Infectious Microbes & Diseases, Publish Ahead of Print
DOI: 10.1097/IM9.0000000000000130

OPEN

The Quest for Understanding Cutaneous Leishmaniasis in Northern Province, Sri Lanka: An Analysis of Clinical Data from the District General Hospital, Vavuniya

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Editor: Stijn van der Veen

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Author contributions: RA and AM contributed to the conception and design of the study, acquisition of the data and literature search; AM and UM conducted all experiments; RA provided samples and clinical data; KPA and AM prepared the manuscript; KPA conducted the

data analysis; KPA and AM were the contributors to the interpretation of the data; all authors substantially contributed to drafting and revising the article, and approved the final manuscript.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Ethics Review Committee of Faculty of Medicine, University of Jaffna (Decision date: December 17, 2015; Ref No. J/ERC/15/68/NDR/0136). Written informed consents were obtained from all study patients.

Data availability: All data generated or analyzed during this study are included in this published article. Additional project information for the current study can be obtained from the corresponding author on reasonable request.

Funding: No funding was received for this work.

Conflicts of interest: The authors reported no conflicts of interest.

Abstract

The study aimed to examine the clinical and epidemiological patterns of cutaneous leishmaniasis (CL) in patients attending the Dermatology Unit, District General Hospital in Vavuniya, Sri Lanka. A total of 77 patients clinically suspected for CL were interviewed through a structured questionnaire and skin-lesion samples were obtained between January 2016 and January 2017. The definitive diagnosis of CL was made through microscopic identification of smears, histopathological examination of biopsies and kDNA PCR. Treatment modalities were chosen based on the location of the lesions on the body and complexity of the lesions. Of 77 suspected patients, 68 were confirmed for CL, with a mean age of 34.6 (±12.7) years, and included 54 males (79.4%) and 14 females (20.6%). Being a male was a significant risk factor (*P*=0.032, OR= 4.82) associated with CL. Lesions were observed mainly on the exposed areas of the body, of which the forearm (22.1%) was the most commonly affected site. Single lesions (75.0%) and ulcerated nodules with central crust (39.7%) were the prominent features among the infected group. The age-group of 21-40 years was significantly associated with healing lesions (*P*=0.028, ME=0.55). However, a significant negative relationship was detected between ulcerated nodular lesions and lesion healing (*P*=0.0436, ME=-0.375). Males are at higher risk of CL. Early diagnosis and specific treatment, along with preventive measures such as protective clothing and sand fly repellents can limit the spread of the disease. There is a need for a comprehensive approach to prevent and control the transmission of CL.

Keyword: *Leishmania donovani*; cutaneous leishmaniasis; kDNA; microscopy; skin lesions; Sri Lanka; Northern Province

Introduction

Leishmaniasis is a neglected tropical vector-borne parasitic disease caused by *Leishmania* parasites. Successful transmission of *Leishmania* parasites to humans is exclusively connected with the bloodsucking behavior of female phlebotomine sand flies.[1,2] Depending on host and parasitic factors, leishmaniasis is manifested in three clinical forms: visceral leishmaniasis (VL), mucocutaneous leishmaniasis and cutaneous leishmaniasis (CL).[1,3]

Over 350 million people from the tropical, subtropical and temperate regions are at risk of contracting leishmaniasis.[4] Each year, approximate numbers of newly emerged cases range from 0.7 to 1.2 million cases of CL and from 0.2 to 0.4 million cases of VL.[4] Most (90%) of the VL patients are reported from India, Nepal, Bangladesh, Sudan, Ethiopia and Brazil, while 90% of the CL patients are reported from Afghanistan, Algeria, Iran, Saudi Arabia, Syria, Brazil, Colombia, Peru and Bolivia.[5]

CL is the most prominent type of clinical form reported in Sri Lanka, which is caused by *Leishmania donovani* zymodeme Mon-37.[6,7] The same species also causes the more fatal VL in South-East Asia, Latin America, Eastern Africa and India.[7,8] The first-confirmed locally acquired cases of CL, mucocutaneous leishmaniasis and VL in Sri Lanka were reported in 1992 from Hambantota, 2005 from Anuradhapura and 2007 from Anuradhapura, respectively.[9–11] Anuradhapura, Hambantota, Polonnaruwa, Kurunegala and Matara are the noticeable endemic areas for 90% of CL cases in Sri Lanka.[7] Old World CL has a range of symptoms from spontaneous healing lesions to chronic non-healing mutilating ulcers that may resemble other implications such as leprosy, lupus vulgaris, warts and simple ulcers.[12] Indeed, laboratory

confirmation by accurate diagnosis seems to be more critical prior to treatment.[12] Direct microscopic identification of *Leishmania* amastigotes is considered to be the primary technique of diagnosis, along with methods like parasite culture, histopathological identification and molecular techniques.[7]

Despite not being an endemic area, the Northern Province of Sri Lanka experienced a rise in CL cases reporting to the District General Hospital (DGH) Vavuniya, a well-equipped hospital with an established Dermatology Unit since 2015, prompting current study to assess risk factors and pattern of the disease among patients at the DGH Vavuniya.

Results

Socio-demographic information of patients

Out of 77 patients suspected of CL (Female = 19, 24.7%; male = 58, 75.3%), 68 (88.3%) were confirmed as true positive for CL and 9 (11.7%) were negative. None of the patients had a history of visits to any endemic foreign countries. The positive CL cases were confirmed if at least one laboratory test was positive for CL (63 by microscopic identification of smears, 61 by PCR and 43 by histopathology). Among the 68 positive cases, 14 (20.6%) were females and 54 (79.4%) were males; age ranged from 5 to 70 years with a mean of 34.6 (±12.7) years. The majority of CL-positive cases (n=41, 60.3%) were reported within the age group of 21-40 years and the highest number of positive cases were referred from Anuradhapura district (n=31, 45.6%, Figure 1A), while only one patient (1.5%) was reported from each of the Badulla, Colombo, Kegalle, and Monaragala districs (Figure 1A). Similarly, when the workplace districts of patients (Figure 1B) were considered, the highest number of patients (n=24, 35.3%) worked in

Anuradhapura district. Analysis of the relationship between residential districts and workstation districts for spatial spread of the disease (Figure 1C) revealed that 75% (n=51) were working in Anuradhapura and Vavuniya districts; and 28 patients were permanently residing in Anuradhapura, which is highly endemic for CL. Military personnel had the highest number of CL-positive cases (n=37, 54.4%) compared to other occupational categories (SDC, Figure S1).

Most of the suspected individuals lived in houses with proper windows, doors, complete roof and cemented floor. However, four CL-positive individuals (5.2%) had a habit of sleeping outdoors. The majority of the CL-suspected population engaged in outdoor occupational activities (48, 62.3%) and among them 43.8% (n=21) stated that they had entered the forest after sunset. Detailed demographic data are presented in Table 1. Being male was a risk factor significantly associated with CL incidence (*P*=0.032, OR= 4.82, CI 95%: 1.14-21.8). The majority of the patients had no past history of CL. The other details, such as contact history, history of sand fly bites and comorbidities are summarized in Table 2.

Clinical manifestation and lesion characteristics

Of the 68 CL-positive patients, 75.00% (n=51) had single lesions, while 25.00% (n=17) had multiple lesions. Lesions were mostly observed on exposed areas of the body (Figure 2A): forearms (n=15, 22.06%), legs (n=12, 17.65%) and arms (n=8, 11.76%). The onset of lesions was mostly observed as either slowly enlarging small papules (n=48, 70.59%) or multiple small papules coalescing to form a plaque (n=15, 22.06%). Based on our observations, nodular lesions were the most prevalent type (n=31, 45.59%), followed by papules (n=24, 35.29%) and plaques (n=13, 19.12%) (Figure 2B, 3A and B). Among the nodular type lesions, 12.90% (n=4) were

typical nodules, 83.87% (n=26) were ulcerated nodules and only one nodule was observed with a central crust (Figure 3A and C). Among papular lesions, 50% (n=12) were observed as typical papular lesions (Figure 3D and E) and 16.67% (n=4) were ulcerated (Figure 3A). The rest of the papules had a central crust (n=6, 25%) with the exception of one appearing as satellite lesion (Figure 3A and F). Plaque type lesions were mostly observed as either typical plaque (n=6, 46.15%, Figure 3G) or ulcerated plaque (n=6, 46.15%, Figure 3A and H). However, the plaque type lesion of one patient presented with a central crust (Figure 3A and I). Overall, the most frequently observed lesion type was the ulcerated nodular lesion with central crust (n=27, 39.71%, Figure 2A). Presence of erythema (n=65, 95.59%), a hypopigmented halo around the lesion (n=43, 63%, Figure 3B and J), and rash with photo distributed area (n=3, 4.41%) were elicited as other lesion characteristics. Although the lesions lasted for up to 6 months (n=54, 79.41%) in the majority of the patients, the duration was extended up to 30 months for a few patients, most of whom had non-healing ulcers (Figure 3K).

Lesion size ranged from 3 mm to 38 mm, and 29.41% (n=20) of the CL patients had 7-10 mm lesion sizes. Comparison of lesions characteristics and the respective grades indicating the parasitic density of the smears obtained from each of the lesion types are summarized in Table 3. However, there was no statistically significant correlation elicited between parasite density and other factors.

Our histopathological examination showed epidermal and dermal changes in all 43 cases examined. The dermal changes were characterized by mononuclear cell infiltration, mostly lymphocytes, histiocytes and plasma cells of varying types, and occasionally accompanied by

neutrophils. Dense/moderate infiltration of lymphocytes, histiocytes and plasma cells without granuloma formation was observed in 26 (60.5%) cases. Additionally, 14 (32.5%) cases exhibited a similar type of dense/moderate infiltration, but with ill-defined granulomatous inflammation, while 27 (62.8%) cases had well-defined granulomatous inflammation. Langerhans-type multinucleated giant cells were noted in two cases (4.65%), but no caseating granulomas or non-caseating necrosis were observed. Notably, no Grenz zone was detected.

Treatment approach

Of the 68 confirmed CL cases, 86.76% (n=59) was treated with intra-lesional sodium stibogluconate (IL-SSG), 8.82% (n=6) with cryotherapy, and one patient received both as a combination. Of all confirmed patients, 63.23% (n=43) achieved complete healing of the lesion without any side-effects and 2.94% (n=2) experienced spontaneous healing. Furthermore, 33.8% (n=23) of the patients responded to treatments with some adverse effects, mostly post inflammatory depigmentation. The time taken for complete healing ranged from 3 to 31 months, and 36.76% (n=25) of the patients achieved complete healing between 3 to 6 month after treatment commenced. Multinomial logistic regression analysis revealed a significant positive association between the age group 21-40 and lesion healing [P=0.028, marginal effect (ME)=0.55] and a significant negative association between ulcerated-nodular lesions (P=0.0436, ME=-0.375) on lesion healing.

Discussion

High incidences of CL in Sri Lanka have prompted the attention of health professionals and researchers.[7] Several demographic factors are associated with a higher risk of CL infections. Our

study revealed that males are more likely to contract CL than females, consistent with previous studies conducted in Sri Lanka[13–16] and Brazil.[17] Since males are more involved in outdoor occupational activities, they are also more frequently exposed to sand fly bites. On the other, females might develop more effective immunity against *Leishmania* parasites due to Th1-type responses induced by upregulation of oestrogen.[17]

Military personnel represented 54.4% (n=37) of the CL-positive group, with the majority (n=25, 67.6%) of them residing in CL-endemic districts (Anuradhapura, Galle, Hambanthota, Polonnaruwa, monaragala, Kandy, Matale, Kurunegala) and working in non-endemic areas (Vavuniya, Killinochchi, Mullathivu, and Mannar). Therefore, continuous deployment may raise the risk of *Leishmania* infection in military personnel, as there is a high chance for exposure to sand fly bites during occupational activities.[18,19] This suggests a possibility of disease transmission from the adjoining endemic areas and also from employees permanently residing in endemic areas. A study conducted in Sri Lanka also revealed a higher prevalence of CL among young military personnel.[20] Similar to previous studies from Sri Lanka,[14,15,21–23] the present study also found that young adults aged 21-35 years were highly prone to CL.

Several studies have inferred that dogs and goats may be reservoir animals for *Leishmania* parasites,[24,25] but this was not supported by the current study as 84.4% of the CL-positive patients were not involved in animal husbandry. Although poor housing conditions was reported as one of the potential risk factors previously,[26] we observed that 32.3% (n=22) of the *Leishmania*-positive individuals from our study lived in substandard housing, with no statistically significant correlation.

Three diagnostic methods, direct microscopy smear examination, PCR diagnosis and histopathological examination of skin biopsies were used simultaneously for discriminating infected individuals. Among the study group, overall smear positivity was 81.8% (n=63) and PCR diagnosis discriminated 79.2% (n=61). Comparatively, a lower proportion of patients (n=43, 55.8%) were discriminated by histopathological examination from biopsy specimens. A previous study also found that routine processing of biopsy specimens during histopathological examination may results in sparse appearance of *Leishmania* parasites compared with direct microscopy.[27] Another study conducted in Canada demonstrated that diagnostic sensitivity for acute lesions (< 3 months) was as high as 78.8%, but significantly lower for chronic lesions (> 12 months). However, sensitivity of PCR diagnosis remained consistent.[28] A similar observation was noticed in our study, suggesting that both direct microscopy and PCR have a similar range of sensitivity. Nevertheless, the combination of these three methods was able to identify 88.3% of suspected cases as CL positive. Although parasitological and microscopic examination are widely used in diagnosis of CL, none of these method are able to reach 100% sensitivity.[29] Therefore, complementation of molecular methods with parasitological and microscopic methods will be useful to improve the overall sensitivity, as previously suggested by Trevisan et al. in 2015.[30] Although the parasite density of many smears ranged from G1+ to G5+ (Table 3), there was no significant association between parasite density and lesion duration.

Our study found that 96.1% (n=74) of the suspected patients had no past history of CL. However, among those infected, 55.9% (n=38) had a contact history with an infected family member or co-worker. Although, a Sri Lankan study reported a significant association between

contact history and positive *Leishmania* infection,[15] several other studies conducted in Sri Lanka and our study did not find any association.[9,21,31–36]

In the present study, single lesions (n=51, 75.0%) were more prominent than multiple lesions (n=17, 25.0%). There was no significant association between lesion number and parasite density. Similar results have been reported in studies conducted in North India[37] and Sri Lanka.[15,21,33] The prominent pathological lesion type observed was ulcerated nodule with central crust (n=27, 39.71%), which is consistent with existing literature,[38–41] followed by the papular type lesions (n=17, 25.0%).

In contrast to our study, a Sri Lankan study noticed a low incidence of papular lesions, which may be due to neglect of the early lesions by the patient due to their small size and asymptomatic nature. They also observed a low number of satellite lesions (n=1) and plaques (n=4), possibly relating to the different immune responses of patients.[42] Although we observed a similar number of satellite lesions (n=1), we found a higher number of plaques (n=13) compared to their observation.

Participants in our study reported that lesions typically appeared as slowly enlarging papules (n=48, 70.6%) before progressing to other types, which is in agreement with previous reports from Sri lanka.[33,43] The type and onset of lesions may vary primarily based on the causative *Leishmania* species and host immune responses.[44,45]

Different treatment modalities have been used to manage CL. Pentavalent antimonial compound has been used extensively for treatment of CL and VL in recent decades, which yields the best outcome for treatment of CL.[46–49] Antimonial resistance and toxicity have become an emerging challenge in the treatment of leishmaniasis.[46] However, several studies have proven the efficacy of IL-SSG as a preferential treatment modality for CL,[48–50] which may minimize the toxicity associated with systemic administration of antimonials. Although lesions may heal spontaneously, treatment is recommended by the Sri Lanka College of Dermatologists in order to minimize the formation of scars and control the disease prevalence.[51] We also observed two cases healing spontaneously without the need for IL-SSG. Since many *Leishmania* species are thermosensitive,[8,52] cryotherapy using liquid nitrogen has been shown to be effective, less expensive and causing less side effects.[8,52] However, it is not advisable to apply cryotherapy on the face due to the risk of scarring.[52] Combination of IL-SSG and cryotherapy has been suggested as an effective treatment for CL.[8,52] In our study, the majority of the patients (n=59, 86.7%) received IL-SSG treatment alone without requiring cryotherapy. Only one patient with complicated lesions and a non-healing ulcer was treated successfully with the combination of both IL-SSG and cryotherapy.

Further, our study revealed a significant association between the age group 21-40 years and complete healing of lesions. This may be because the majority of the CL-positive CL patients were within the age group 21-40. The major limitation of the present study was the small sample size as the cohort was limited to suspected patients referred to the Dermatology Unit, DGH Vavuniya, Sri Lanka.

Military personnel constitute the majority of the *Leishmania*–infected group in this study. Most of them resided permanently at CL-endemic areas, but had been deployed to non-endemic areas in Northern Province. Men, who are more likely to be involved in outdoor occupational activities, were significantly more prone to *Leishmania* infections than women, and a higher number of lesions were observed on exposed areas of the body, highlighting the importance of using protective clothing and applying sand fly repellents. Prompt diagnosis and treatment coupled with vector surveillance and control strategies may facilitate control of leishmaniasis in both endemic and non-endemic areas. Alternative treatments, such as cryotherapy, immunotherapy and amphotericin B should be considered due to the potential risk of resistance to antimonial compounds.

Materials and methods

Study site and design

This prospective clinical-epidemiological study included 77 suspected CL patients referred to the Dermatology Unit, DGH Vavuniya from peripheral hospitals in Northern Province, Sri Lanka, between January 2016 and January 2017. The protocol was reviewed by the Institutional Review Board for Human Subject Research of the University Faculty of Medicine of University of Jaffna (Decision date: December 17, 2015; Ref No.: J/ERC/15/68/NDR/0136). Written informed consents were obtained from all study patients.

Data collection

Socio-demographic data and clinical history of all suspected CL patients were documented via interviewer-administered questionnaires.

Sample collection and diagnosis

Needle aspirates and biopsy specimens were collected for microscopic, histopathological and kDNA PCR investigations for laboratory confirmation. A positive result was confirmed if at least one of these three tests turned out positive. Multiple samples were obtained from the patients with multiple lesions.

Lesion aspirate

Sterile saline (0.5-1.0 mL) was injected into the active lesion edges using a 1- mL syringe with a 26G needle and suction was applied while performing rotator and fro movements. Aspirate obtained from the lesions was used to prepare thin and thick smears for microscopic and PCR analysis, respectively.

Microscopic examination of smears and smear grading

Air-dried smears were transported to the laboratory at the Department of Parasitology, Faculty of Medicine, University of Jaffna for further investigations. Smears were fixed with 100% methanol, stained with Giemsa stain, and examined under high power (×100) light microscopy (Zeiss AxioscopeA1 microscope with AxioCam MRc5 CCD camera) to identify amastigotes. Smears were also graded for the parasite density from G0 to G6+, according to WHO guidelines, 2010[53] (SDC, Table S1).

Histopathological examination of biopsies

Punch biopsy (2-3 mm) was obtained from the active lesion margin under local anesthesia. Biopsy samples were dehydrated, embedded in paraffin, sliced into 4-6 µm thick sections, and

stained with hematoxylin and eosin (H&E). Tissue smears were examined under a microscope for *Leishmania* bodies and associated histopathological changes in the Histopathology Laboratory at DGH Vavuniya, Sri Lanka.

Molecular identification

DNA extraction. Molecular identification of *L. donovani* was performed at the Parasitology Laboratory, Faculty of Medicine, University of Jaffna. Air-dried thick smears were utilized for extracting DNA of *Leishmania* parasites as a simple cost-effective method.[54] Briefly, thick smears on glass slide were scraped using a sterile scalpel blade and sample materials were collected into a 1.5 mL microcentrifuge tubes containing 500 µL of nucleus-free water and homogenized by vortex for 10 minutes. Subsequently, DNA was extracted manually using the phenol-chloroform method.[54] The ethanol-precipitated DNA was reconstituted in 50 µL TE buffer and stored at -20 ^{o}C.

PCR amplification of DNA. A 120-bp fragment from the conserved region of kinetoplastic minicircle DNA (kDNA) from *Leishmania* spp. was amplified by PCR using the primers (Forward: 5′-GGCCCACTATATTACACCAACCCC-3′ and Reverse: 5′-GGGGTAGGGGCGTTCTGCGAA-3′) as described previously.[55] Resulting PCR products were separated on 1% agarose gels and examined under UV. The DNA extracted from the promastigote form of cultured parasites was used as the positive control.

Treatment

Patients were treated with IL SSG (a dose of 0.5-5 mL injected into the base and margins of the lesion to produce complete blanching every week[53]), cryotherapy (15 seconds double freeze-thaw cycles every two weeks), or a combination of both based on individual cases. Total disappearance of the lesion, resolution of the induration or complete re-epithelization of the ulcerated lesion and absence of inflammatory signs or erythema were considered as clinical cure and the end point of treatment.

Statistical analysis

Data was analyzed using R version 4.2.3. Binomial logistic regression was used to analyze significant risk factors associated with CL-incidence, and multinomial logistic regression was used to analyze factors associated with treatment outcomes. The value of 0.05 was considered as the cutoff for significance.

Acknowledgments

The authors would like to express our sincere gratitude to the staff attached to the Dermatology Clinic, District General Hospital, Vavuniya, for giving their tremendous support. We also thank all the technical staff members of Department of Parasitology, Faculty of Medicine, University of Jaffna.

Supplemental digital content

Supplemental materials are available at

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Figure legends

Figure 1. Geographical locations of cutaneous leishmaniasis (CL)-positive cases referred to the General Hospital, Vavuniya. A: Residential districts of CL-positive patients referred to the dermatology clinic of Vavuniya General Hospital. B: Workstation districts of CL-positive patients referred to the dermatology clinic of Vavuniya General Hospital. C: Joining classification of each residential district of CL-positive patients to their workstation districts.

Figure 2. Lesion types and site of the lesions of cutaneous leishmaniasis (CL)-positive patients. A: Number of cases with lesions at different sites. B: Number of cases with different types of lesions.

Figure 3. Clinical manifestations of the cutaneous leishmaniasis (CL)-positive patients. A: Comparison of the three main types of lesions with their characteristics. B: Comparison of three main types of lesions with the presence of hypopigmentation. C: Nodular lesion with central crust. D: Papular lesion. E: Paired papules. F: Papular nodule (satellite) lesion. G: Plaque type lesion. H: Ulcerated plaque type lesion. I: Plaque type lesion with central crust. J: Hypo-pigmented nodular lesion with central crust. K: Non-healing ulcer.

Figure 1

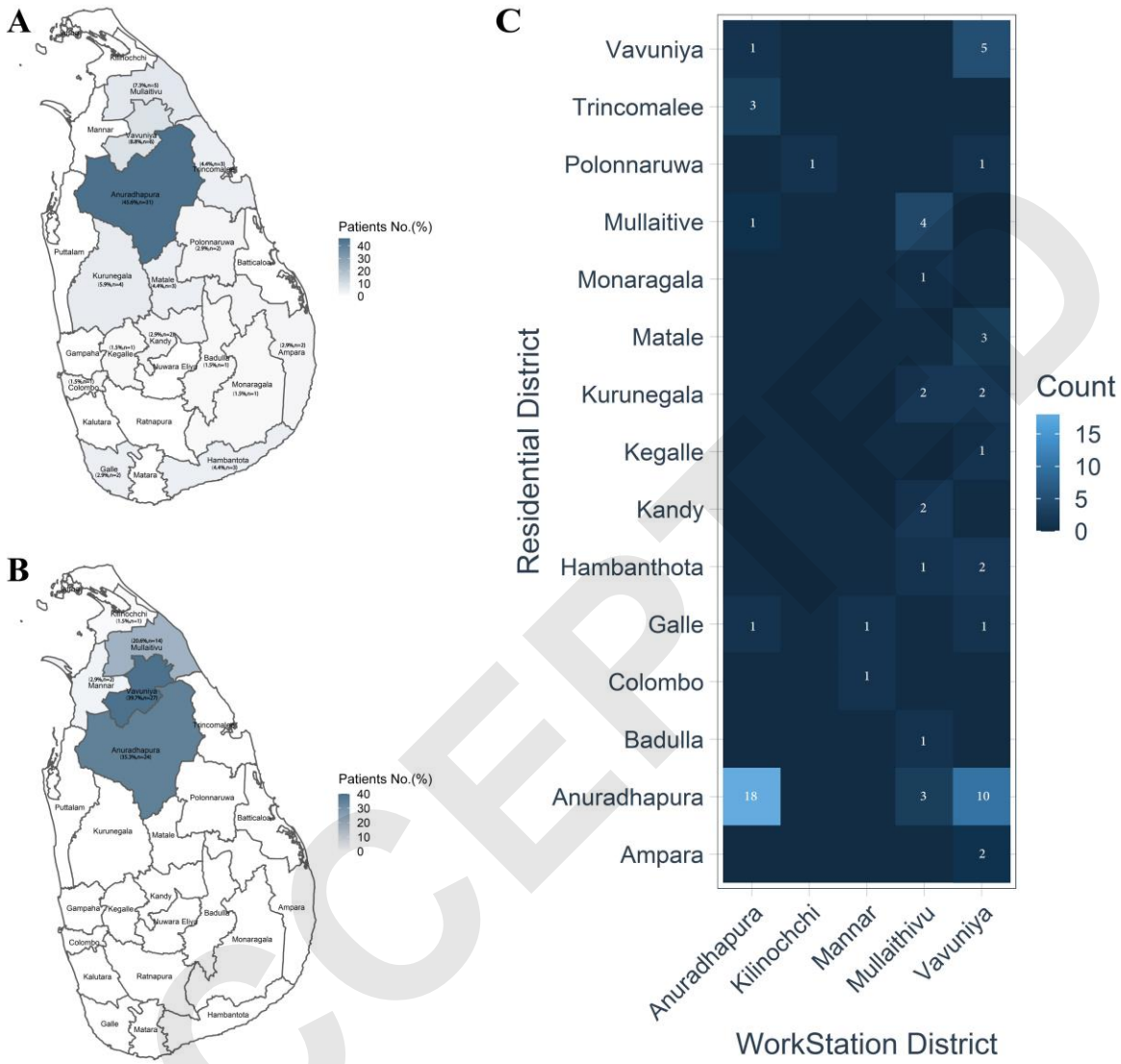


Figure 2

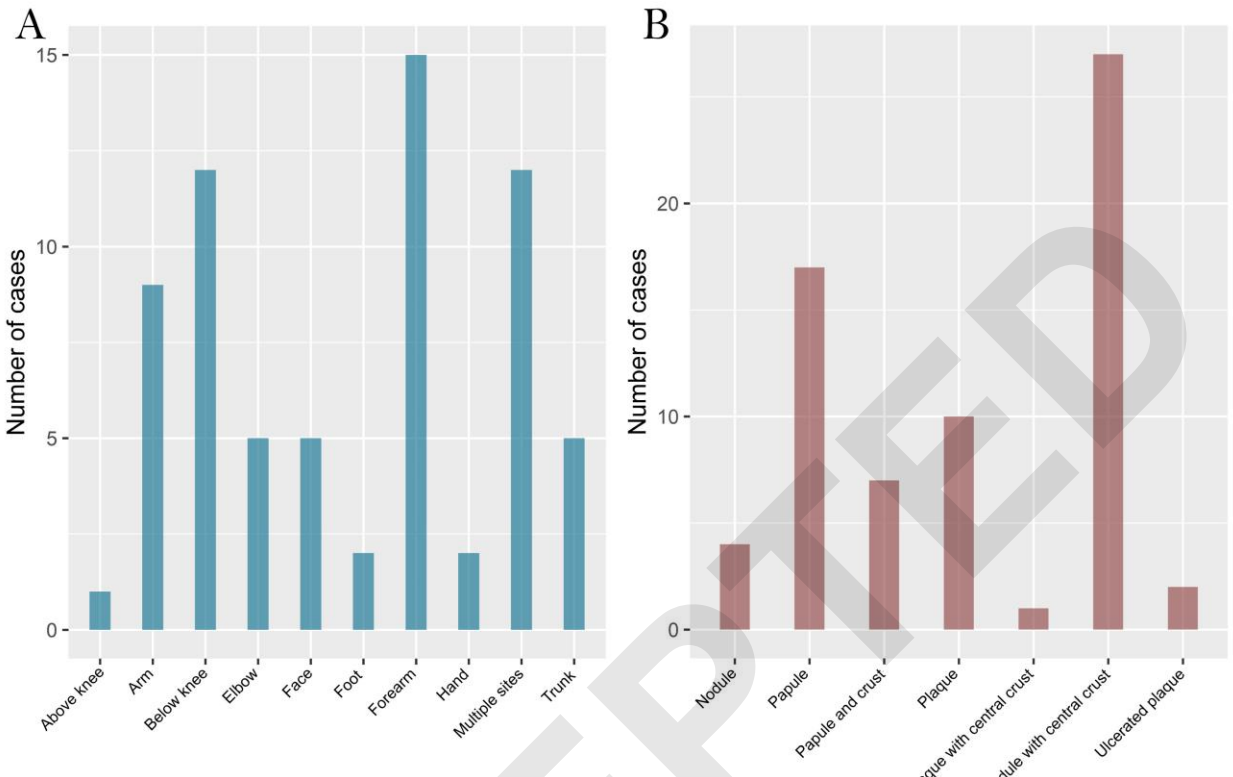
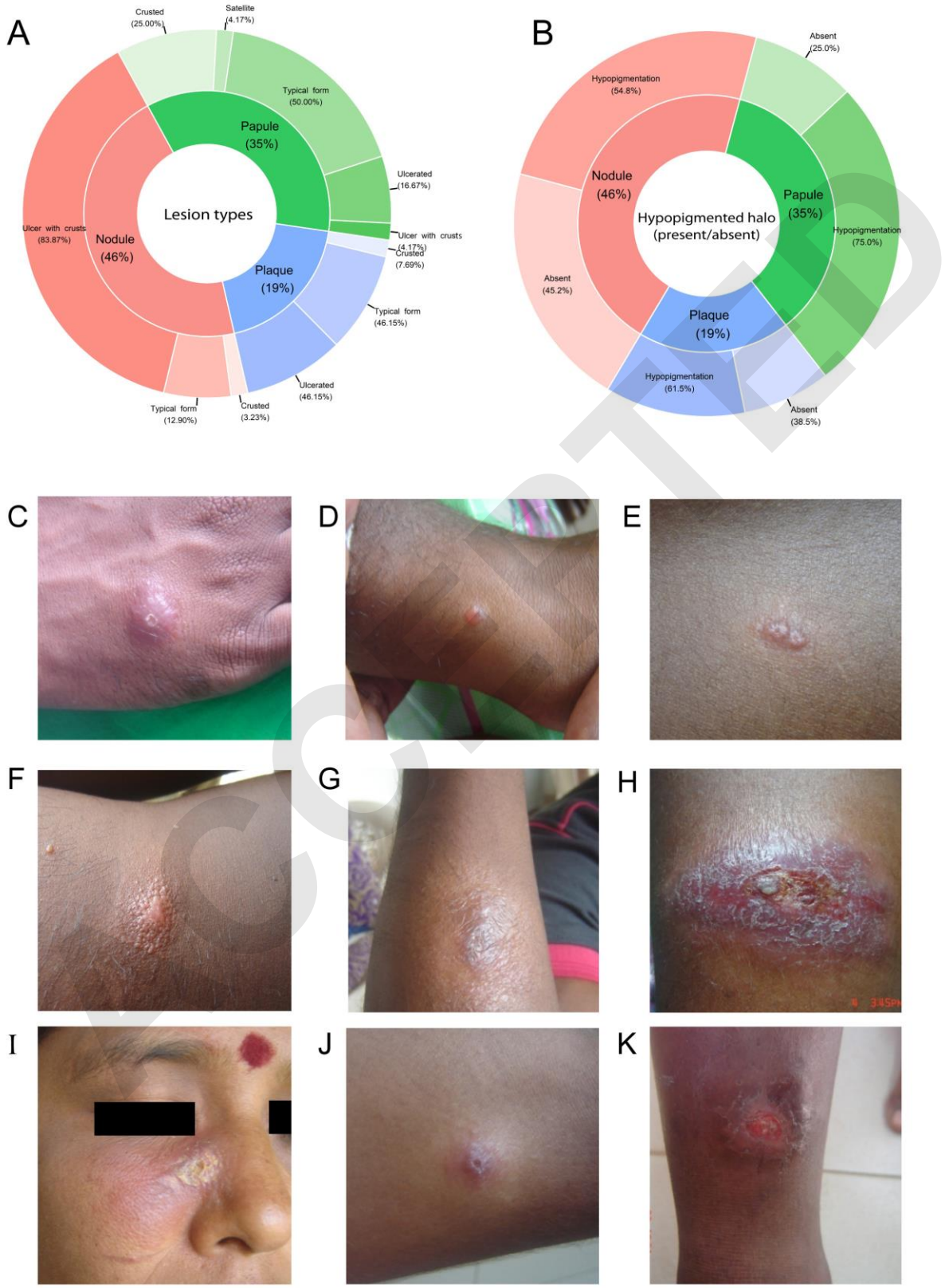


Figure 3



Tables

Table 1 Suspected cutaneous leishmniasis (CL) patients with socio-demographic factors and exposure-related risk factors.

Factor (number of patients)	Category of response (n, %)	CL category	
		CL-positive (%)	CL-negative (%)
Socio-demographic factors			
Gender (77)	Female (19, 24.7%)	14 (73.3%)	5 (26.3%)
	Male (58, 75.3%)	54 (93.1%)	4 (6.9%)
Age groups (77)	<20 (10, 13.0%)	7 (70.0%)	3 (30.0%)
	21-40 (45, 58.4%)	41 (75.0%)	4 (25.0%)
	41-60 (18, 23.4%)	17 (94.4%)	1 (5.6%)
	>61 (4, 5.2%)	3 (75.0%)	1 (25.0%)
Level of education (77)	<Grade_5 (6, 7.8%)	4 (66.7%)	2 (33.3%)
	Grade_5 (26, 33.8%)	23 (88.5%)	3 (11.5%)
	<O/L (8, 10.4%)	7 (87.5%)	1 (12.5%)
	O/L (2, 2.6%)	2 (100%)	0 (0%)
	A/L (35, 45.4%)	32 (91.4%)	3 (8.6%)
Economic status (77)	Poor (66, 85.7%)	58 (87.9%)	8 (12.1%)
	Middle (11, 14.3%)	10 (90.9%)	1 (9.1%)
Overseas travel history (77)	No (77, 100%)		
House condition			
House condition (77)	Substandard (24, 31.2%)	22(91.7%)	2 (8.3%)

	Standard (53, 68.8%)	46 (86.8%)	7 (13.2%)
House maintenance (77)	Poor (19, 24.7%)	17 (89.5%)	2 (10.5%)
	Regular(58, 75.3%)	51 (87.9%)	7 (12.1%)
Wall condition (77)	No cracks (68, 88.3%)	60 (88.2%)	8 (11.8%)
	Presence of cracks (9, 11.9%)	8 (88.8%)	1 (11.1%)
Surrounding environmental conditions			
Pet animal (76)	No (20, 25.9%)	19 (95.0%)	1 (5.0%)
	Yes (56, 72.7%)	49 (87.5%)	7 (12.5%)
Animal husbandry (77)	No (65, 84.4%)	57 (87.7%)	8 (12.3%)
	Yes (12, 15.6%)	11 (91.7%)	1 (8.3%)
Animals within the house (77)	No (38, 49.3%)	36 (94.7%)	2 (5.3%)
	Yes (39, 56%)	32 (82.1%)	7 (17.9%)
Animals in peridomestic area (77)	No (31, 40.2%)	27 (87.1%)	4 (12.9%)
	Yes (46, 59.7%)	41 (89.1%)	5 (10.9%)
Presence of shrub jungle (76)	No (38, 49.3%)	36 (94.7%)	2 (5.3%)
	Yes (38, 49.3%)	32 (84.2%)	6 (15.8%)
Presence of forest (76)	No (66, 85.7%)	59 (89.4%)	7 (10.6%)
	Yes (10, 13.0%)	9 (90.0%)	1 (10.0%)
Behavioral factors			
Outdoor sleeping (77)	Always (2, 2.6%)	2 (100%)	0 (0%)
	During hot weather (1, 1.3%)	1 (100%)	0 (0%)

	Nighttime only (1, 1.3%)	1 (100%)	0 (0%)
	No (70, 90.9%)	62 (88.6%)	8 (11.4%)
	Other (3, 3.9%)	2 (66.7%)	1 (33.3%)
Sleeping surface (77)	Bed (60, 77.9%)	53 (88.3%)	7 (11.7%)
	Floor (16, 20.8%)	14 (87.5%)	2 (12.5%)
	Bed/floor (1, 1.3%)	1 (100%)	0 (0%)
Sleep under bed nets (77)	No (9, 11.7%)	9 (100%)	0 (0%)
	Yes (68, 88.3%)	59 (86.8%)	9 (13.2%)
Insecticide spraying (77)	No (74, 96.1%)	65 (87.8%)	9 (12.2%)
	Outside and vicinity (3, 3.9%)	3 (100%)	0 (0%)
Outdoor occupational activities (77)	No (29, 37.7%)	25 (86.2%)	4 (13.8%)
	Yes (48, 62.3%)	43 (89.6%)	5 (10.4%)
Entering the forest for outdoor-work after sunset (77)	No (55, 71.4%)	48 (87.3%)	7 (12.7%)
	Yes (22, 28.6%)	20 (90.9%)	2 (9.1%)

A/L, General Certificate of Education advanced level examination in Sri Lanka; O/L, General Certificate of Education ordinary level examination in Sri Lanka.

Table 2 Clinical history of the suspected individuals

Factor (number of patients)	Category of response (n, %)	CL category	
		CL-positive (%)	CL-negative (%)
Clinical history			
Past history of CL (77)	No (74, 96.1%)	67 (90.5%)	7 (9.4%)
	Yes (3, 3.9%)*	1 (33.3%)	2 (66.6%)
Past treatment (3)	No (1, 1.3%)	0 (0%)	1 (100%)
	IL-SSG (2, 2.6%)	1 (50.0%)	1 (50.0%)
Response to past treatment (2)	Nonresponse (1, 1.3%)	1 (100%)	0 (0%)
	Responded (1, 1.3%)	0 (0%)	1 (100%)
Other comorbid conditions (77)	No (68, 88.3%)	63 (92.6%)	5 (7.3%)
	Diabetes mellitus (3, 3.9%)	2 (66.7%)	1 (33.3%)
	Eczema (1, 1.3%)	1 (100%)	0 (0%)
	Hypertension (4, 5.2%)	2 (50.0%)	2 (50%)
	Immunosuppressive drugs (1, 1.3%)	0 (0%)	1 (100%)
CL in family members or coworkers (77)	No (37, 48.0%)	30 (81.1%)	7 (18.9%)
	Current infection (22, 28.6%)	21	1 (4.5%)

		(95.4%)	
	Past infection (18, 23.4%)	17 (94.4%)	1 (5.5%)
Relationship (40)	Coworkers (28, 36.4%)	26 (92.8%)	2 (7.1%)
	Family members (7, 9.1%)	7 (100%)	0 (0%)
	Neighbors (4, 5.2%)	4 (100%)	0 (0%)
	Relatives (1, 1.3%)	1 (100%)	0 (0%)
Treatment received (23)	No (4, 5.2%)	4 (100%)	0 (0%)
	Yes (19, 24.7%)	19 (100%)	0 (0%)
Relations outcome (21)	Not cured (5, 6.5%)	5 (100%)	0 (0%)
	Cured (16, 20.8%)	15 (93.7%)	1 (6.2%)
History of sand fly bites (77)	No (75, 97.4%)	66 (88.0%)	9 (12.0%)
	Yes (2, 2.6%)	2 (100%)	0 (0%)
Place where sand fly bite happened (2)	At work place (2, 2.6%)	2 (100%)	0 (0%)
Time of sand fly bite (2)	Daytime (1, 1.3%)	1 (100%)	0 (0%)
	Late evening (1, 1.3%)	1 (100%)	0 (0%)

CL, cutaneous leishmniasis; IL-SSG, intra-lesional sodium stibogluconate.

Table 3 Comparison of lesion characteristics and smear grading.

Lesion type	Number of lesions	Duration (Months)	Grade						
			G0+	G1+	G2+	G3+	G4+	G5+	G6+
Papule (n=17, 25.00%)	Single (n=11, 64.71%)	<6			2	4	1	1	
		13-18			1				
		19-24	1						
	Multiple (n=6, 35.29%)	<6			3	2	1		
Papule with central crust (n=7, 10.29%)	Single (n=4, 57.14%)	<6		2			1	1	
	Multiple (n=3, 42.86%)	<6		1				1	
		7-12	1						
		25-30						1	
Nodule (n=4, 5.88%)	Single (n=3, 25%)	<6					1	1	
		7-12				1			
	Multiple (n=1, 75%)	<6					1		
Ulcerated nodule with central crusts (n=27, 39.71%)	Single (n=20, 74.07%)	<6		4	2	2	3	2	
		7-12	2					2	
		13-18		1	2				
	Multiple (n=7, 25.92%)	<6			2	2		2	
		13-18		1					
Plaque (n=10, 14.71%)	Single (n=10,100.00%)	<6	1	3	2	1	1	1	
		7-12				1			

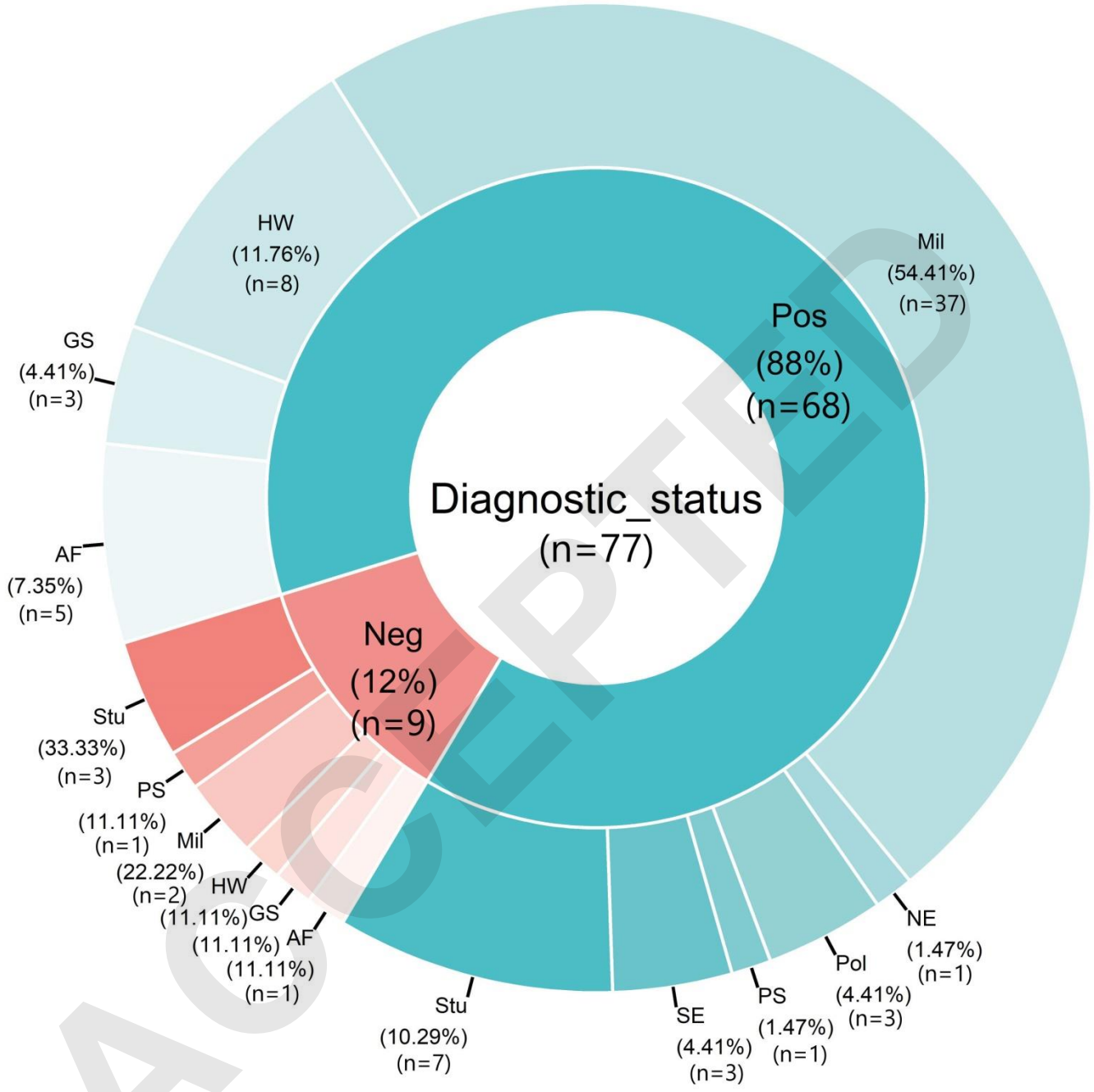
Plaque with central crust (n=1, 1.47%)	Single (n=1,100.00%)	<6		1				
Ulcerated Plaque (n=2, 2.94%)	Single (n=2,100.00%)	<6			1	1		
Total	68		5	13	15	14	9	12

Supplemental digital content (SDC)

SDC, Table 1 ***Grading of Leishmania amastigote numbers based on the World Health Organization guidelines***

Grade	Average parasite density[a]
6+	>100 parasites/field
5+	10-100 parasites/field
4+	1-10 parasites/field
3+	1-10 parasites/10 field
2+	1-10 parasites/100 field
1+	1-10 parasites/1000 field
0	0 parasites/1000 field

[a] Using 10× eyepieces and 100× oil-immersion lens



SDC, Figure 1 ***Comparison of smear positivity with patients' occupations. Neg: cutaneous leishmaniasis negative patients; Pos: cutaneous leishmaniasis positive patients; AF: agricultural farmer; GS: government sector; HW: house wife; Mil: military; NE: non-employee; Pol: police; PS: private sector; SE: self-employee; Stu: student.***