Leishmania tropica* as the predominant species (54–63% of cases), with *Phlebotomus sergenti* as the dominant vector [20].

Multivariate analysis identified three independent predictors of severe disease: adult age, rural residence, and poor house condition. The attenuation of male sex and mud-built construction after adjustment indicates confounding by socioeconomic status; male agricultural Workers reside disproportionately in rural, substandard housing. The persistence of house condition (adjusted OR 1.38, $p < 0.01$) after controlling for residence suggests that structural defects operate beyond mere rurality, likely by providing microhabitats for sandfly resting and breeding [34]. This finding has immediate vector control implications: targeted housing improvement, specifically crack-plastering and structural sealing, may reduce transmission intensity even without broad environmental management.

The temporal dynamics in the study such as 67.0% of lesions < 6 months duration, 87.0% <10 months, suggest relatively prompt healthcare-seeking in this Population, Contrary to assumptions about access barriers in conflict zones. However, the 13.0% with prolonged lesions (> 10 months) and the 27.9%

meeting severity criteria indicate substantial diagnostic and therapeutic delays for a clinically significant minority. The predominance of small lesions (< 15 mm in 92.7%) may reflect either early presentation or intrinsic characteristics of *L. tropica* infection in this setting.

Limitations and Future Directions

Our findings must be interpreted within constraints. The cross-sectional design precludes incidence estimation and causal inference regarding risk factors. The absence of species-level identification, whether *L. tropica* versus *L. major*, limits vector-specific transmission pathway characterization, though prior regional studies strongly suggest *L. tropica* predominance [20]. Species-level identification by polymerase chain reaction (PCR) could not be employed due to limited laboratory infrastructure, restricted cold-chain logistics for reagent storage, and operational insecurity in this conflict-affected setting, where sustained military operations and infrastructure degradation preclude molecular diagnostic capacity at scale. The KAP (knowledge, attitudes, practices) data on CL [35], while suggestive of health literacy gaps, were not collected with validated instruments, highlighting the need for future implementation of standardized KAP questionnaires in this setting.

From a policy perspective this study establishes South Waziristan as a CL hyperendemic zone requiring prioritized intervention. The concentration of 39% of cases in Birmil alone suggests that targeted Vector control such as indoor residual spraying with pyrethroids, insecticide-treated bed nets, environmental management of breeding sites, and housing improvements like crack-plastering should be implemented in regional programs. Personal protective measures during crepuscular periods and community health education for early lesion recognition are also essential.

The independent effect of house condition on severity, combined with the 92% prevalence of mud construction, indicates that integrated housing improvement programs could yield dual benefits: immediate structural protection and long-term transmission reduction. The Age-stratified clinical patterns suggest differential service delivery: school-based screening for pediatric facial lesions, and enhanced case management capacity for adult severe disease. Given cross-border transmission dynamics with Afghanistan, coordinated bilateral initiatives through the Angur Ada corridor are warranted.

Conclusion

This study identifies South Waziristan, Pakistan as a CL hyperendemic zone with *L. tropica* transmission sustained by cross-border connectivity and traditional rural ecology. Independent predictors of severe disease (adult age, rural residence, poor housing) and extreme geographic clustering (95% of cases in five subdivisions) provide clear targets for intervention. These findings establish baseline evidence to guide policy and resource allocation in this conflict-affected setting.

Declarations

Ethical Declaration

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethical Committee at Gomal Medical College, Khyber Pakhtunkhwa, Pakistan (Approval No. 18/GJMC/JC; Approval date: 19 January 2023). Written and verbal informed consent was obtained in Urdu and/or Pashto from all participants or legal guardians for minors. Clinical photography consent was obtained separately with anonymization measures. In case of facial lesions, patient eyes were masked to protect anonymity; only unmasked facial lesions are shown for clinical demonstration purposes.

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Conflicts of Interest

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available to ensure the privacy of the study participants, but are available from the corresponding author on reasonable request.

Author Contribution

ZU and AZ conceptualized and designed the study and drafted the initial manuscript. ZU was responsible for patient recruitment, data collection, and primary data management. ZU, JA (Junaid Asghar), MSA, SSS, AA, SU, SAR, JA (Jamil Ahmad), AASB, and SG designed the study methodology, performed data analysis and interpretation. JA (Junaid Asghar) critically revised the manuscript for important intellectual content. AZ supervised the project and provided oversight throughout the research process. All the authors approved the final version for submission.

References

1. World Health Organization. Leishmaniasis. (2023). https://www.who.int/news-room/fact-sheets/detail/leishmaniasis

2. Soong, L. Subversion and utilization of host innate defense by *Leishmania amazonensis*. *Front. Immunol.* **3**, 58. https://doi.org/10.3389/fimmu.2012.00058 (2012).
3. Gupta, G., Oghumu, S. & Satoskar, A. R. Mechanisms of immune evasion in leishmaniasis. *Adv. Appl. Microbiol.* **82**, 155–184. https://doi.org/10.1016/B978-0-12-407679-2.00005-3 (2013).
4. Jha, B. et al. In and out: Leishmania metastasis by hijacking lymphatic system and migrating immune cells. *Front. Cell. Infect. Microbiol.* **12**, 941860. https://doi.org/10.3389/fcimb.2022.941860 (2022).
5. Reithinger, R. et al. Cutaneous leishmaniasis. *Lancet Infect. Dis.* **7**, 581–596. https://doi.org/10.1016/S1473-3099(07)70209-8 (2007).
6. Bailey, F. et al. A new perspective on cutaneous leishmaniasis—Implications for global prevalence and burden of disease estimates. *PLoS Negl. Trop. Dis.* **11**, e0005739. https://doi.org/10.1371/journal.pntd.0005739 (2017).
7. Burza, S., Croft, S. L. & Boelaert, M. *Leishmaniasis Lancet* **392**, 951–970. https://doi.org/10.1016/S0140-6736(18)31204-2 (2018).
8. Bennis, I. et al. Psychosocial burden of localised cutaneous leishmaniasis: a scoping review. *BMC Public. Health*. **18**, 358. https://doi.org/10.1186/s12889-018-5260-9 (2018).
9. Bilgic-Temel, A., Murrell, D. F. & Uzun, S. Cutaneous leishmaniasis: A neglected disfiguring disease for women. *Int. J. Womens Dermatol.* **5**, 158–165. https://doi.org/10.1016/j.ijwd.2019.01.002 (2019).
10. Kayani, B. et al. Cutaneous leishmaniasis in Pakistan: a neglected disease needing one health strategy. *BMC Infect. Dis.* **21**, 622. https://doi.org/10.1186/s12879-021-06327-w (2021).
11. Brooker, S. et al. Leishmaniasis in refugee and local Pakistani populations. *Emerg. Infect. Dis.* **10**, 1681–1684. https://doi.org/10.3201/eid1009.040179 (2004).
12. Karmaoui, A. et al. A systematic review and global analysis of the seasonal activity of *Phlebotomus (Paraphlebotomus) sergenti*. *PLoS Negl. Trop. Dis.* **16**, e0010886. https://doi.org/10.1371/journal.pntd.0010886 (2022).
13. Cosma, C. et al. Leishmaniasis in humans and animals: A One Health approach. *Trop. Med. Infect. Dis.* **9**, 258. https://doi.org/10.3390/tropicalmed9110258 (2024).
14. Ghatee, M. A., Taylor, W. R. & Karamian, M. Geographical distribution of cutaneous leishmaniasis agents in Iran. *Front. Public. Health*. **8**, 11. https://doi.org/10.3389/fpubh.2020.00011 (2020).
15. Khan, N. H. et al. Cutaneous leishmaniasis in Khyber Pakhtunkhwa Province. *Am. J. Trop. Med. Hyg.* **95**, 1106–1114. https://doi.org/10.4269/ajtmh.16-0343 (2016).
16. Khan, A. et al. Epidemiological and pathological characteristics of cutaneous leishmaniasis from Baluchistan. *Parasitology* **148**, 591–597. https://doi.org/10.1017/S0031182020002413 (2021).
17. Ullah, Z. et al. Characterizing cutaneous leishmaniasis in North Waziristan. *Turk. J. Med. Sci.* **53**, 1767–1775. https://doi.org/10.55730/1300-0144.5746 (2023).

18. Reithinger, R., Mohsen, M. & Leslie, T. Risk factors for anthroponotic cutaneous leishmaniasis. *PLoS Negl. Trop. Dis.* **4**, e639. https://doi.org/10.1371/journal.pntd.0000639 (2010).
19. Malik, M. S. et al. Disease status of Afghan refugees and migrants in Pakistan. *Front. Public. Health*. **7**, 185. https://doi.org/10.3389/fpubh.2019.00185 (2019).
20. Hussain, M. et al. Epidemiology of cutaneous leishmaniasis outbreak. *Waziristan Emerg. Infect. Dis.* **24**, 159–161. https://doi.org/10.3201/eid2401.170358 (2018).
21. World Health Organization. Global leishmaniasis surveillance updates 2024: consolidating gains and new initiatives. World Health Organization. (2025). https://www.who.int/publications/i/item/who-wer10045-535-552
22. Akhoundi, M. et al. A historical overview of the classification, evolution, and dispersion of *Leishmania*. *PLoS Negl. Trop. Dis.* **10**, e0004349. https://doi.org/10.1371/journal.pntd.0004349 (2016).
23. Carrer, D. C., Papera, F. & Ríos, D. N. Cutaneous leishmaniasis: epidemiology and translational challenges. *Front. Microbiol.* **16**, 1588311. https://doi.org/10.3389/fmicb.2025.1588311 (2026).
24. Carew, J. et al. Vector-borne diseases and the Syrian conflict: a systematic review. *PLoS Negl. Trop. Dis.* **19**, e0013721. https://doi.org/10.1371/journal.pntd.0013721 (2025).
25. El Saik, I. Transmission patterns of *Leishmania tropica*. *PLoS Negl. Trop. Dis.* **16**, e0010009. https://doi.org/10.1371/journal.pntd.0010009 (2022).
26. World Health Organization. Control of cutaneous leishmaniasis in Afghanistan: achievements and challenges. (2018). https://wkc.who.int/resources/publications/i/item/who-wer9317
27. Messaoudene, F. et al. Human cutaneous leishmaniasis in North Africa. *Microorganisms* **11**, 2608. https://doi.org/10.3390/microorganisms11102608 (2023).
28. Wijerathna, T. et al. Socioeconomic and demographic factors associated with cutaneous leishmaniasis. *Parasit. Vectors*. **13**, 244. https://doi.org/10.1186/s13071-020-04122-1 (2020).
29. Ashraf, A. et al. Molecular profiling and risk factors of cutaneous leishmaniasis in Punjab. *Parasitol. Res.* **124**, 126. https://doi.org/10.1007/s00436-025-08581-2 (2025).
30. Faiman, R. et al. Studies on the flight patterns of sand flies. *Acta Trop.* **120**, 110–114. https://doi.org/10.1016/j.actatropica.2011.06.015 (2011).
31. Tonelli, G. B. et al. Sand fly behavior: much more than weak-flying. *Mem. Inst. Oswaldo Cruz*. **116**, e210230. https://doi.org/10.1590/0074-02760210230 (2021).
32. Carvalho, A. M. et al. Impaired Th1 response and therapeutic failure in cutaneous leishmaniasis. *J. Infect. Dis.* **223**, 527–535. https://doi.org/10.1093/infdis/jiaa374 (2021).
33. Serrano-Coll, H. et al. Cutaneous leishmaniasis: immunological insights and clinical challenges. *Colomb Med.* **56**, e3006750. https://doi.org/10.25100/cm.v56i3.6750 (2025).
34. Calderon-Anyosa, R. et al. Housing characteristics and leishmaniasis: a systematic review. *Am. J. Trop. Med. Hyg.* **99**, 1547–1554. https://doi.org/10.4269/ajtmh.18-0037 (2018).

35. Rahimi, B. A. et al. Knowledge, attitudes, and preventive practices of cutaneous leishmaniasis. *Sci. Rep.* **16**, 8605. https://doi.org/10.1038/s41598-026-37902-3 (2026).
36. Soomro, B. et al. Prevalence of cutaneous leishmaniasis in South Waziristan. *Open. Access. Public. Health Health Adm. Rev.* **3** (2), 171. https://doi.org/10.59644/oaphhar.3 (2025).
37. Al-Shehari, W. A. et al. Epidemiology of cutaneous leishmaniasis in Yemen. *BMC Infect. Dis.* **26**, 306. https://doi.org/10.1186/s12879-025-11144-6 (2026).
38. Kaya, O. M., Serarslan, G. & Dirican, E. Clinical characteristics of pediatric cutaneous leishmaniasis patients. *Postepy Dermatol. Alergol*. **37**, 229–233. https://doi.org/10.5114/ada.2018.79729 (2020).
39. Deniz, M. et al. Evaluation of pediatric patients with cutaneous leishmaniasis. *Trop. Med. Infect. Dis.* **11**, 71. https://doi.org/10.3390/tropicalmed11030071 (2026).
40. Piscopo, T. V., Mallia, A. C. & Leishmaniasis *Postgrad. Med. J.* **82**, 649–657. https://doi.org/10.1136/pgmj.2006.047340 (2006).
41. Reithinger, R. & Dujardin, J. C. Molecular diagnosis of leishmaniasis. *J. Clin. Microbiol.* **45**, 21–25. https://doi.org/10.1128/JCM.02029-06 (2007).
42. EBSCO Research. Pakistan's Federally Administered Tribal Areas (FATA). (2023). https://www.ebsco.com/research-starters/religion-and-philosophy/pakistans-federally-administered-tribal-areas-fata
43. Bureau Report. South Waziristan divided into two districts. *Dawn com.* (2022). https://www.dawn.com/news/1714876

Figures

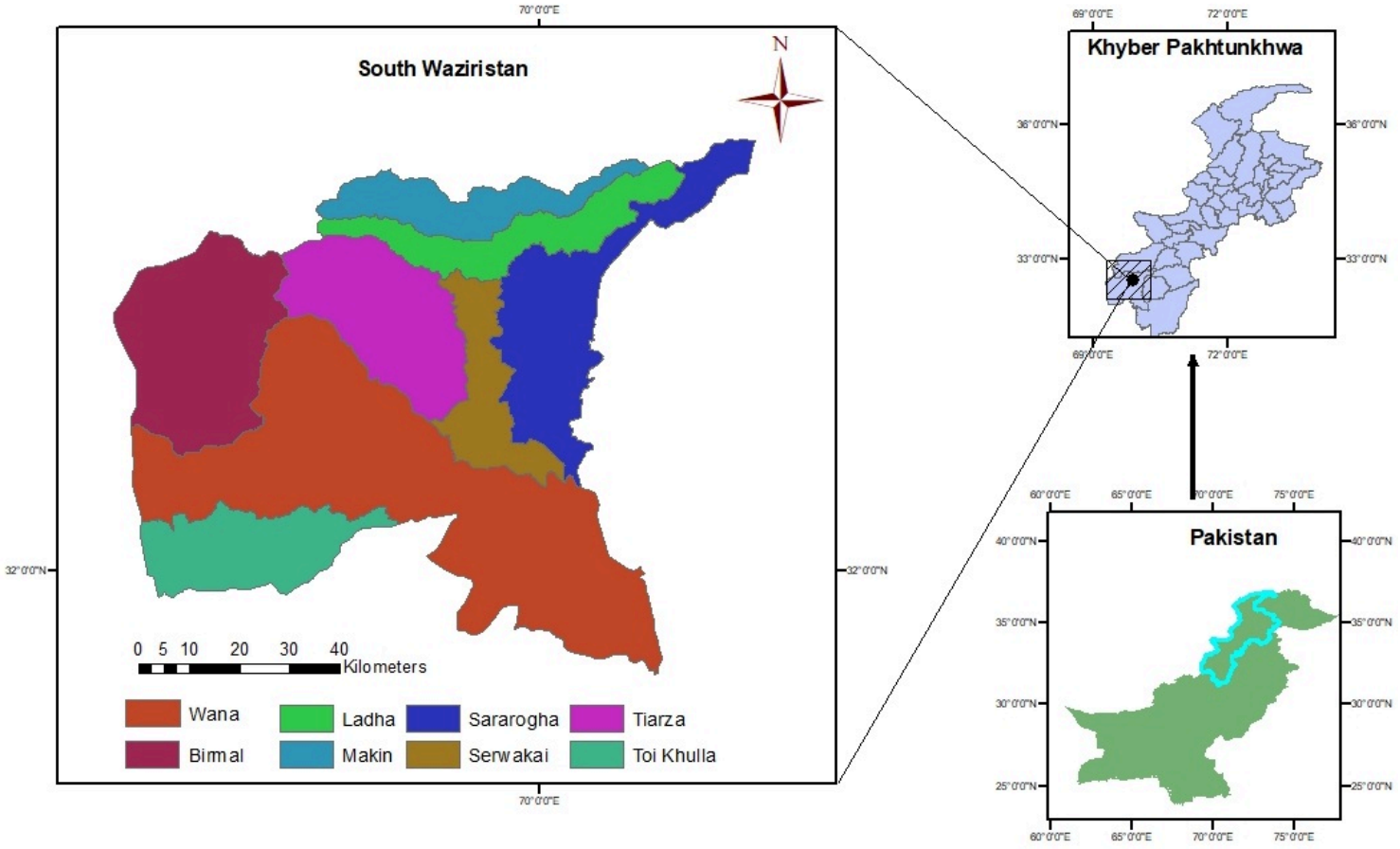


Figure 1

Figure 2.1 Geographic map of South Waziristan district illustrating eight subdivisions (Makin, Ladha, Wana, Tiarza, Sararogha, Toi Khulla, Birmil, and Serwakai) and bordering districts/countries.

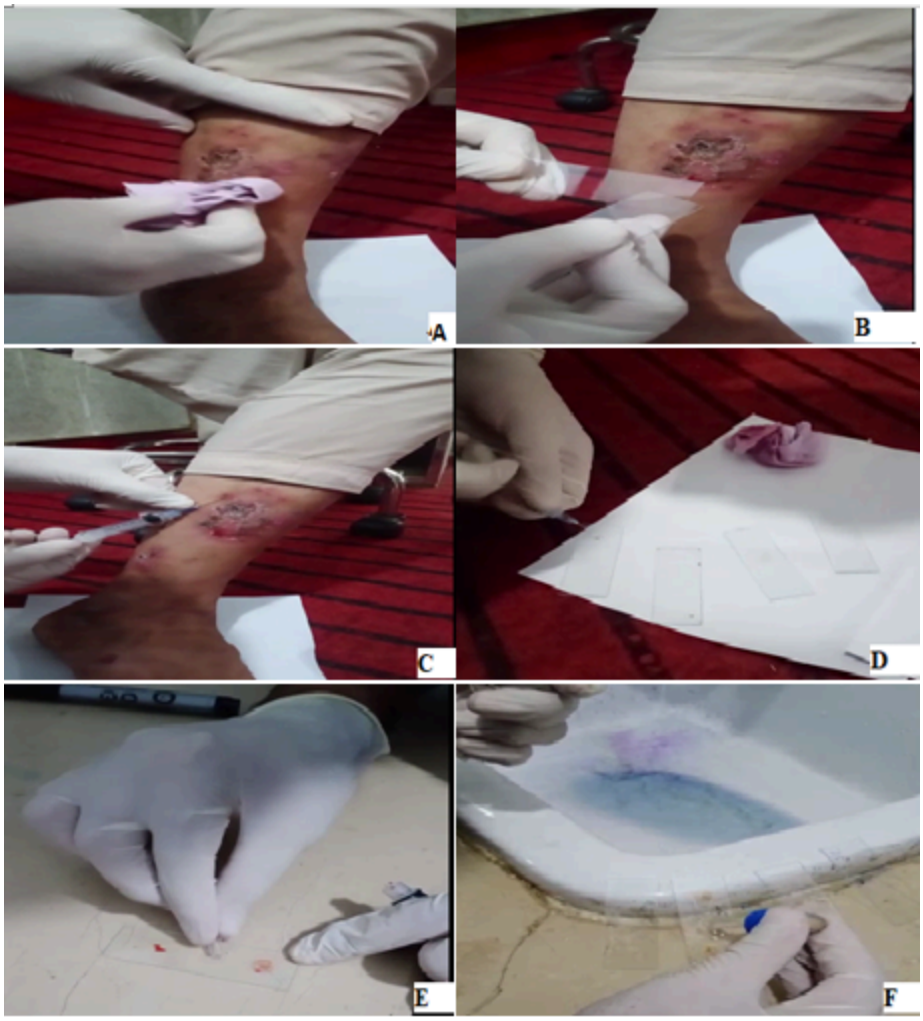


Figure 2

Figure 2.2 Sample collection and processing workflow. (A) 70% alcohol sterilization of perilesional skin; (B) lesion scraping for slide preparation; (C) tissue aspiration with sterilized syringe; (D) placement of aspirate on slide; (E) smear preparation; (F) alcohol fixation of smear.

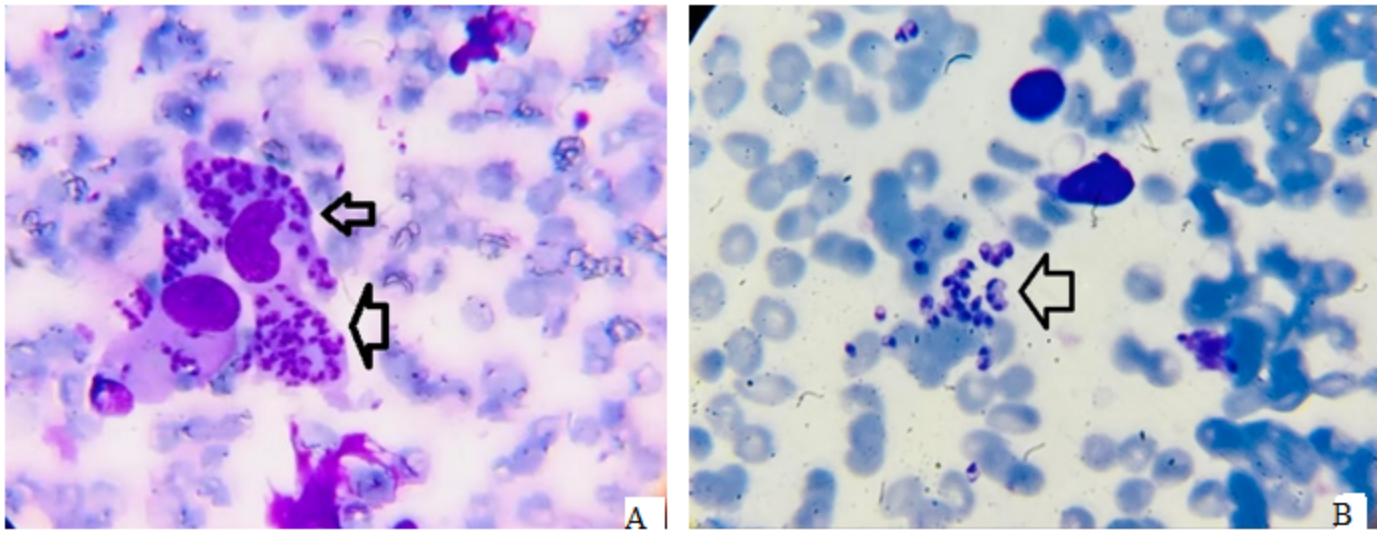


Figure 3

Figure 2.3 Microscopic detection of *Leishmania* amastigotes. (A) Intracellular amastigotes within phagocytic cells; (B) extracellular amastigotes.

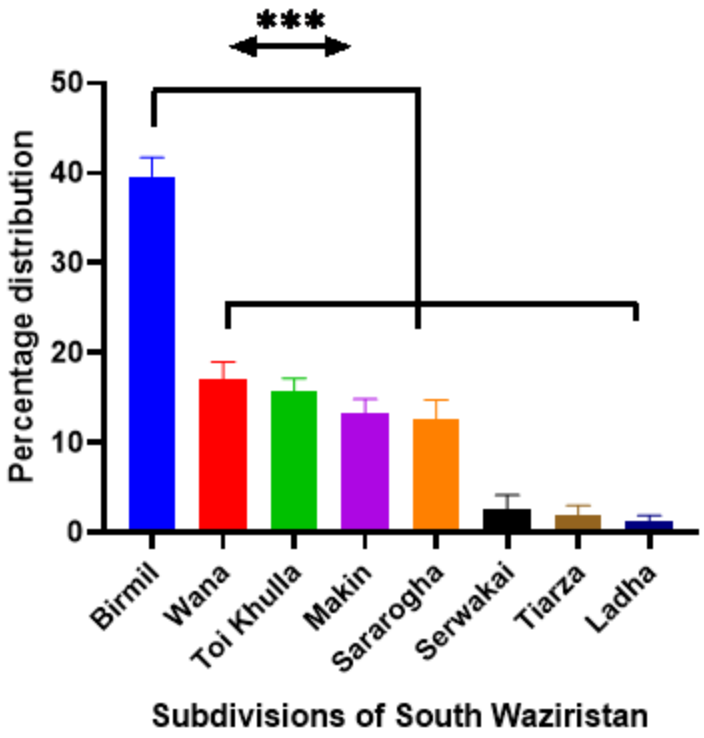


Figure 4

Figure 3.1 Analysis of the five high-burden subdivisions revealed marked geographic clustering in cutaneous leishmaniasis distribution. Birmil accounted for the highest proportion of total cases (39.0%, 95% CI: 37.3–40.8%), followed by Wana (17.0%, 95% CI: 15.7–18.4%), Toi Khulla (14.0%, 95% CI: 12.8–15.3%), Makin (13.0%, 95% CI: 11.8–14.3%), and Sararogha (12.0%, 95% CI: 10.9–13.2%). Together, these five subdivisions concentrated 95.0% of all confirmed cases (n=2,924), indicating extreme geographic concentration.



Figure 5

Figure 3.2 Clinical manifestations of cutaneous leishmaniasis (CL) lesions affecting the hands, lower extremities, and face in study participants. Images were obtained with written informed consent from patients or legal guardians (parents/caregivers) prior to photography; for facial images, patient eyes were masked to protect anonymity while preserving visibility of lesions for clinical demonstration.