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# Clinicoepidemiological Study of Cutaneous Leishmaniasis: A Hospital Based Study

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## Abstract

**Introduction:** Cutaneous leishmaniasis is a tropical disease transmitted by the bite of an intracellular parasite infected sandfly. Visceral leishmaniasis is common in Nepal. However, cutaneous leishmaniasis remains rare with only limited number of cases documented in Nepal.

**Objectives:** To describe the clinical and epidemiological distribution of cutaneous leishmaniasis in Karnali province of Nepal.

**Materials and Methods:** All cases clinically diagnosed from direct microscopic Giemsa-stain smears, histopathology or fine needle aspiration cytology as cutaneous leishmaniasis were enrolled in the study for one year. Demographic data and clinical details were recorded on a printed proforma. Statistical analysis was done using STATA/SE version 15.0 for MacBook.

**Results:** Only 46 cases with age ranging from 2 to 75 years were included in the study. Mean age was 23±2.45 years. Majority of cases below 20 years (47.83%) were infected. Male to female ratio was 0.70:1. Mean duration of the disease was 4.96±0.47 months. Single lesions (73.91%) were mostly present. Single lesions were particularly presented on the face (73.53%). Cutaneous leishmaniasis (95.65%) outnumbered mucocutaneous one on lips (4.35%). Ulcerative-papulonodular type lesions (58.70%) were mostly presented in the cases. Kalikot district (56.52%) had the majority of cutaneous leishmaniasis cases in Karnali province. Majority of the cases presented during the months of February, March, April, August, November, and December (65.22%).

**Conclusion:** The study concluded that the increase in cutaneous leishmaniasis is alarming in the Kalikot district of Karnali province in Nepal. The result of this study would continue contributing to the existing data in the literature and encourage early diagnosis and treatment of cutaneous leishmaniasis.

**Key words:** Cutaneous Leishmaniasis; Diagnosis; Fine-needle Aspiration Cytology; Giemsa-stain Smears; Histopathology; Nepal

## Introduction

Cutaneous leishmaniasis (CL) is a vector-borne and rare parasitic infection prevalent in tropics and subtropics caused by different species of *Leishmania*.[1] *Leishmania* species mainly results in cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis. Visceral leishmaniasis is commonest in Terai region however, CL is the rarest form of leishmaniasis in Nepal. CL is mainly transmitted from a bite of an infected female sandfly

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of the genus phlebotomus in the old world and the genus Lutzomyia in the new world.[2] CL is endemic in 70 countries, with the majority of cases reported from Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria, and currently expanding to newer locations, including the Indian subcontinent. It is estimated that 600,000 to 1 million new cases of CL occur worldwide annually.[3] CL presents through multiple stages of papule, nodule, central crusting, ulceration, and scar formation at the site of inoculation. Incubation period of CL is 2 weeks to 6 months. Differential diagnosis of CL consists of lupus vulgaris, tuberculosis, deep fungal infection, sarcoidosis, leprosy etc. To demonstrate Amastigotes or Leishman-Donovan (LD) bodies, a simple test in direct microscopic Giemsa-stained smears, skin biopsy, or fine needle aspiration cytology (FNAC) from infected skin may help to confirm the diagnosis. After reporting the first CL case in 1998, only limited cases of CL are documented in Nepal till date.[4] However, the prevalence of CL is increasing. The study aimed to describe the clinicoepidemiology aspect of CL in Karnali province of Nepal which may add data to the existing English literature.

## Materials and Methods

This was a cross-sectional hospital-based study conducted between July 2021 to June 2022. All cases visiting the Dermatology and Venereology outpatient department of Province Hospital, Karnali province, Birendranagar, Surkhet, Nepal, including cases with non-healing ulcer, were enrolled in the study during the study period. Each patient's demographic data (age, sex, and area of residence) and clinical details (site and duration of the lesion) were duly recorded. All cases of both genders in all ages with clinical manifestation and laboratory diagnosis of CL were included in our study. The cases that failed to determine CL in laboratory examination were excluded from the study. All those willing to participate were explained the procedure and the reason for photography before taking their written informed consent. Demographic and detailed clinical data, including site, size, and duration were recorded in a preset proforma. The travel history of thepatient was also documented. Laboratory examinations with either direct microscopic Giemsa-stained smears, skin biopsy, and FNAC confirmed the presence of LD bodies in the smears to confirm the diagnosis of CL. The affected site of the skin was disinfected and squeezed firmly between the index finger and thumb to give 3-4 mm incision of 3 mm depth. Then imprints were prepared directly from the lesion for direct microscopic Giemsa staining to examine leishmania. The skin lesions to be punch biopsied were selected, disinfected, and anesthetized yielding a cylindrical core of tissue extracted after rotating down through epidermis and dermis. The biopsied site was sutured if necessary. The diagnosis of CL was confirmed by standard histopathological examination (Giemsa stain). FNAC was taken from base of the ulcer using 23-gauze needle and 10 ml needle for suctioning then the dried smears were stained with Giemsa stain to examine leishmania. All the multiple lesions of the cases were documented and a smart phone was used to take photographs. We performed the descriptive analysis of the data, and different variables were presented as percentages and/or frequencies as appropriate. Statistical analysis was done using STATA/SE version 15.0 for MacBook.

An Approval letter from the institutional review board was obtained before resuming the study.

## Results

A total of 46 cases were enrolled in the study over one year with CL diagnosed by either direct microscopic Giemsa-stained smear, histopathology or FNAC. A total of 46 cases ranging from 2 to 75 years with a mean age of 23±2.45 years were diagnosed during one year. The youngest case was 2 years old female, and the oldest one was 75 years old male. Mostly 22(47.83%) cases were below 20 years old. 11(23.91%), 7(15.22%), 3(6.52%), and 3(6.52%) cases were between 21-30, 31-40, 41-50 and more than 50 years of age respectively. There were 19(41.30%) males and 27(58.70%) female cases (Table 1). Male: female ratio was 0.70:1. Most of the 26(56.52%) cases were from Kalikot district. Surkhet, Dailekh, Dolpa, Humla, and Jajarkot district presented with 14(30.43%), 3(6.52%), 1(2.17%), 1(2.17%), and 1(2.17%) cases, respectively in Karnali province of Nepal.

Duration of the disease at the time of presentation ranged from 1 to 12 months, with a mean duration of 4.96±0.47 months. The majority of the cases gave a history of insect bites.

Majority, 34 (73.91%) cases had a single lesion. Multiple lesions, 12(26.09%) were present among the remaining cases. Among 12(26.09%) multiple lesions cases, 8(17.39%), 2(4.35%) and 2(4.35%) cases presented with two, three, and four number of lesions respectively. Majority of 25(73.53%) cases presented with single lesions on the face. 6(17.65%), 1(2.94%), 1(2.94%) cases presented with single lesions on upper extremities, lower extremities, trunk and abdomen respectively. Facial lesions were more concentrated over the nose, cheeks, forehead, chin, and submandibular region. Mucocutaneous lesions involving the lower lips were only seen in two cases (4.35%) whereas cutaneous lesions were seen in 44(95.65%) cases.

Majority of the cases were presented during the month of 5(10.87%) February, 5(10.87%) March, 5(10.87%) April, 5(10.87%) August, 5(10.87%)

November, and 5(10.87%) December. 3(6.52%), 3(6.25%), 3(6.25%), and 3(6.25%) cases were presented during the month of May, July, September, and October, respectively. 2(4.35%) and 2(4.35%) cases were presented during the month of January and July, respectively.

Lesions were either ulcerative papulonodular type in 27(58.70%) cases (Figure 1) or non-ulcerative plaque type in 19(41.30%) cases (Figure 2).

All the cases were positive for LD bodies in either histopathology 23(50%) (Figure 3), Giemsa-stained smear 18(39.13%) or FNAC 5(10.87%) (Figure 4).

| Variable | Category | Percentage of cases, % (Number of cases/Total cases) |
|---|---|---|
| Sex | Male | 41.30 (19/46) |
| | Female | 58.70 (27/46) |
| Age category (years) | ≤ 20 | 47.83 (22/46) |
| | 21-30 | 23.91 (11/46) |
| | 31-40 | 15.22 (7/46) |
| | 41-50 | 6.52 (3/46) |
| | > 50 | 6.52 (3/46) |
| Lesion number | Single | 73.91 (34/46) |
| | Multiple | 26.09 (12/46) |
| Lesion distribution | One | 73.91 (34/46) |
| | Two | 17.39 (8/46) |
| | Three | 4.35 (2/46) |
| | Four | 4.35 (2/46) |
| Lesion type | Ulcerative papulonodular | 58.70 (27/46) |
| | Non-ulcerative plaque | 41.30 (19/46) |
| Laboratory examinations (Diagnosis) | Histopathology | 50 (23/46) |
| | Direct Microscopic Giemsa-stained smear | 39.13 (18/46) |
| | Fine needle aspiration cytology (FNAC) | 10.87 (5/46) |

**Table 1 :** Socio-demographic factors and frequency distribution of CL cases (N=46)

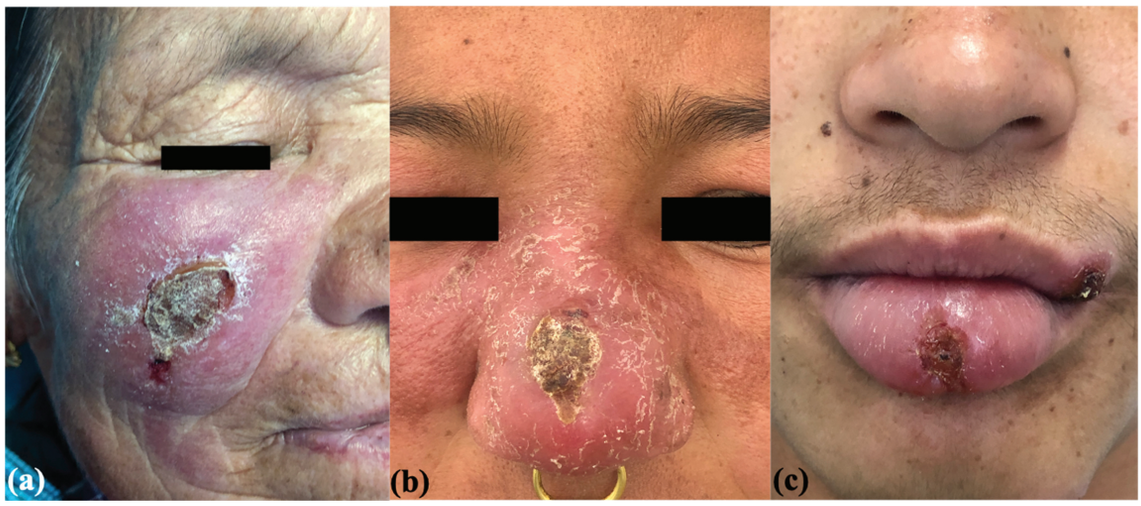


Figure 1: Clinical and morphological manifestation ofwell-defined erythematous ulcer presented over (a) right cheek, (b) nose, and (c) lower lip, demonstrating ulcerative papulonodular type of lesions.

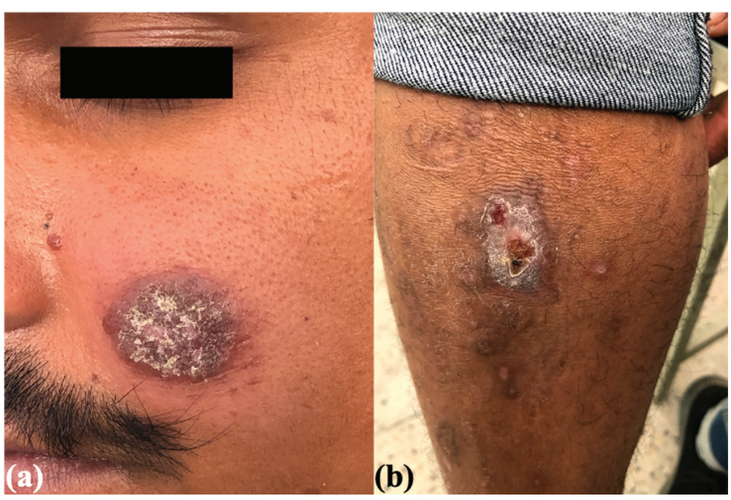


Figure 2: Clinical and morphological manifestation ofcircumscribed erythematous lesion presented over (a) left check, and (b) right leg revealing nonulcerative plaque type of lesions.

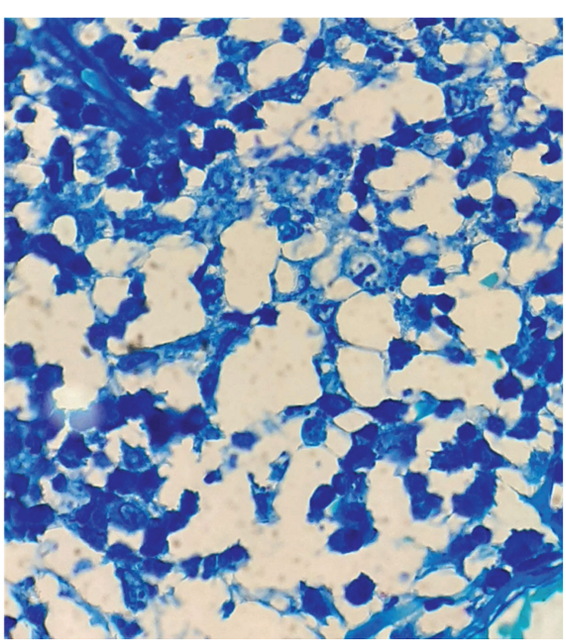

Figure 3: Histopathology showing numerous intracellular amastigotes of Leishmania species on Giemsa stain (original magnification × 100)

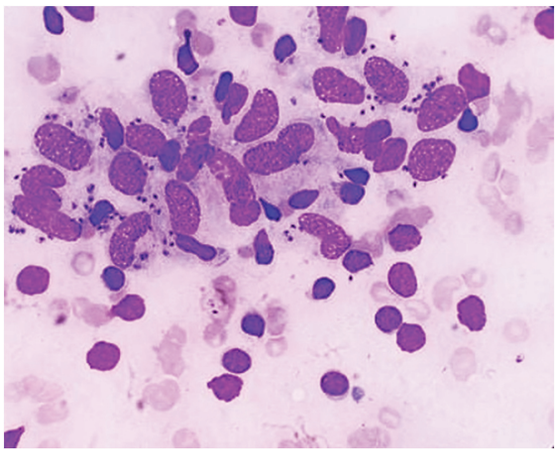

Figure 4: Multiple intracellular and extracellular LD bodies in Direct microscopic examination (Giemsa stain, original magnification × 100)

## Discussion

Cutaneous leishmaniasis is a significant health issue transmitted by an infective parasite in developing countries such as Nepal. The disease is endemic mainly in places of favorable dry, hot, and humid climates suitable for the breeding of sandflies. Visceral leishmaniasis is endemic in 12 districts of low land Terai region, and 63 districts are non-endemic in Nepal.[5] The vector-borne diseases namely, malaria, dengue, Japanese encephalitis, and kala-azar (leishmaniasis) are endemic in the Terai districts of Nepal, covering half of the population.[3] According to Epidemiology and Diseases Control Division (EDCD), in Nepal, only 42 CL cases are reported from 26 different hospitals, followed by a few sporadic CL cases.[3] Recently, there has been an increase in the number of reports on CL from the far-western, midwestern, and central parts of Nepal, which borders Himachal Pradesh in India, where the disease is endemically determining its source.[2] Karnali province is the largest province with ten districts in Nepal, was chosen for our study due to the high number of (9) CL cases reported in 2021.[3] Many factors play an important role in the presence and distribution of CL in Karnali province, including the presence of animal reservoirs such as rodents and dogs; the presence of marshes; and the use of clay to build houses in the villages. Visceral leishmaniasis is common in Nepal. However, to date only few cases of CL have been reported, especially in Karnali province, Nepal.

In the present study, all age groups were affected. However, the highest number of cases were below 20 years of age group, which is similar to studies by Aara N *et al*.[6], Sharma NL *et al*.[7], Yemisen M *et al*.[8], and Gurel*et al*.[9] In contrast, Gunathilaka N *et al*. found a higher incidence in the age group 26-35.[10] Similarly, Ghimire PG *et al*.[11] and Galgamuwa LS *et al*.[12] found a higher incidence in the age group 21-40 years. People below 40 years are at higher risk of exposure due to their high activity levels, occupation, and education.[12] However, Pandey K *et al*.[3] found a higher incidence in the age group above 40 years. CL was rare as the age increased due to long-term acquired immunity in most older people who may have been infected during their early childhood in the high prevalence.[9]

In this study, the disease was more common in female with male to female ratio of 0.70:1. This is consistent with earlier studies done by Sharma NL *et al*.[7], Yemisen M *et al*.[8], Gurel MS *et al*.[9] and Virath R *et al*.[13] In contrast, higher incidence of CL was found in males.[3,6,10-12,14] In Nepal, males are mostly indulged to outdoor works and females to household works. However, due to foster of equality to outdoor works in both females and males both have higher chances of sandfly bites.[11] Also, more significant cosmetic concern in females may have led to female preponderance in our study.[13] The cattle kept close to the residential houses by people in rural areas are also known to attract both anthropophilic and zoophilic vectors.[15]

The mean duration of CL in our study from the onset of lesion to diagnosis was 4.96±0.47 months. Pandey *et al*. reported that in most of the CL case, 31% presented to the hospital within 1-6 months of the onset of lesions, while 21.4% and 9.5% of cases presented to the hospital between 7 and 12 months and after one year of symptom onset respectively.[3] Aara N *et al*. reported that the mean duration of CL was 3.7 months.[6] Sharma NL *et al*. described that the mean duration of the majority of the cases reported within 1-6 months was 7.8 months.[7] Yemisen M *et al*. demonstrated that average duration of CL was 3.6 months.[8] Gurel MS *et al*. disclosed that the average duration was 3.4 months.[9] Ghimire PG *et al*. revealed that mean duration of CL was 5.3±4.4 months.[11] Galgamuwa LS *et al*. expressed that the mean duration ranging from 6 weeks to 4 years was 6.0±4.3 months.[12] Virath R *et al*. noted that the median duration ranging from 15 days to 1 year was 11.25 months.[13] Due to the misdiagnosis, lack of laboratory facilities and transport infrastructure in the remote areas like Karnali province of Nepal to seek medical advice may be the cause for the delay in the presentation of CL.

The single lesion of CL was mostly seen in 73.91% of the cases in our study, which is consistent with the findings of Pandey K *et al*.[3], Aara N *et al*.[6], Sharma NL *et al*.[7], Yemisen M *et al*.[8], Gurel MS *et al*.[9], Gunathilaka N *et al*.[10], Ghimire PG *et al*.[11], Galgamuwa LS *et al*.[12], and Virath*et al*.[13] In contrast, multiple lesions were mostly seen in the study by AlSamarai*et al*.[14] Face was the most common location of single lesion in our study. Similar findings were reported by Pandey K *et al*.[3], Sharma NL *et al*.[7], Yemisen M *et al*.[8], Gurel MS *et al*.[9], Ghimire PG*et al*.[11], and Virath R *et al*.[13] In contrast, Aara N *et al*.[6], Gunathilaka*et al*.[10], Galgamuwa LS *et al*.[12], and AlSamarai AM *et al*.[14] reported most common lesions in upper limbs. Lesions presented mainly on exposed parts of the body suggests vectors and sandflies could easily access those areas with short sleeved upper clothing items as their vestment and due to the high population density of sandflies in those areas.[3,11] Phlebotomoussp. attack exposed parts of the body to suck blood, making it frequently to appear on the hands, face, and legs.[14] Moreover, while sleeping, the face and upper extremities are generally the only uncovered site of the body where the vector can easily reach. Thus, they were the most commonly involved sites, and the ulcerated form was the most common form in our study.[8]

Our study mostly presented with the ulcerative papulonodular type of CL lesion consistent with the study by Sharma NL *et al*.[7], Gurel MS *et al*.[9], and Galgamuwa LS *et al*.[12] Ulcerative plaque was commonly seen in the study by Aara N *et al*.[6], Ghimire PG *et al*.[11], and AlSamarai AM *et al*.[14] Crusted plaque variant was presented in a study by Virath R *et al*.[13] Most commonly presented ulcerated lesions were reported by Yemisen M *et al*.[8] and Gunathilaka N *et al*.[10] Among the various morphologic form of CL ulcerative-nodular form (oriental sore) is the most commonly described lesion which is consistent to our study.[16]

Majority number of new CL cases, 5(10.87%) in each month of February, March, April, August, November, and December was observed in our study. However, minimum number of CL cases, 3(6.52%) in each month of May, July, September, and October was observed in our study. Similarly, 2(4.35%) cases in each month of January and July were also observed in our study. This kind of monthly distribution failed to report previously from Nepal.[3,11] Majority of CL cases were found in the month of February (32.1%) in a study done by AlSamarai AM *et al*.[14] Sharma NL *et al*. determined that most CL cases appeared during summer months which suggested higher seasonal vector activity during warmer months, of the year.[7] Gurel MS *et al*. reported a higher incidence of cases from November to March, with a maximum reported in March and reaching a peak in the wintertime, then declining to a minimum between August and October.[9] Aara N *et al*. suggested that the majority of CL cases were determined during winter (November-April) than during summer (May-October), where maximum cases were reported in January (181 cases) and lowest in October (58 cases).[6] Increased incidence in winter may be due to the fact that many persons in that region sleep in the open during summer months and are exposed to the bites of infected sandflies, which are most abundant during May-September.[6] And where lesions might develop in these persons after 2-3 months which might enlarge and ulcerate in approximately six months when winter is at its peak.[17] Yemisen M *et al*. also showed a typical increase in CL cases between November and May, but the highest incidence rates were seen between February and May due to the incubation period and the delay (15.8 weeks) for admittance and decrease in the summer.[8] This can be attributed to the sandfly life cycle and the incubation period (2-3 months) of CL.[8] In contrary, Toprak and Ozer found that June, July, and September are the most abundant months for sandflies.[18] The variation in seasonal peaks could be due to the existence of various dominant reservoir species in each study area as well as to the activity of the sand flies. The differences in the monthly distribution of CL cases might also be related to the development of female insects and their blood requirement during their life cycle for the maturation and development of eggs, especially in the spring season. The lapse between when the patient was bitten, and the appearance of skin lesions might be related to the long incubation period of leishmaniasis (two to four months).[14]

In this study majority of the cases were from Kalikot district 26(56.52%) of Karnali province, which is in line with the study done in the Midwestern region by Ghimire PG *et al*.[11] The factors of climate, population, and living condition may be the result in increasing cases of CL in Kalikot district. Pandey K *et al*. in 2021 found second highest CL cases from Karnali Province (9) after Sudurpaschim province (11).[3] This is probably the first instance that a bulk number of CL cases were found in Karnali province in Nepal, which used to be a very rare incidence in the past.[3,11] Bastola A *et al*. from Nepal reported a case that had stayed in the Dolpa district of Karnali province for 18 months before being infected.[5]

The majority of the laboratory diagnosis of CL in our study was performed by histopathology 23(50%) followed by direct microscopic Giemsa-stained smear 18(39.13%) and FNAC 5(10.87%). Majority of the cases, 958(69%) was confirmed as CL by Giemsa-stained skin slit smears, and histopathology was positive in 193(45.8%) of 421 patients in a study by Aara N *et al*.[6] Sharma NL *et al*. visualized the rate of direct tissue smears were positive in 37% where the majority 75% of positive smears were from 1-6 month-old lesions.[7] Histopathology is not regarded as diagnostic unless the parasite is demonstrated in histologic sections where organisms appear to be scarce and difficult to identify in hematoxylin and eosin-stained section.[7] Yemisen*et al*. used Giemsa stain to observe amastigotes in 69% of the cases.[8] Gurel*et al*. also confirmed the cases of CL by laboratory demonstration in a Giemsa-stained smear from the lesion.[9] Gunathilaka*et al*. confirmed the majority of 92.1% cases by slit skin smear and tissue immersion smear than lesion aspirate (LA) microscopy

of 46%.10 Histopathological diagnosis was confirmed only in 80% of the suspected individuals.[10] Galgamuwa LS *et al*. confirmed the cases of CL with Giemsa stained smears, biopsy, DNA extraction and polymerase chain reaction (PCR).12Virath R *et al*. performed a slit skin smear, which was positive in all his cases.[13] Modern diagnostic methods include immunofluorescence, use of monoclonal antibodies, DNA probes, polymerase chain reaction, and electron microscopy with limited value as these are expensive and time-consuming and are not routinely employed.[13] In contrast, skin smears can rapidly demonstrate amastigotes for diagnosis of CL in endemic areas.[13] AlSamarai*et al*. reported that the majority of Giemsa stains had a detection rate of 73% in clinically diagnosed cases, while culture had a detection rate of 43%.[14] Among the diagnostic methods available, fine-needle aspirate culture was reported to be the most sensitive method in Iraq.[14] However, direct microscopic examination of lesion scraping continuesto be the diagnostic method most widely applied due to the ease of performance, low cost and speed.[14] Ghimire PG *et al*. detected CL in all 33 cases using fine-needle aspiration cytology.[11] In his study Jha A *et al*. also detected CL using FNAC which is easier, less painful, and more cost-effective than conventional skin biopsy.[19] In contrast, in their recent study Pandey K *et al*. detected LD bodies in 83.3%(35/42) using Giemsa stained smears and 16.7%(12/28) were rK39 positive.[3]

All our cases were positive for LD bodies. Thus, invasive procedures like culture and expensive PCR techniques were not done.

## Conclusion

To the best of our knowledge, this is the first study in a significant respondent investigating the clinico-epidemiological pattern of CL in Karnali province, Nepal. This study highlights the issue of important rare diseases which may draw the attention of the policymakers to formulate a protocol for effective surveillance, health education, control, rapid diagnosis, and training expansion of health personnel in the efficient management of CL. We highly recommend that future research and control program efforts focus on CL in both endemic and non-endemic districts to achieve elimination goals. CL should be kept as a high suspicion of non-healing ulcerative and granulomatous lesion to avoid misdiagnosis in both endemic and non-endemic area. Therefore, implementing vector control programs in insecticide applications, sanitation, health education focused on the transmission, preventive methods of CL, and improving living conditions for the population are necessary to limit the spread of this infection to other regions.

## Limitation

The study population was small and only focused on one hospital. We could not succeed in the identification of the species of *Leishmania* due to lack of resources, funding, and search for any animal reservoir. Also, culture and serology were not performed. We could not determine the prevalence of this infection in Karnali province, Nepal since we did not carry out active case detection. Furthermore, only suspected and laboratory based diagnosed cases were enrolled in the study, and we could have missed non-suspected CL cases.

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