

Study of Histopathological Spectrum of Leprosy Patients with Special Reference to Conventional PCR for Detection of *Mycobacterium leprae* in Skin Biopsy Samples: A Prospective Observational Study

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ABSTRACT

Introduction: Leprosy, caused by *Mycobacterium leprae*, is a chronic infectious disease affecting the skin and peripheral nerves. The clinical presentation varies, from tuberculoid to lepromatous forms due to differences in immune response. Although slit skin smears (SSS) and histopathological analysis are standard diagnostic methods. Modified Ziehl–Neelsen staining is a widely used technique, but molecular methods like polymerase chain reaction (PCR) offer superior sensitivity and diagnostic utility. The aim of the study was to evaluate histological findings, and PCR results in skin biopsies from leprosy patients and to correlate histopathological and molecular findings.

Material and Methods: A 4 mm skin punch biopsy was obtained from 55 clinically diagnosed leprosy patients, preserved in formalin, and processed for histopathology. Staining was performed using hematoxylin and eosin (H&E) and modified Ziehl–Neelsen stains. PCR testing was conducted for molecular detection of *Mycobacterium leprae*.

Results: The majority of patients were aged between 21–40 years, with 31 males (56.4%) and 24 females (43.6%). Borderline lepromatous leprosy was the most prevalent type. Of the 55 cases, 45.45% tested positive with modified Ziehl–Neelsen staining, whereas PCR demonstrated a higher positivity rate of 54.54%.

Conclusion: PCR is a valuable adjunct for diagnosing leprosy in cases where modified Ziehl–Neelsen staining results are negative. Despite its advantages, modified Ziehl–Neelsen staining remains essential in low-resource settings due to its accessibility and cost-effectiveness. Incorporating PCR in cases where Ziehl–Neelsen staining shows negative results, it can enhance the overall diagnostic yield.

Keywords: Leprosy, *Mycobacterium leprae*.

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INTRODUCTION

Leprosy remains a significant public health concern globally. It is a chronic granulomatous infectious disease caused by *Mycobacterium leprae*, which primarily affects the skin and peripheral nerves. Complications of leprosy include loss of feeling, pain and heat receptors, ulcers, infertility in men and corneal insensitivity. The clinical presentation varies depending on bacterial load and the host's immunological profile, spanning a spectrum from tuberculoid to lepromatous leprosy. Ridley and Jopling classified leprosy into five categories: tuberculoid (TT), borderline tuberculoid (BT), mid borderline (BB), borderline lepromatous (BL), and lepromatous leprosy (LL).^{1,2}

For therapeutic purposes, the World Health Organization (WHO) adopted a simplified classification based on the number of skin lesions. Paucibacillary (PB) leprosy refers to cases with five or fewer lesions, treated with a two-drug regimen for six months. In contrast, multibacillary (MB) leprosy involves six or more lesions, requiring a three-drug regimen for one year.^{3,4}

Despite extensive efforts to eliminate leprosy, including multidrug therapy (MDT), active transmission persists. In 2016, the global prevalence was reported as 171,948 cases, with a rate of 0.23 per 10,000 population. India alone accounted for 114,451 new cases in 2019–2020, contributing to 80% of cases in Southeast Asia.⁵ However, by 2020–21, the National Leprosy Eradication Programme (NLEP) reported a decline in new cases to 65,147, reflecting an annual new case detection rate of 4.56 per 100,000 population.

The Global Leprosy Strategy 2016–2020, “Accelerating Towards a Leprosy-Free World,” aimed

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to strengthen government partnerships, eliminate leprosy-related disabilities, and promote social inclusion. The strategy emphasized the early detection and diagnosis of leprosy, a priority that remains unmet due to the lack of reliable diagnostic tools, especially in the disease's early stages.⁶

Innovative diagnostic approaches, including the use of nanotechnology and fluorescence microscopy, have shown potential in improving sensitivity. For instance, light-emitting diode (LED)-based fluorescence microscopy offers higher sensitivity and is feasible even in remote laboratories without culture facilities.^{7,8} This aim is to study of histopathological spectrum of leprosy patients with special reference to conventional PCR for detection of *mycobacterium leprae* in skin biopsy samples.

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted at the Department of Pathology and central research laboratory, SRMS IMS, Bareilly. Ethical approval for the study was obtained from the Institutional Ethics Committee (#SRMC IMS/ECC/2022/127). Written informed consent was obtained from all participants prior to inclusion.

Study Duration

The research spanned 1.5 years, providing adequate time for data collection, processing, and analysis.

Study Population

The study population consisted of patients diagnosed with leprosy. Participants were recruited using simple random sampling.

Sample Size

Simple Random sampling technique was used to select the sample.

The sample size (n) calculated as 50.68, an additional 10% for non response was added to give a sample size, thus sample size chosen for study was 55.

Biopsy Procedure and Histopathological Analysis

A 4-mm skin punch biopsy was obtained fixed in 10% formalin and submitted to the Department of Pathology for processing.

Histological sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H&E) for histological examination. Modified Ziehl-Neelsen (ZN) staining was performed to detect acid-

fast bacilli (*Mycobacterium leprae*). The Ridley and Jopling classification system was applied to categorize histopathological findings.

Molecular Analysis Using PCR

PCR analysis was employed to detect *Mycobacterium leprae* DNA in paraffin-embedded tissue. Sections of 5–10 µm thickness were prepared, with the initial exposed sections discarded to minimize contamination. These sections were then transferred to central research laboratory for PCR analysis. DNA extraction involved deparaffinization, lysis with proteinase K, and purification using the QIAamp DNA formalin fixed paraffin embedded (FFPE) Mini Kit as per procedure. PCR amplification targeted specific genomic regions of *Mycobacterium leprae*, ensuring high sensitivity and specificity.

Statistical Analysis

Data were entered into a Microsoft Excel spreadsheet and analysed using SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Numerical variables were summarized as mean and standard deviation, while categorical variables were summarized as counts and percentages. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

A study was conducted to analyze the distribution and diagnostic characteristics of 55 leprosy patients, focusing on clinical, histological, and molecular findings. The majority of participants (45.5%) were aged between 21 and 40 years). Among the 55 participants, 56.4% were male (n=31), while 43.6% were female (n=24).

The most common histological type was Borderline Lepromatous Leprosy (BLL), which accounted for 29.15% of the cases. The baseline characteristics of the patients presented is detailed in Table 1. The association between histological findings and Modified Ziehl-Neelsen (ZN) staining results is shown in Table 2. Overall, 45.5% of the total cases tested positive for ZN, and statistical analysis revealed a significant association between ZN staining and histological types ($\chi^2 = 17.884$, $p = 0.007$). The relationship between PCR results and histological classification is summarized in Table 3. The PCR positivity rates varied by histological type, with LL exhibiting the highest PCR positivity rate at 90%, followed by BLL at 56.3%. Overall, 54.5% of the 55 cases were PCR positive, while 45.5% were negative. Statistical analysis ($\chi^2 = 9.788$, $p = 0.134$) (table 3). Figure 1 showing different histopathology of different type of leprosy. Figure 2 showing (PCR) Agarosegel electrophoresis of PCR products of 372bp. obtained by using primers to the *mycobacterium leprae* repetitive sequence.

Table 1: Patient characteristics

Age (in years)	Frequency	Percent
0-20	5	9.1%
21-40	25	45.5%
41-60	18	32.7%
61-80	6	10.9%
81-100	1	1.8%
Total	55	100.0%
Mean $\pm$ Std.	38.90 $\pm$ 16.73	
Gender	Frequency	Percent
Male	31	56.4%
Female	24	43.6%
Total	55	100.0%
Histological Findings	Frequency	Percent
Tuberculoid Leprosy (TL)	7	12.7%
Borderline Tuberculoid Leprosy (BTL)	13	23.6%
Mid Borderline Leprosy (MBL)	5	9.1%
Borderline Lepromatous Leprosy (BLL)	16	29.1%
Lepromatous Leprosy (LL)	10	18.2%
Histoid Leprosy (HL)	1	1.8%
Indeterminate Leprosy (IL)	3	5.5%
Total	55	100.0%

DISCUSSION

Leprosy remains a significant global public health concern. This chronic granulomatous infectious disease, caused by *Mycobacterium leprae*, primarily affects the skin and peripheral nerves. The clinical manifestations of leprosy vary based on the host's immune response and bacterial load, resulting in a spectrum of presentations ranging from tuberculoid to lepromatous forms.

The diagnosis of leprosy hinges on identifying characteristic lesions, thickened peripheral nerves, and sensory loss. Due to the inability to culture *M. leprae* in vitro, diagnosis is often challenging, particularly

for inexperienced practitioners, as it may mimic other dermatological conditions.^{7,9,10} Accurate diagnosis requires a combination of clinical examination, histopathological evaluation, and the detection of bacteria in skin biopsies.

In our cohort, the majority of patients (45.5%) belonged to the 21–40 years age group, followed by 32.7% in the 41–60 years range. Smaller proportions were noted in the 0–20 years (9.1%), 61–80 years (10.9%), and 81–100 years (1.8%) categories, with a mean age of 38.90 $\pm$ 16.73 years. Similar findings were reported in studies by Mathur *et al.*, and Kaur *et al.*, where the majority of cases also fell within the 21–40 age group.^{11,12}

The male-to-female ratio in our study was 1.3:1, with males constituting 56.4% of cases. This male predominance aligns with findings from Mathur *et al.*, Kaur *et al.*, and Moorthy *et al.*, among others.¹¹⁻¹⁵ The disparity may reflect gender-specific sociocultural factors, including differences in healthcare access and disease awareness, as also noted in previous literature.¹⁶⁻¹⁸

Borderline lepromatous leprosy (BLL) was the most frequently observed type (29.1%), followed by borderline tuberculoid leprosy (BTL) (23.6%), and lepromatous leprosy (LL) (18.2%). Tuberculoid leprosy (TL) accounted for 12.7% of cases, with mid-borderline leprosy (MBL), indeterminate leprosy (IL), and histoid leprosy (HL) comprising smaller proportions. The predominance of borderline leprosy forms is consistent with studies by Moorthy *et al.* and Giridhar *et al.*^{13,14,19,20}

The highest clinical-histopathological concordance was observed in LL (93.75%) and TT (78.5%), while the lowest was in IL (27.78%). Borderline forms demonstrated variable concordance, consistent with findings from Moorthy *et al.* and Bijjaragi *et al.*, highlighting the diagnostic complexity within the borderline spectrum.^{13,19}

PCR positivity was highest in LL (90%) and HL (100%), with borderline types showing moderate positivity. No significant correlation between PCR results and

Table 2: Association of Histological findings with Modified Ziehl Neelsen-staining

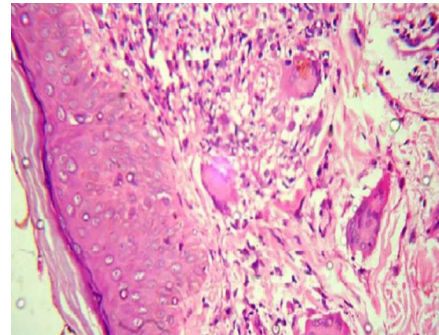
Histological Finding	ZN stain		Total	Chi square test	p- value
	Modified Ziehl Neelsen-Stain Positive (%)	Modified Ziehl Neelsen-Stain Negative (%)			
Tuberculoid leprosy (TL)	1 (14.3%)	6 (85.7%)	7		
Borderline tuberculoid leprosy (BTL)	3 (23.1%)	10 (76.9%)	13		
Mid borderline leprosy (MBL)	2 (40.0%)	3 (60.0%)	5		
Borderline lepromatous leprosy (BLL)	9 (56.3%)	7 (43.8%)	16	17.88	0.00
Lepromatous leprosy (LL)	9 (90.0%)	1 (10.0%)	10	4	7
Histoid leprosy (HL)	1 (100.0%)	0 (0.0%)	1		
Indeterminate leprosy (IL)	0 (0.0%)	3 (100.0%)	3		
Total	25 (45.5%)	30 (54.5%)	55		

Table 3: Association of Histological findings with PCR

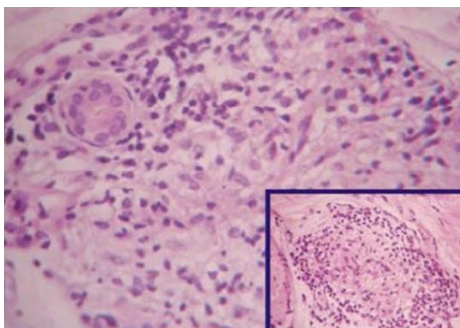
<i>Histological Findings</i>	<i>PCR</i>		<i>Chi square test</i>	<i>p-value</i>
	<i>PCR Positive</i>	<i>PCR Negative</i>		
Tuberculoid Leprosy (TL) (N=7)	2 (28.6%)	5 (71.4%)	9.788	0.134
Borderline Tuberculoid Leprosy (BTL) (N=13)	5 (38.5%)	8 (61.5%)		
Mid Borderline Leprosy (MBL) (N=5)	2 (40.0%)	3 (60.0%)		
Borderline Lepromatous Leprosy (BLL) (N=16)	9 (56.25%)	7 (43.75%)		
Lepromatous Leprosy (LL) (N=10)	9 (90.0%)	1 (10.0%)		
Histoid Leprosy (HL) (N=1)	1 (100.0%)	0 (0.0%)		
Indeterminate Leprosy (IL) (N=3)	2 (66.7.0%)	1 (33.3%)		
Total(N=55)	30(54.5%)	25(45.5%)		



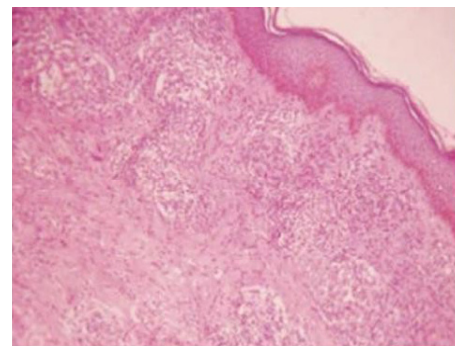
Tuberculoid Leprosy Numerous Langhans Giant Cells With Lymphocytic Infiltrate (H&E, 10X)



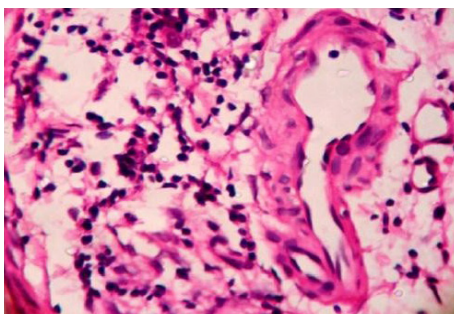
Tuberculoid Leprosy Epithelioid Granuloma With Langhans Giant Cells (H&E, 40X)



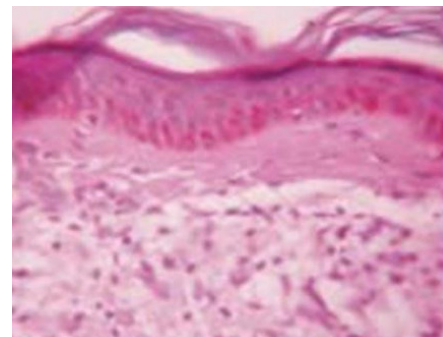
Borderline Tuberculoid Leprosy–(Lymphohistiocytes Around Adnexa (H&E,40X)



Mid Borderline Leprosy–(Showing Both Lymphohistiocytes and Foamcells (H&E,10X)

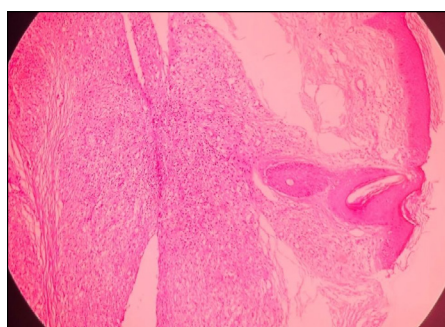


Borderline Lepromatous Leprosy Lymphohistiocytic Infiltrate Around Blood Vessels (H & E, 40X)

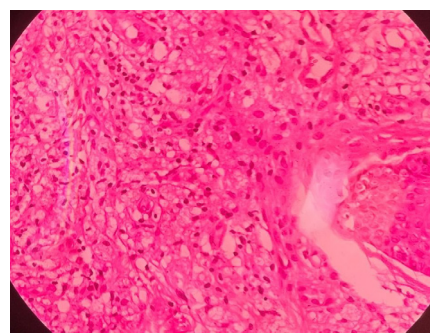


(Lepromatous Leprosy) Grenz Zone (H&E,40X)

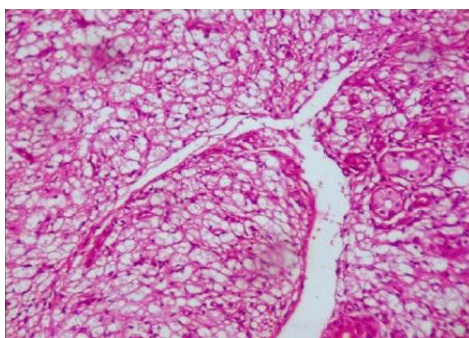
Figure 1: Showing different Histopathology



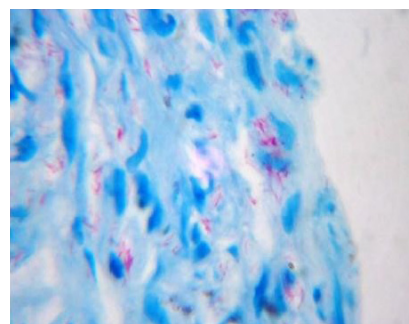
Lepromatous Leprosy (H & E, 10X)



Lepromatous Leprosy (H & E, 40X)



Histoid Leprosy Sheets of Lepra Cells (H & E, 40X)



Lepromatous Leprosy Afb in Small Clusters (Modified ZN Stain, 100X)

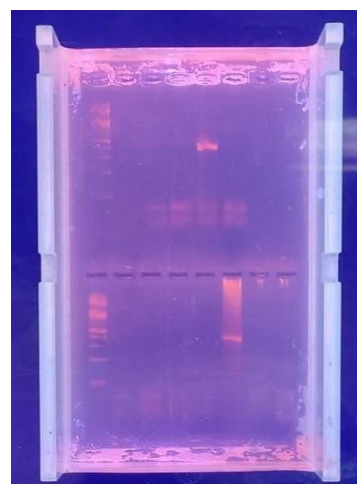
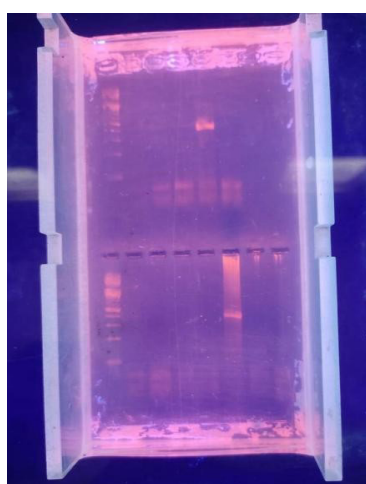


Figure 2: (PCR) Agarosegel electrophoresis of PCR products of 372bp. obtained by using primers to the mycobacterium leprae repetitive sequence.

histopathological forms was observed ($\chi^2 = 9.788$, $p = 0.134$). This aligns with studies like Tatipally *et al.*, which emphasized the need for more reliable genetic markers to enhance PCR sensitivity.^{21,22}

CONCLUSION

In conclusion, the present study highlights the potential of PCR as an effective adjunctive tool in the diagnosis of leprosy, complementing the modified Ziehl–Neelsen (ZN) stain. While PCR enhances diagnostic accuracy, particularly in cases with negative ZN staining or inconclusive histopathology, it cannot replace the

modified ZN stain due to its limited accessibility in resource-constrained settings. Thus, integrating PCR with conventional methods can facilitate earlier diagnosis and treatment, improving patient outcomes.

LIMITATIONS

Single centre study with limited number of cases.

ETHICAL STATEMENT

The study received approval from the Institutional Ethics Committee (Approval Number: SRMS IMS/ ECC/2022/127). Informed consent was obtained from all

participants prior to their inclusion in the study, ensuring adherence to ethical standards and respect for participant autonomy.

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