

Histopathological Evaluation of Skin Biopsies of Leprosy at a Tertiary Care Centre of Western Rajasthan, India: A Cross-sectional Study

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ABSTRACT

Introduction: Leprosy is a slowly progressive chronic granulomatous disease caused by *Mycobacterium Leprae* (*M. leprae*) and predominantly affects skin and peripheral nerves. Leprosy is a leading cause of physical disability in India and poses a major public health challenge for the country. Histopathological evaluation and demonstration of Lepra bacilli by special stain is gold standard for definitive diagnosis and subtyping of leprosy.

Aim: To study the common histological subtypes of leprosy on the basis of microscopic features and bacteriological index.

Materials and Methods: A cross-sectional study was conducted in the Department of Pathology at Dr. S.N. Medical College, Jodhpur, Rajasthan, India. The duration of the study was two years and 11 months, from January 2019 to December 2021. A total of 55 skin biopsies which were histologically diagnosed as different types of leprosy were included. Patients data of age, gender, skin examination and other investigations were collected from the requisition forms and analysed. After proper fixation and processing of biopsy samples, Haematoxylin and Eosin (H&E) stained sections were examined for epidermal atrophy, presence of grenz zone, granulomas, infiltrates of lymphocytes, histiocytes, foam cells, infiltration of nerves and adnexa. The sections stained with Fite-Faraco stain were studied for the assessment of bacteriological index. On the basis of microscopic features, cases were categorised into tuberculoid leprosy,

borderline tuberculoid leprosy, indeterminate leprosy, borderline leprosy, lepromatous leprosy and histoid leprosy as per Ridley-Jopling classification and percentage is calculated for each category. Data was entered and analysed by using Microsoft Excel version 2008 and Statistical Package for Social Sciences (SPSS) version 23.0.

Results: The mean age of the study participants was 39.6 years. Male to female ratio was 2.92:1. There were 33 (60%) cases with erythematous lesions followed by 15 (27.27%) hypopigmented lesions and 7 (12.73%) nodules. Out of 55 there were 21 (38.18%) cases of tuberculoid leprosy followed by 11 (20%) cases of borderline tuberculoid leprosy, 8 (14.55%) cases of indeterminate leprosy, 6 (10.91%) cases of borderline leprosy, 5 (9.09%) cases of lepromatous leprosy and 4 (7.27%) cases of histoid leprosy. Bacteriological index was negative in 35 (63.64%) cases followed by 2+ in 6 (10.91%) cases, 5 (9.09%) had index of 4+, 4 (7.27%) cases had index of 3+, 3 (5.45%) had index of 1+, and 1 (1.82%) case had index of 5+ and 6+ each.

Conclusion: Skin biopsy is easy, simple, inexpensive and outpatient procedure which provides adequate material for confirmation of the clinical diagnosis and further management. Histopathological examination including microscopic features of H&E stained sections, along with assessment of bacteriological index on Fite-Faraco stained sections remain the gold standard for diagnosis and subtyping of leprosy cases.

Keywords: Chronic, Fite-faraco stain, *Mycobacterium leprae*, Physical disability

INTRODUCTION

Leprosy is a granulomatous skin lesion caused by *M. leprae* that predominantly affects cooler tissues such as skin and peripheral nerves. The bacilli are shed from nose, upper respiratory tract, and skin [1]. It is a slowly progressive, chronic infectious disease which can express itself in different clinicopathological forms depending on immune status of host [2]. The mode of transmission is not clearly known although, person-to-person spread through nasal droplets is believed to be the main route [3]. India has succeeded in bringing the national prevalence down to "elimination as a public health problem" of less than 1/10,000 in December 2005 and even further down to 0.66/10,000 in 2016. Despite the above successes, India continues to account for 60% of new cases reported worldwide each year and is among the 22 "global priority countries" that contribute 95% of world numbers of leprosy cases warranting more efforts to bring the numbers down [4].

Physical disabilities caused due to leprosy often evoke severe social stigma, that leads to prejudice against patients and their families [5]. Infection with *M. leprae* leads to chronic granulomatous inflammation in the skin and peripheral nerves. The type of leprosy that patients develop is determined by their cell-mediated immune

response to infection. Types may be categorised according to the Ridley-Jopling classification [6]. The clinical classification describes only the gross appearances of the lesions, the histopathological classification is based on microscopic features and also takes into account the immunological manifestations, which enable it to successfully bridge the pitfalls in leprosy diagnosis. Suspicious cases which can be missed in clinical practice and epidemiological studies can be confirmed histopathologically. It is a valuable aid to reach confirmatory diagnosis of leprosy, its subtyping, prognosis assessment of regression of the disease in patient under treatment and also for research purpose [7].

The aim of the present study was to study the common histopathological types of leprosy based on microscopic features along with bacteriological index. Objectives of study were to evaluate frequency in relation with the age and gender and to study different types of skin lesions of leprosy.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology at Dr. S.N. Medical College, Jodhpur, Rajasthan, India. The duration of the study was two years and 11 months, from January

2019 to December 2021. The study was approved by Institutional Ethics Committee by reference number SNMC/IEC/467.

Inclusion criteria: All specimens of skin biopsies received in pathology department for histopathological examination after clinical diagnosis or suspicion of leprosy were included in the study.

Exclusion criteria: Inadequate and autolysed biopsy samples were excluded from the study.

Study Procedure

After obtaining telephonic consent, patients data of age, gender, skin examination and other investigations were collected from the requisition forms. Microscopic features were analysed from slides. Skin biopsies for the study were obtained by incisional or punch biopsy, which was performed by the Dermatologist. These biopsies were kept in 10% formalin and a detailed clinical history, examination findings indicating signs and symptoms of the skin lesions and provisional clinical diagnosis was sent to the Department of Pathology. Following adequate fixation for about 12-24 hours, the tissues were submitted for routine processing, following which the paraffin embedded serial sections of 4-5 μ thickness, were obtained. These were stained with H&E for morphological assessment and with Fite-Faraco staining for identification of the lepra bacilli.

The sections were examined for epidermal atrophy, presence of grenz zone, granulomas, infiltrates of lymphocytes, histiocytes, foam cells, infiltration of nerves, blood vessels and adnexa. The sections stained with Fite-Faraco stain were observed under oil immersion using 100x objectives for the assessment of bacteriological index. Following was the scale used to calculate the bacteriological index [8]:

1+=1-10 bacilli in 100 Oil Immersion Field (OIF)-examine 100 OIF

2+=1-10 bacilli in 10 OIF-examine 100 OIF

3+=1-10 bacilli in 1 OIF-examine 25 OIF

4+=10-100 bacilli in 1 OIF-examine 25 OIF

5+=100-1000 bacilli in 1 OIF-examine 25 OIF

6+=> 1000 bacilli in 1 OIF-examine 25 OIF

After studying the histopathological features and noting the bacteriological index, the diagnosis of leprosy was confirmed and classified according to Ridley-Jopling classification [6].

STATISTICAL ANALYSIS

Data were entered and analysed by using Microsoft Excel version 2008 and SPSS version 23.0. Continuous variables were summarised as mean and Standard Deviation (SD) and analysed by Analysis of Variance (ANOVA). Nominal/categorical variables were summarised as percentage and analysed by Chi-square test, p-value <0.05 was considered statistically significant.

RESULTS

Total 55 cases were analysed. There were 41 (74.55%) males and 14 (25.45%) females with male:female ratio of 2.92:1. The mean age of cases was 39.6 years. In present study, 33 (60%) cases were in age group 21 years-40 years followed by 15 (27.27%) cases were in age group 41 years-60 years [Table/Fig-1].

Age groups (in years)	Number of cases n (%)	Mean \pm SD
≤ 20	1 (1.82)	39.6 \pm 14.24
21-40	33 (60.00)	
41-60	15 (27.27)	
≥ 61	6 (10.91)	
Total	55 (100.00)	

[Table/Fig-1]: Distribution according to age (N=55).

Clinically, Leprosy presents with a spectrum of findings that may include hypopigmented macules, erythematous plaques and/or nodules often found on the face, extensor surfaces of the limbs, buttocks, and trunk. In the present study, 33 (60%) cases had erythematous lesions, followed by 15 (27.27%) cases had hypopigmented lesions and 7 (12.73%) cases had nodules [Table/Fig-2,3a-c].

Type of lesions	Number of cases (n)	Percentage (%)
Erythematous lesions	33	60
Hypopigmented lesions	15	27.27
Nodules	7	12.73
Total	55	100

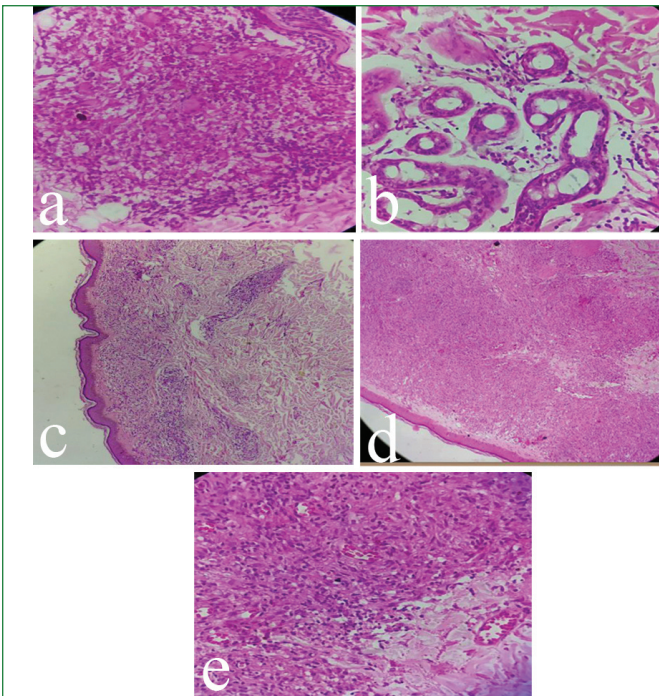
[Table/Fig-2]: Distribution according to types of lesion (N=55).



[Table/Fig-3a-c]: a) Well-defined erythematous lesions of leprosy over back. b) Hypopigmented patches of leprosy on thigh. c) Skin-coloured nodular lesions of leprosy over back and extensor surface of upper limb.

Out of the total 55 cases, 21 (38.18%) were of tuberculoid leprosy followed by 11 (20%) were of borderline tuberculoid leprosy, 8 (14.55%) were of indeterminate leprosy, 6 (10.91%) were of borderline leprosy, 5 (9.09%) were of lepromatous leprosy and 4 (7.27%) were of histoid leprosy [Table/Fig-4a-e,5]. The mean age of cases having borderline leprosy was 38.83 years, cases having borderline tuberculoid leprosy was 40.91 years and cases having tuberculoid leprosy was 37.52 years [Table/Fig-5]. There was statistically no significant difference in mean age among different type of lesions (p-value=0.192, ANOVA test).

Lepra bacilli index was negative in 35 (63.64%) cases, followed by 2+ in 6 (10.91%) cases, 5 (9.09%) had index of 4+, 4 (7.27%) had index of 3+, 3 (5.45%) had index of 1+, and 1 (1.82%) case had index of 5+ and 6+ each [Table/Fig-6a-f,7]. The mean age of cases having lepra bacilli index 1+ was 46.67 years and index 2+ was 30.83 years, and cases having lepra bacilli index negative was 39.86 years [Table/Fig-7]. There was statistically no significant difference in mean age among different types of lepra bacilli index (p-value=0.174, ANOVA test).

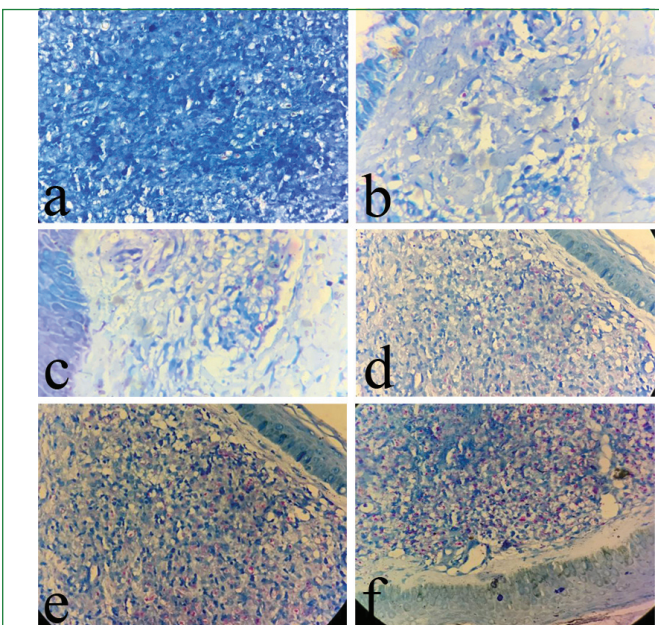


[Table/Fig-4a-e]: a) Borderline tuberculoid leprosy (H&E, 40X)-shows diffusely form granuloma with langhans giant cells. b) Indeterminate leprosy (H&E, 40X)- shows lymphocytic infiltration around glands. c) Lepromatous leprosy (H&E, 10X) shows flattened epidermis, with characteristic grenz zone. d) Histoid leprosy (H&E, 10X) shows flattened epidermis and spindle like cells in a storiform pattern. e) Histoid leprosy (H&E, 40X) shows spindle like cells in dermis.

Histopathological diagnosis	Number of cases n (%)	Age (in years)	p-value
		Mean±SD	
Tuberculoid leprosy	21 (38.18)	37.52±12.89	0.192
Borderline tuberculoid leprosy	11 (20.0)	40.91±14.57	
Indeterminate leprosy	8 (14.55)	45.13±16.63	
Borderline leprosy	6 (10.91)	38.83±20.92	
Lepromatous leprosy	5 (9.09)	43.00±13.86	
Histoid leprosy	4 (7.27)	32.15±2.22	

[Table/Fig-5]: Distribution of cases according to histopathological diagnosis and mean age for different subtypes (N=55).

n: Number of cases; %: Percentage



[Table/Fig-6a-f]: a) Fite-Faraco stain (100x oil immersion) shows Acid Fast Bacilli (AFB) (bacteriological index 1+). b) Fite-Faraco stain- 100X shows AFB (bacteriological index 2+). c) Fite-Faraco stain-100X shows AFB (bacteriological index 3+). d) Fite-Faraco stain-(100x) shows AFB (bacteriological index 4+). e) Fite-Faraco stain (100x) -shows AFB (bacteriological index 5+). f) Fite-Faraco stain- 100X shows AFB (bacteriological index 6+) in case of histoid leprosy.

Lepra bacilli index	Number of cases n (%)	Age (in years)	p-value
		Mean±SD	
1+	3 (5.45)	46.67±22.55	0.174
2+	6 (10.91)	30.83±5.60	
3+	4 (7.27)	45.5±24.45	
4+	5 (9.09)	41.6±13.24	
5+	1 (1.82)	33±0	
6+	1 (1.82)	35±0	
Negative	35 (63.64)	39.86±13.77	
Total	55 (100)		

[Table/Fig-7]: Distribution of cases according to lepra bacilli index and mean age for different categories (N=55).

n: Number of cases; %: Percentage

DISCUSSION

Skin lesions of leprosy are common in both males and females. The mean age of cases was 39.6 years with majority of cases (60%) were in age group 21-40 years followed by 27.27% cases were in age group 41-60 years. In consistency with results of present study Basir B et al., studied 50 patients and reported that, largest age group was 21-30 years with 14 patients contributing to 28% [9]. In the study by Patel C and Nishal A, maximum cases were in 21-30 years age group which constitutes about 28.33% [10]. In a recent study done in 2022 by Naik SM et al., which included 100 cases of skin biopsy of leprosy, in which 35% case were in 21-30 years age group [11]. There were 74.55% males and 25.45% females with male:female ratio were 2.92:1. In concordance with results of present study Basir B et al., reported that, overall incidence among males and females was 64% and 36%, respectively [9]. In a study by Jain N and Gupta K, found an incidence of 67% among males and 33% among females [12]. In the study by Sinha R et al., the incidence of disease in males was about 71% and in females was about 29% [Table/Fig-8] [9-14]. There were 60% cases with erythematous lesions followed by 27.27% hypopigmented lesions and 12.73% nodules in present study.

Yadav N et al., reported erythematous patches and hypopigmented macules in 67.5%, and 32.5% cases, respectively [14]. Jain N and Gupta K, found hypopigmented lesions (76%) to be the most common clinical finding [12]. Majority of cases had tuberculoid leprosy (38.18%) followed by 20% had borderline tuberculoid leprosy, 14.55% had indeterminate leprosy, 10.91% had borderline leprosy, 9.09% had lepromatous leprosy and 7.27% had histoid leprosy. Basir B et al., reported among the 50 patients, 16 (32%) had borderline tuberculoid, 13 (26%) patients had borderline leprosy, 7 (14%) had tuberculoid leprosy, 4 (4%) had lepromatous leprosy and 3 (6%) patients had intermediate leprosy [9]. According to study by Naik SM et al., 52 patients had borderline tuberculoid, 20 patients had Borderline leprosy, 13 patients had Lepromatous leprosy, eight patients had Tuberculoid leprosy and three patients had mid borderline [11]. In a study by Jain N and Gupta K, out of 100 cases, highest number of cases found were of borderline tuberculoid leprosy (43%) followed by tuberculoid leprosy (12%), lepromatous leprosy (9%), mid borderline (7%) and least common was borderline leprosy (3%) [12]. In the present study, majority of cases (63.64%) were negative for lepra bacilli index followed by 10.91% had index of 2+, 9.09% had index of 4+, 7.27% had index of 3+, 5.45% had index of 1+, 1.82% had index of 5+ and 6+ each. Naik SM et al., reported out of 100 cases, 67 cases turned positive for Acid Fast Bacilli (AFB) on Wade-Fite staining [11]. Lepra bacilli index was negative in 37% cases followed by 22% had index of 1+, 13% had index of 2+, 5% had index of 3+, 10% had index of 4+, 8% had index of 5+ and 5% cases had index of 6+.

Histopathological examination remains the gold standard for diagnosis and helpful for subtyping of leprosy, especially using Ridley-Jopling

Studies	Basir B et al., [9]	Patel C and Nishal A [10]	Jain N and Gupta K [12]	Sinha R et al., [13]	Yadav N et al., [14]	Naik SM et al., [11]	Present study
Year	2018	2019	2019	2019	2019	2022	2021
Place	Assam	Surat	Jaipur, Rajasthan	Patna	Uttarakhand	Pune	Jodhpur, Rajasthan
Total cases	50	60	100	200	62	100	55
Age group (in years) (%)	21-30 (28)	21-30 (28)	31-40 (21)	11-30 (23)	11-20 (25)	21-30 (35)	21-40 (60)
Gender predominance (%)	Male (64)	Male (76.6)	Male (67)	Male (71)	Male (61)	Male (69)	Male (74.5)
Histological diagnosis (%)	Borderline tuberculoid (32)	Lepromatous leprosy (30)	Borderline tuberculoid (43)	Borderline lepromatous leprosy (43)	Lepromatous leprosy (38)	Borderline tuberculoid (52)	Tuberculoid leprosy (38.18)

[Table/Fig-8]: Comparison of present study with other studies [9-14].

classification [6,15,16]. Spectrum of leprosy presentation is very wide and no single criterion can be used to diagnose leprosy conclusively. Cases where lepra bacilli were not demonstrated in slides and cases classified in indeterminate leprosy were difficult to diagnose. Therefore, correlation of clinical and histopathological features along with bacteriological index is more useful for diagnosis and accurate subtyping of leprosy [17,18].

Limitation(s)

Proper biopsy technique is very crucial to yield adequate amount of tissue for histopathological examination. Many characteristic microscopic features are not visible in cases with inadequate biopsies.

CONCLUSION(S)

Skin biopsy is easy, simple, inexpensive and outpatient procedure, which provides adequate material for confirmation of the clinical diagnosis. Dermatological lesions of leprosy are heterogeneous with wide age distribution, clinical and histopathological spectrum. In present study, tuberculoid leprosy was the commonest type and need to be carefully reported. Demonstration of lepra bacilli yields a better report.

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