Original Article

DOI: 10.21276/APALM.3125

Clinicohistopathological Correlation in Leprosy Lesions: Study in a Tertiary Care Institute in Chhattisgarh

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ABSTRACT

Background: Leprosy is a chronic granulomatous infectious disease, caused by Mycobacterium leprae. Early diagnosis is important to prevent morbidity caused by leprosy. However, accurate clinical or histopathological diagnosis is challenging especially in early skin lesions and in mid-borderline leprosy which shows overlapping features. Hence clinicohistopathological correlation is of utmost importance for accurate diagnosis. This study was conducted to evaluate the predominant subtype of leprosy in patients visiting for treatment in a tertiary care hospital, Chhattisgarh during the post-elimination phase in India.

Methods: A retrospective study of 49 skin biopsies received from June 2019 to October 2020 was conducted in the Department of Pathology and Lab Medicine, AIIMS Raipur. Clinically suspected cases, outside diagnosed cases, and new lesions in treated cases of leprosy were included in this study. Clinical diagnosis was correlated with histopathological examination.

Results: Among 49 skin biopsies, 34 cases were diagnosed as leprosy on histopathological examination. There was no evidence of leprosy in the remaining 15 cases. The most common type was borderline tuberculoid (35.3%), followed by Erythema nodosum leprosum (20.6%). Three cases were associated with type 1 lepra reaction. Among 34 confirmed cases of leprosy, 17(50.0%) cases were positive for acid-fast bacilli by Fite Faraco stain on histological sections. Maximum cases belonged to the age group of 31-40 years and the male to female ratio was 5.8:1.

Conclusion: Histopathological examination of skin lesion along with Fite Faraco stain and clinical correlation is equally efficient for categorization and adequate treatment to achieve leprosy free world.

Keywords: *Fite-Faraco Stain, Histomorphology, Immunological Spectrum, Subtypes of Leprosy*

Introduction

Leprosy is caused by Mycobacterium leprae which was discovered by Hansen in 1837 [1,2,3]. It is a slow-growing organism with an incubation period that varies from 2-12 years [4]. It is a chronic debilitating granulomatous disease, and most of the patients do not present with clinical disease [2,4]. The immune system of the host determines the manifestation of the disease [2,3,5,6]. Person-to-person transmission can occur through the nasal droplet; however, it is not yet proven [3,4]. The sequel of reactivation of past infection is not yet understood. Though leprosy is one of the oldest debilitating diseases, still there are no tools to diagnose subclinical infections and the bacteria cannot be cultured [1,2,4]. It is a public health problem carrying a social stigma for the patients affected though, prevalence has been fallen in the last 50 years [1,2,4]. Among the total world's leprosy cases, India alone represents more than 50% of cases [3]. Undertreatment may lead to further transmission of disease if the disease is not correctly classified [6].

Skin lesions in the form of macules, papules, and nodules, thickened nerve, and sensory loss is the known features of leprosy [3]. It can cause permanent damage to skin, nerves, limbs, and eyes [1,2]. Finding acid-fast bacilli (AFB) inside the nerve is diagnostic of leprosy [4]. Leprosy is classified based on the standard research classification of Ridley and Jopling which is based on immunopathological features like granuloma cell type, presence or absence of the subepidermal grenz zone, pathological changes in nerve, and encroachment of epidermis [1,2]. The cell-mediated immune response to infection is the basis for the development of a particular type of leprosy [2,4]. The classification of leprosy is as per descending order of their immune status from tuberculoid (TT) to lepromatous (LL) and in between TT and LL, there are borderline tuberculoid (BT), midborderline (BB), and borderline lepromatous (BL) type. BB is the most labile form which presents overlapping features [2,7]. Indeterminate leprosy (IL) includes the type that does not fit into any of the five categories, so it is excluded from standard Ridley and Jopling classification but included in Indian classification [1,8]. The ICD-10 however uses Ridley-Jopling classification and also adds an indeterminate or 'I' category [2]. Histoid leprosy (HL) is an uncommon type of LL with an estimated incidence of 2.79 to 3.60% in India [1,8].

This is the retrospective study conducted to review the importance of histopathological examination and to confirm various histological types of leprosy in a tertiary care hospital in Chhattisgarh to emphasize the importance of clinical correlation for exact subtyping of leprosy, to expedite the precise mode of therapy and regular follow-up of patients in this institute to prevent undesirable complications. It also helps to assess the immune status of the patient by histopathological examination like type 1 and type 2 reactions [7]. Histopathological examination of skin lesions also helps to rule out other granulomatous inflammation. Clinico- histopathological correlation has a value for early and accurate subtyping of the lesion and to decrease the global caseload [7,9].

Materials and Methods

This is a retrospective study of 49 skin biopsies received from the Department of Dermatology. This study is approved by the ethics committee of AIIMS Raipur. Cases include clinically suspected and follow-up cases of leprosy received in the department of pathology, AIIMS Raipur from June 2019 to October 2020. These cases were selected regardless of their age, sex, and socioeconomic status. Clinical history, site and morphological examination of skin lesions, clinical diagnosis, and drug treatment were retrieved from histopathological requisition form. Biopsies were fixed in 10% formalin and processed. Serial sections of 5µ thickness were cut and stained with routine Haematoxylin and Eosin. Fite Faraco stain was studied for demonstration of acid-fast bacilli. Fite Faraco stain was performed on deparaffinized tissue sections in two changes of xylene- peanut oil for 6 minutes each and wash in warm running tap water for 3 minutes. Then the sections were stained in carbolfuchsin stain at room temperature for 25 minutes. Then drain excess water from slides vertically on a paper towel. The differentiated with 1% hydrochloric acid for 1 minute till faint pink color appears, again washed in tap water till 3 minutes, then counterstained in methylene blue by one quick dip so that section should look pale blue and then washed in running tap water till excess methylene blue rinse out. Then allowed it to dry before a quick dip in xylene to mount the slide. Under the microscope, acid-fast bacilli appear as a bright red rod against blue background [14]. Conventional Ziehl-Neelsen stains were also studied to find out mycobacterium tubercular infection in suspected cases. Clinical and histopathological diagnosis was done according to the Ridley and Jopling classification.

Results

Out of 49 skin biopsies, 34 cases (69.4%) were confirmed leprosy cases on histopathological examination. The remaining 15 cases showed no evidence of leprosy. Among the total 34 cases, 29 (85.3%) were male and 5 (14.7%) were female with the male to female ratio was 5.8:1. The age group of patients ranged from 11 to 70 years. The majority of cases (10 cases, 29.4%) belonged to the age group of 31-40 years followed by in the age group of 21-30 years (9 cases, 26.5%).

Among 34 diagnosed cases of leprosy, the majority of cases were presented with single or multiple erythematous lesions (79.4% cases) in the form of nodules, macules, and papules and few cases presented with the hypopigmented patch (20.6% cases). The predominant site of lesions found was the upper extremity (35.29%), followed by trunk (32.35%), lower extremity (26.47%), and face (5.88%). [**Figure 1**]

All cases were examined for acid-fast bacilli by Fite Faraco stain. Among 34 histopathological proven cases of leprosy, 17(50.0%) cases showed the presence of acid-fast bacilli in histological sections [**Table 1**]. No acid-fast bacillus could be demonstrated in any of the cases of TT and IL. Amongst cases of BT, only one out of 12 (8.3%) cases showed the presence of few acid-fast bacilli. All (100%) histologically diagnosed cases of BL, LL and HL showed the presence of AFB. Few suspected cases were also examined by conventional Ziehl Neelsen stain. Among a total of 17 positive cases on Fite Faraco stain, 3 cases (17.6%) were also positive for AFB on Ziehl Neelsen stain which includes one case each of LL, ENL, and histoid leprosy.

Amongst the confirmed cases of leprosy, the majority of cases presented with erythematous plaques and nodules (27 cases) contributed mostly by BT and ENL as compared to hypopigmented patch (7 cases) found in borderline tuberculoid. Nerve thickening was seen in 9 patients (26.5%). Ulnar nerve involvement was common, followed by the posterior tibial nerve. The remaining cases showed multiple nerve involvement, including lateral popliteal nerve and common peroneal nerve. No case of pure neuritic leprosy was observed in the study. Visible deformity with grade 2 disability was noted in 3 (8.8%) cases.

Among 34 cases, a clinical and histopathological agreement was seen with 21 cases (48.7%). A maximum percentage of agreement was noted with ENL type (100%). This was followed by BT (37.5%), BL and HL (33.3% each), and LL (20 %). [**Table 2, Figure 2**]

Discussion

Leprosy may lead to severe morbidity if not diagnosed and treated accurately. The clinicohistopathological correlation remains the most powerful tool to approach the lesion correctly and to assess the immune status of the patient [3,4,10]. Such correlation also helps in assessing follow-up

Table 1: Showing positivity of AFB in a different type of leprosy.

Type of leprosy	No. of cases	Positive		Negative	
		No. of cases	percentage	No. of cases	Percentage
TT	2	0	0	2	100
BT	12	1	8.4	11	91.6
BB	2	1	50	1	50
BL	4	4	100	0	0
LL	4	4	100	0	0
Histoid	1	1	100	0	0
IL	2	0	0	2	100
ENL	7	6	85.7	1	14.3
Total	**34**	**17**	**50**	**17**	**50**

Table 2: showing the percentage of parity between clinical and histopathological diagnosis

Subtypes of leprosy	Clinically suspected	Histopathological classification									Agreement	Percentage of parity
		TT	BT	BB	BL	LL	HL	IL	ENL	No evidence		
TT	0	-	-	-	-	-	-	-	-	-	-	-
BT	24	2	9	0	1	0	0	2	1	9	9/24	37.5
BB	1	0	1	0	0	0	0	0	0	0	0/1	0
BL	9	0	2	0	3	1	0	0	1	2	3/9	33.3
LL	5	0	0	2	0	1	0	0	0	2	1/5	20.0
HL	3	0	0	0	0	2	1	0	0	0	1/3	33.3
IL	0	-	-	-	-	-	-	-	-	-	-	-
ENL	7	0	0	0	0	0	0	0	5	2	7/7	100.0
Total	**49**	**2**	**12**	**2**	**4**	**4**	**1**	**2**	**7**	**15**	**21/49**	**42.8**

Table 3: Comparison of the spectrum of leprosy in different studies.

Type	The present study (%)	Roy P et al	Ramesh A et al	Nadia S et al	Prabha V et al
	5.9	16	-	14.4	14.3
BT	35.3	36	50	34.7	16.9
MB	-	-	-	-	1.3
BB	5.9	-	-	16.1	0.0
BL	11.8	8	18.8	5.9	25.3
LL	11.8	12	29.54	21.2	26.6
Histoid	2.9	8	2.27	3.4	1.9
IL	5.9	8	-	4.2	1.3
ENL	20.6	12	-	-	12.3a

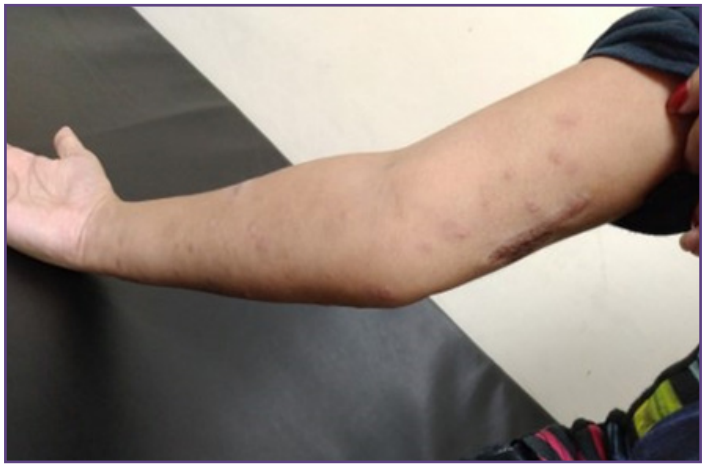

Fig. 1: Skin lesions in Erythema nodosum leprosum (ENL).

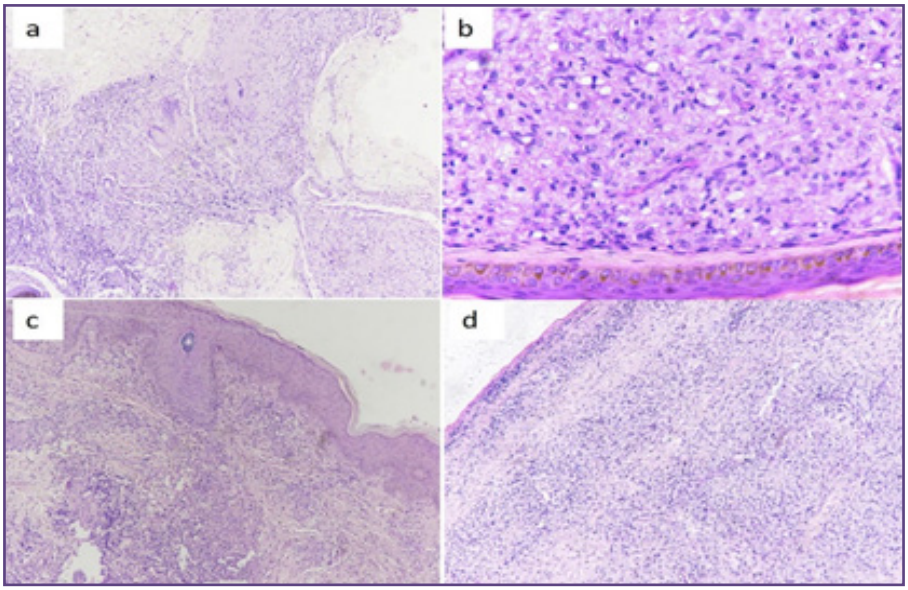

Fig. 2: Microphotograph showing a) Epithelioid granulomas in BT leprosy with type 1 lepra reaction (x100, H & E); b) Foamy macrophages in Lepromatous leprosy (x400, H & E); c) ENL (x100, H & E); d) Histoid leprosy (x100, H & E).

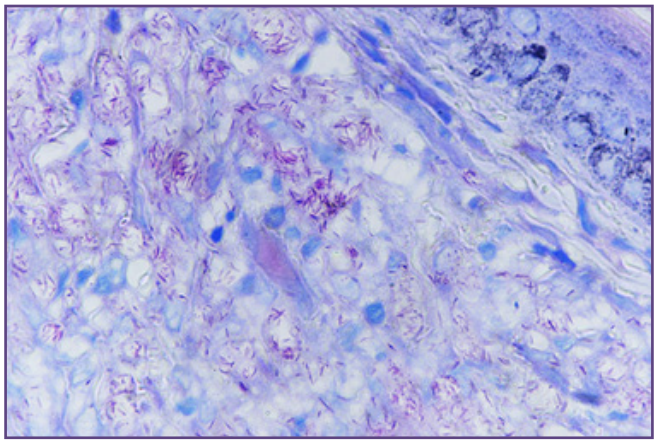

Fig. 3: Lepromatous leprosy with acid fast bacilli (x1000, Fite Faraco stain).

lesions or new lesions erupted despite treatment to study the activity or inactivity of the disease [11,12]. Histopathological examination of skin lesions is essential to confirm the cause, as it mimics other autoimmune disorders which present with bone deformity and skin lesions like rheumatoid arthritis or deforming polyarthritis.

Though there is a substantial decrease in the incidence of leprosy due to the implementation of MDT for leprosy, it is still not eradicated [13]. The purpose of this study was to highlight the significance of histopathological examination and clinical correlation.

Among 34 cases, 85.3% (29 cases) patients were males and 14.7% (5 cases) were females with a male-to-female ratio (M: F) was 5.8:1. In a study conducted by R. Prerona et al in 2019, the male: female ratio was 4.5: 1. Similar findings were also observed in other studies [4,7]. This might be attributed to occupational factors as more chances of exposure in males whereas less reporting of cases in females in India [11]. However, in a study conducted by Vasikar MS et al in 2017, the male to female ratio was 0.8: 1[9].

The age of patients in this study varied between 11-70 years. Maximum numbers of cases occurred in the 3rd decade (29.4%) followed by in the age group of 21- 30 years (26.5%) which is similar to other studies as well [1]. Ramesh A et al found most of their cases (50%) to be between the ages of 30-50 years [5]. Incidence in the younger age group could be due to the endemic nature of leprosy and more chances of noticing lesions in overexposed areas and coming for treatment at this age [1].

In the present study, erythematous plaques/ nodules were the predominant lesions observed (79.4%) than hypopigmented patch which is different from other studies [2,13]. In the present study, out of 34 diagnosed cases, around 19 cases (55.8%) were diagnosed outside and on treatment and presented in OPD with reaction. A hypopigmented patch over skin with loss of temperature sense and numbness is a characteristic feature seen in leprosy. But in our study, we found loss of sensation in the erythematous lesion as well, which might be due to the occurrence of type 1 lepra reactions in these patients. Among 11 cases of lepra reactions in the present study, 7 cases were diagnosed with type 2 (ENL) and 4 cases with type 1 reaction. Two known cases of LL on treatment were histologically confirmed as BB and BL which indicates upgrading reactions. One known case of BT was histologically confirmed as BL which is suggestive of downgrading reaction.

One case clinically suspected as borderline tuberculoid leprosy, on histopathological examination was diagnosed as Lichen planus pigments. 1 case clinically suspected as borderline tuberculoid, on histopathology were diagnosed as Diabetic vasculopathy. One case clinically suspected as ENL or lepromatous leprosy, on histopathology was diagnosed as urticaria. 2 cases clinically suspected as histoid leprosy, on histopathology both were diagnosed as lepromatous leprosy. These discrepancies were probably due to misinterpretation and overdiagnosis of erythematous papules or nodules as leprosy.

Clinically and histopathologically, BT was the commonest type we found in our study. Similar findings were noted in other studies as well [3,5,7]. Maximum parity was seen with ENL. Parity for each type of leprosy was found to be BT (37.5%), BB (00%), BL (33.3%), LL (20.0%), HL (33.3%) and ENL (100%). No cases of TT and IL were clinically suspected; however, both were diagnosed histopathologically [**Table 2]**. In the present study, complete parity between clinical type and histopathological type was noted in 42.8% of cases. The discrepancy might be due to misinterpretation of skin conditions presenting with the hypopigmented or erythematous patch as leprosy. Hence histopathological examination of such lesion is necessary. Selection of the site for biopsy plays an important role in the histopathological diagnosis since clinically dissimilar lesions biopsied from the same patient can show different types of histopathology.

In other studies, observed parity was maximum with LL because it shows fixed clinical and histopathological features. However, in our study parity was maximum with ENL because these cases were diagnosed outside, on treatment, hence might be presented with reaction. Such disparity may occur due to variability in CMI in the population of the different regions [7,11]. In addition, clinically 4 suspected cases of LL / ENL showed no evidence of leprosy histologically. This might be a non-representative biopsy or overdiagnosis of lesions.

In the present study, 4 clinically diagnosed cases of BT were histologically confirmed as 2 cases of TT and 2 cases of IL. However, no cases of IL were suspected clinically. IL cases are difficult to diagnose due to ambiguous histopathological features [11]. IL is not categorized under the standard Ridley Jopling classification. In this study, these 2 cases were presented with erythematous/ hypopigmented lesions with hypoesthesia and the clinical diagnosis was leprosy. On histological examination, it showed a lack of granuloma but presented with lymphocytic infiltrate at the perivascular and periadnexal area. Hence the diagnosis of indeterminate leprosy was given [2]. Prerona Roy et al, V Prabha, and Nadia et al were also noted similar findings [1,3,4] [**Table 3]**. Maximum discordance was seen with midborderline

leprosy (BB), which might be due to showing overlapping features, and many factors like biopsy site and depth of biopsy, duration of lesion, morphology of the lesion, immunological and treatment status of the patient which influenced the histopathological diagnosis, hence clinical correlation is necessary to diagnose the lesion accurately [7,11]. Mid borderline leprosy is also the most unstable form of leprosy with a very frequent shift to either BL or BT spectrum.

Histoid leprosy shows epidermal atrophy, subepidermal 'Grenz zone' immediately below the epidermis and dermis show diffuse involvement of spindle-shaped histiocytes with plenty of acid-fast bacilli. These cases can occur after inadequate dapsone therapy or occasionally denovo [8]. In the present study, the patient was a 53 years old male, presented with a relapse of multinodular lesions and had taken MB-MDT 1 year before for lepromatous leprosy. H & E stained section showed features of histoid leprosy and heavy load of lepra bacilli demonstrated on Fite Faraco stain. Two clinically suspected cases of histoid leprosy were turned out as lepromatous leprosy histologically.

In the present study, 50.0% of cases (17 cases) showed acid-fast bacilli and 50.0% (17 cases) cases were negative for acid-fast bacilli on Fite Faraco stain [**Figure 3**]. Lepra bacilli can be detected well on biopsy sections than on slit skin smear (SSS) as they are mostly located in the deep reticular dermis which is inaccessible to SSS [7]. Out of positive cases, three cases were also positive for acid-fast bacilli on Ziehl- Neelsen stain. Coinfection of leprosy and tuberculosis has been noted in both immunocompetent and immunosuppressed patients [15,16]. It has been found that in such coinfected cases tuberculosis is more severe [15]. Such leprosy-tuberculosis coinfection is rare due to cross-immunity and different reproduction rates of mycobacteria [16]. In our study, for patients with concomitant tuberculosis and untreated leprosy, standard antitubercular therapy (category 1) was given for 6 months. During these 6 months daily dapsone 100mg and clofazimine 50mg with monthly once 300mg clofazimine was added as antileprosy treatment. Monthly rifampicin was not added as the patient will be receiving daily rifampicin as part of an anti TB treatment. After 6 months with the completion of anti-TB treatment, for the next 6 months, the patient was given monthly 600mg rifampicin in addition to dapsone and clofazimine in the doses described above to complete 1 year of antileprsy treatment. One case was diagnosed with untreated lepromatous Leprosy with recurrent severe type 2 lepra reaction with lymph node tuberculosis. She was given 6 months of the standard regimen of ATT as per NTEP guidelines with the addition of clofazimine 300 mg tapered to 50 mg daily later. She was not given dapsone as she was anemic. After 6 months she was given Rifampicin 600 mg monthly once with daily 50 mg of clofazimine. At 12 months Minocycline 100 mg was added with the above-mentioned regimen for the next 12 months. She was treated with oral corticosteroids for type 2 lepra reaction initially but as she was developing repeated episodes of type 2 reaction and was developing Cushingoid features, she was started on Thalidomide for type 2 reaction after detailed counseling. Recently such tubercular coinfection is detected in patients who were initially diagnosed with either borderline or lepromatous leprosy and were on corticosteroid for type 1 reversal reaction [16]. Hence screening for tuberculosis is important in diagnosed cases of leprosy before starting Rifampicin monotherapy as there is the risk of developing rifampicin-resistant tubercular strain in a leprosy patient. In such cases, minocycline can be given in place of rifampicin till TB screening results to prevent fatal outcomes and it is necessary to treat both infections at the same time [16].

The differences in positivity for lepra bacilli in different studies can be attributed to regional variation, different socioeconomic and immune statuses in the population studied. The inactive bacilli may persist for years in nerves of borderline or indeterminate cases even after complete treatment. Hence regular follow-up of the patients after treatment and screening for bacillary load is important before marking them as disease-free [11].

Here we concluded that histopathological examination along with Fite Faraco stain is the gold standard for accurate diagnosis and typing of leprosy. Late diagnosis may lead to an increased risk of disability and continued transmission. Delay can occur either due to late presentation by patient or delay in making diagnosis by health services. Varied clinical manifestations can be seen even in established leprosy and that on treatment, so individual lesions may differ histologically which causes clinical and histopathological disparities. A biopsy is a minimally invasive and easy method as well, hence it should get done in every suspected case of leprosy to achieve the goal of leprosy free world.

Abbreviations

Acid-fast bacilli (AFB); Tuberculoid leprosy (TT); Lepromatous leprosy (LL); Borderline tuberculoid (BT); Midborderline (BB); Borderline lepromatous (BL); Indeterminate leprosy (IL); Histoid leprosy (HL); Erythema Nodosum Leprosum (ENL); Multidrug therapy (MDT); Multibacillary (MB).

Acknowledgment

None

Funding
None

Competing Interests
None declared

Statement of Informed Consent
This is a retrospective study and patient identity has not been revealed, hence written consent is not applicable here.

Statement of Human and Animal Rights
Not applicable in this study

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Financial or other Competing Interests: None.

Date of Submission : 04/09/2021
Date of Final Revision : 01/10/2021
Date of Acceptance : 26/10/2021
Date of Publication : 31/10/2021