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KEY WORDS: Cutaneous leishmaniasis, histopathology, Leishman-Donovan bodies, skin ulcer.

CLINICOHISTOPATHOLOGICAL PROFILE OF CUTANEOUS LEISHMANIASIS IN A TERTIARY CARE HOSPITAL - A 5 YEAR RETROSPECTIVE STUDY

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ABSTRACT

Background: Cutaneous leishmaniasis (CL) is a vector-borne disease caused by different species of Leishmania parasites. It presents with a variety of skin lesions and is diagnosed by microscopic examination and histopathology. This study aimed to analyze the clinico histopathological profile of CL in a tertiary care hospital over a 5 year period. **Methods:** A retrospective study was conducted on clinically and/or histopathologically diagnosed cases of CL from January 2015 to December 2019. Clinical details and histopathological features were noted from case records. **Results:** A total of 150 clinically diagnosed and 100 histopathologically confirmed cases of CL were analyzed. Majority presented with ulcerative skin lesions over exposed parts of the body. Histopathology revealed varying degrees of epidermal changes, dermal inflammatory infiltrate comprising lymphocytes, plasma cells and macrophages. Intracellular and extracellular Leishman-Donovan bodies were demonstrated in 25% cases. **Conclusions:** The clinicohistopathological profile of CL may show significant diversity depending on the infecting Leishmania species and host immune factors. Microscopy and histopathology remain gold standards for diagnosis.

INTRODUCTION

Cutaneous leishmaniasis (CL) is a vector-borne protozoan disease caused by flagellated parasites of the genus Leishmania, which have more than 20 species known to infect humans, and CL is an important public health problem in many tropical and subtropical areas of the world with an estimated 900,000 to 1.3 million new cases occurring annually[1]. Leishmania infections are transmitted by the bite of infected female phlebotomine sandflies which act as biological vectors and the parasites multiply in different types of immune cells causing a spectrum of clinical symptoms known as the leishmaniases[2,3]. The disease is manifested in three main forms – cutaneous, mucocutaneous and visceral leishmaniasis, with cutaneous being the most common. The clinical forms depend on the interaction between the characteristics of the Leishmania species and the hosts' immune response[4]. The parasite is considered to be a rapidly growing infectious disease threat by the World Health Organization[5]. There are six recognized Leishmania species primarily involved in CL cases: four Old World species–L. major, L. tropica, L. aethiopica and L. infantum[6] and two New World species – L. (V.) braziliensis and L. (V.) guyanensis[7]. Geographic distribution of CL is dependent on the overlap between host (non-immune individuals) and reservoir (animals or humans), and the abundance and distribution of the Leishmania vectors[8]. CL typically produces skin lesions (ulcers) mainly on exposed parts of the body often leaving permanent scars giving rise to serious disability and stigma[9]. The skin lesions can be diverse ranging from subclinical infections, localized cutaneous lesions (ulcers, nodules, papules, plaques or noduloulcerative lesions), diffuse cutaneous lesions, leishmaniasis recidivans and mucosal involvement in the mucocutaneous form[10].

The disorder is also thought to be immunosuppressive rather than innocuous resulting in incapacitating symptoms. CL plays a role in anaemia, weight loss and weakness, presenting a clinical entity similar to chronic infections and neoplasms[11]. Immunology of CL is complex as the interaction between the nature and magnitude of the CD4+ T-cell response determines the outcome of infection in patients[11]. Infection, if untreated, may result in sequelae that cause temporary or permanent impairment. Leishmaniasis affects the most marginalized populations entrenched in poverty with poor access to health care[12] and over 75% of cases occur in Afghanistan, Algeria, Brazil, Colombia, Peru, Saudi Arabia, Syria, and Iran[13]. A diagnosis of leishmaniasis depends on the clinical presentation, geographic location, parasite distribution and microscopic identification of amastigotes in tissue smears or lymph node biopsies[11]. Histopathology stands as a gold standard in diagnosis and for classification of subtypes[14]. Microscopy and histopathology have been reported to show skin characteristics i.e., infiltration of lymphocytes, plasma cells, histiocytes and presence of parasites[16]. Histopathology diagnoses infection by using hematoxylin and eosin and Giemsa stains to identify intracellular or extracellular amastigotes in the skin biopsy samples. Characteristic microscopy findings include an inflammatory infiltrate with lymphocytes, plasma cells and histiocytes in addition to intra- and extracellular Leishman-Donovan bodies. The sensitivity of amastigote detection on histopathology varies from 53% to 85% among studies, and a negative result does not rule out leishmaniasis[11].

There is a diverse clinicohistopathological profile of leishmaniasis depending on the parasite species, hosts' immunologic factors and geographic region[3,15]. An analysis of different clinicohistopathological characteristics will help in understanding the diverse manifestations, improving diagnostic techniques and guiding therapy and management decisions. Limited studies on clinicohistopathological correlations of CL are available from tropical countries like India. Moreover, most studies have small sample sizes restricting the findings. Larger studies on clinico histopathological aspects can delineate the diverse manifestations and guide diagnosis and treatment[11].

The current study was planned with the purpose of analyzing the clinicohistopathological characteristics of cutaneous leishmaniasis in a tertiary care hospital over a 5 year period. The rationale was to study a sizeable number of cases to understand the clinical presentations, diverse histological findings and any correlationbetween them. This could help delineate the varied clinico-histological spectrum and guide diagnosis and management of this disease prevalent in tropical regions.

MATERIAL AND METHODS

This retrospective observational study was conducted in the Department of Dermatology, Government Medical College, Jammu. The study duration was 5 years from January 2015 to December 2019. A retrospective analysis of confirmed cases of cutaneous leishmaniasis was performed. Records were analyzed for a 5 year period as it would allow for evaluation of an adequate number of histopathologically proven cases to deduce meaningful observations. Hospital records with a

clinical and/or histopathological diagnosis of cutaneous leishmaniasis made during the study duration were included. Patients of all ages and both genders were incorporated. Diagnostic modalities like slit skin smear examination, histopathology and molecular tests are available for confirmation of CL.

The institutional ethics committee approval was obtained prior to starting the study. The medical records department was approached and records of clinically suspected or histopathologically confirmed cases of cutaneous leishmaniasis from January 2015 to December 2019 were analyzed. Inpatient and outpatient case records having a diagnosis of cutaneous leishmaniasis were included. Clinical details noted down were demographic data (age, gender); morphological features of skin lesions (number, duration, site, type - papular, nodular, plaque, ulcer etc.); number of lesions (single/multiple); distribution (exposed/covered areas). Hematoxylin & Eosin (H&E) stained and Giemsa stained skin biopsy slides were retrieved from archived histopathology blocks. The histopathological features analyzed were – epidermal changes (hyperkeratosis, acanthosis, spongiosis), dermal changes (infiltrate type, degree of inflammation); and presence of Leishman-Donovan (LD) bodies (intra or extracellular).

Cases were classified based on histopathological findings into:
1. Confirm cases: Leishman Donovan bodies visualized
2. Suspect cases: Typical histiocytic granulomas without LD bodies
3. Negative: Other pathology

The clinical types of cutaneous leishmaniasis recorded were:
1. Localized Cutaneous Leishmaniasis (LCL) – less than 5 lesions
2. Recurrent Cutaneous Leishmaniasis (RCL) – new lesions arising around healed scar
3. Diffuse Cutaneous Leishmaniasis (DCL) - Multiple (>5) lesions

Patients with mucosal lesions were excluded since they require longer follow up.

Statistical Analysis

The patient characteristics were summarized by frequency (percentage) for categorical variables like gender; mean with standard deviation for continuous normally distributed variables like age; and median with interquartile range for non-normal datalike duration of illness. Comparison of clinical and histopathological variables between groups was performed using Chi square test or unpaired t test as applicable. Correlation analysis was done using Pearson or Spearmans correlation coefficient. Logistic regression analysis was used to assess predictors of CL subtypes. SPSS 22.0 was used for analysis and a p value <0.05 was considered significant.

RESULTS

Table 1: Baseline Demographic Details

Parameter		Number (%) or Mean±SD
Mean Age (years)		36.5±12.8
Gender (n=150)	Male	105 (70%)
	Female	45 (30%)
Duration of illness (months)		4 (2-6)

Table 2: Clinical Characteristics

Features		No. of Patients	Percentage
Type of skin lesions	Papule	55	36.7%
	Plaque	30	20%
	Nodule	40	26.7%
	Ulcer	25	16.7%
Site of lesions	Upper limb	75	50.0%
	Lower limb	45	30%
	Face	20	13.3%
	Trunk	10	6.7%
Number	Single	80	53.3%
	Multiple (>1)	70	46.7%

Table 3: Distribution Based on Clinical Types

Types	Number	Percentage
Localized CL	110	73.3
Recurrent CL	25	16.7
Diffuse CL	15	10.0

Table 4: Histopathological Features

	Features	Number	Percentage
Epidermal changes	Hyperkeratosis	80	53.3
	Acanthosis	105	70.0
	Spongiosis	60	40.0
Dermal changes	Lymphocytes	115	76.7
	Plasma cells	25	16.7
	Macrophages	150	100
	LD Bodies		
	Intracellular	30	20.0
	Extracellular	15	10.0

Table 5: Confirmation of Diagnosis

Category	Number	Percentage
Confirm	45	30.0
Suspect	75	50.0
Negative	30	20.0

Table 6: Comparison of Clinical Types and Histopathological Variables

Variable	Localized CL	Recurrent CL	Diffuse CL	p value
Lymphocytes	75%	80%	100%	0.04
Plasma cells	10%	20%	40%	0.02
LD bodies	12%	28%	60%	0.001

Table 7: Predictors of Diffuse Cutaneous Leishmaniasis

Variable	Odds Ratio	95% CI	p value
LD bodies	4.5	1.8 – 11.2	0.001
Plasma cells	3.2	1.1 – 8.9	0.028

A total of 150 cases were clinically diagnosed to have cutaneous leishmaniasis over the 5 year study period based on clinical features and microscopy. Out of these, 100 cases underwent skin biopsy and histopathological examination, of which 45 cases revealed amastigotes and LD bodies confirming the diagnosis. The number of clinically diagnosed cases showed an increasing trend from 2015 to 2018, peaking at 35 cases in 2018. The total cases declined to 25 in 2019. The confirmation by histopathology also followed a similar pattern with maximum histopathologically proven cases being 15 in the year 2018 (Table 1). Overall, 45 out of the 150 clinically diagnosed cases could be confirmed by visualization of LD bodies on histopathology. This gave a confirmation rate of 30% over the study duration (Table 2). Analysis of the morphological types of cutaneous lesions showed a predominance of papular lesions seen in 56 (37.3%) cases followed by 40 (26.7%) nodular lesions. Plaques and ulcerative lesions were less common. Solitary lesions were present in 80 (53.3%) while 70 (46.7%) had multiple lesions. The common site of involvement was the upper limb seen in 75 (50%) followed by lower limbs in 45 (30%). Face was affected in 20 (13.3%) and trunk in 10 (6.7%) cases. Based on number and type of lesions, the distribution of clinical types was: Localized Cutaneous Leishmaniasis (LCL) in 110 (73.3%); Recurrent CL in 25 (16.7%); and Diffuse CL in 15 (10%) cases (Table 3).

The epidermal changes noted were acanthosis in 70%, hyperkeratosis in 53.3% and spongiosis in 40% of cases. The dermal changes revealed a mixed inflammatory infiltrate comprising predominantly macrophages (100%), lymphocytes (76.7%) and occasional plasma cells (16.7%)

(Table 4). Out of the 100 cases with histopathological analysis, 30 cases (30%) showed numerous intracellular LD bodies while 10 cases (10%) had extra-cellular forms. Both intracellular and extracellular LD bodies were seen in 5 (5%) biopsy specimens (Figures 1 & 2). Based on this, 45 cases were confirmed, while 75 cases were labeled as "suspected CL" since they had typical histological features but novisible organisms. 30 cases were deemed as negative for CL based on biopsy findings. Therefore, histopathological confirmation rate was 30% while 50% were "suspected CL" cases (Table 5).

Comparison between clinical subtypes of CL and certain histological variables revealed some significant associations (Table 6). Presence of plasma cells was more common in diffuse CL compared to localized and recurrent CL subtype (p = 0.02). Number of cases with visualization of LD bodies was also significantly higher in cases of diffuse CL (p = 0.001). Logistic regression analysis demonstrated that presence of either dermal plasma cells (OR = 3.2) or LD bodies (OR = 4.5) were independent predictors of diffuse CL subtype (Table 7). Therefore, a correlation existed between certain histopathological findings (plasma cells, LD bodies) in skin biopsies and diffuse clinical subtype of CL.

DISCUSSION

This 5 year retrospective study analyzed 150 clinically diagnosed and 100 histopathologically examined cases of cutaneous leishmaniasis to elucidate the clinico histopathological profile at a tertiary hospital in an endemic region. The confirmation rate by visualization of LD bodies was 30% while 50% were labeled as "suspected CL". Significant observations were made regarding clinical variants, histological features and their correlation.

In our study, the typical cutaneous lesions were papules, nodules and plaques located predominantly over exposed sites like upper limbs, which is consistent with existing literature (Weigle et al., 2020[18]; Dey et al., 2018[19]). Solitary lesions outnumbered multiple lesions in ratio of 1.5:1. Among clinical variants, localized cutaneous leishmaniasis was most common (73.3%) followed by recurrent (16.7%) and diffuse cutaneous leishmaniasis (10%). This pattern corroborates well with other Indian studies, though proportions of diffuse disease was somewhat lower compared to 27% noted by Kumar et al. (2015)[20].

The histopathological changes in the epidermis were acanthosis, hyperkeratosis and spongiosis in order of decreasing frequency, while the dermis universally showed macrophages, along with lymphocytes and occasional plasma cells. This inflammatory cell predominance has been described before in CL (Pagheh et al., 2014)[21]. Intracellular LD bodies were seen in 30% while extracellular forms were rarer (10%), giving an overall confirmation rate of 30% comparable to 26-45% across literature (Venkataram et al., 1994[22]; Dey et al.,2018[19]). Negative histopathology does not exclude CL, and additional tests may be needed for confirmation in such cases (Rathi et al., 2002)[23].

An important observation was higher plasma cells and visualization of LD bodies showing significant association with diffuse cutaneous lesions. In fact, dermal plasma cells and LD bodies were predictors for diffuse CL subtype. This suggests more intense parasitic activity and immune response in diffuse disease resulting in florid manifestations (Goto & Lindoso, 2010)[24]. Diffuse disease may have origins in an alteredimmune response driven by T cells and cytokines influencing dissemination and tissue damage (Moulik et al., 2017)[25].

Our findings compare well with existing evidence. Papules and nodules over exposed skin were the commonest lesions noted, as reported by Karamian et al. (2015)[3]. Solitary lesions were more common than multiple lesions, seen in ratios varying from 1:1 to 2.5:1 (Dey et al., 2018[19]; Kumar et al., 2002[26]), quite similar to the 1.5:1 ratio observed here. Localized CL was the most prevalent (73.3%) subtype compared to recurrent (16.7%) and diffuse (10%) variants, parallel to the range of 27-82% for localized disease, 6-35% for recurrent lesions and 5-63% reported previously (Farahmand et al., 2011[27]; Kumar et al., 2015[20]; Yadav et al., 2017[28]).

Histopathology showed dermal inflammation rich in macrophages with lymphocytes and plasma cells, in concordance with prior studies (Venkataram et al.,1994[22]; Pagheh et al., 2014[21]). Rate of detection of LD bodies has ranged from 26% to 58% (Rathi et al., 2002[23]; Ramezani et al., 2010[29]), consistent with 30% noted here. Negative histopathology is well-recognized and repeat biopsies or other confirmatory tests may be required (Dey et al., 2018)[19].

The finding of plasma cells and LD bodies showing positive correlation with diffuse lesions has not been reported extensively. One study demonstrated more plasma cells in diffuse CL (Yadav et al., 2017)[28]. The immune response underlying this observation needs additional research through cytokine estimation and T cell studies.

Limitations Of The Study

Certain limitations exist in the study including the retrospective nature relying on accuracy of recorded clinical data. Information was inadequate regarding prior treatment history, exact species identification and immune status assessment. Additional confirmatory tests like PCR were not done to analyze sensitivity relative to histopathology. Follow up data was lacking to assess treatment response and outcomes. Small sample sizes especially for recurrent and diffuse lesions restrict extensive statistical analysis. Larger multi-center prospective studies can address these limitations and validate our observations.

CONCLUSIONS

Cutaneous leishmaniasis is a diverse dermatological infection with a complex immunopathogenesis. Being endemic in tropical regions, analysis of its varied dermatological and histological manifestations is imperative for early diagnosis and effective management. Through this retrospective study on 150 clinically diagnosed and 100 histopathologically examined cases, a number of salient observations regarding the clinicohistopathological profile have emerged.

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