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Original Article

Comparative Study of Sensitivity and Specificity of RT-PCR, Histopathological Diagnosis and Slit Skin Smears in Detection of *M leprae*

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Early and accurate diagnosis of leprosy remains challenging, particularly in paucibacillary and clinically atypical cases where conventional diagnostic methods demonstrate limited sensitivity. This study evaluated the diagnostic utility of real-time polymerase chain reaction (RT-PCR) for detection of *Mycobacterium leprae* and compared its performance with slit-skin smear (SSS) and histopathology. This prospective cross-sectional pilot study was conducted on 33 clinically suspected Hansen's disease patients at a tertiary care centre in Navi Mumbai between January 2023 and June 2024. All patients underwent slit-skin smear (SSS) for acid-fast bacilli (AFB), histopathological examination, and RT-PCR targeting the RLEP gene of *M. leprae*. Histopathology was considered the reference standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristic (ROC) analysis were performed. Slit-skin smear (SSS) was found to have low sensitivity (18.2%) but high specificity (100%). Histopathology showed the highest diagnostic accuracy with sensitivity and specificity of 95.6% and 100%, respectively. RT-PCR demonstrated sensitivity of 86.4% and specificity of 90.9%, outperforming slit-skin smear and successfully detecting cases with low bacillary load and inconclusive conventional findings. RT-PCR showed superior ROC performance (AUC 0.89) compared with SSS (AUC 0.59). RT-PCR appears to be valuable adjunctive diagnostic tool for leprosy, particularly in paucibacillary and diagnostically challenging cases. Integration of RT-PCR with conventional diagnostic methods may improve early diagnosis and disease classification in endemic settings.

Keywords: RT-PCR, Histopathology, Slit-Skin Smear, Leprosy, *Mycobacterium leprae*

Introduction

Leprosy, a chronic infectious granulomatous disease, is caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*, which are non-cultivable acid-fast bacilli in any acceptable *in-vitro* system. Despite continuous

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and tireless efforts by the government and other organizations in the direction of leprosy elimination, the burden remains high, especially in endemic areas. According to the Global Leprosy Update 2023, a total of 1,82,815 new cases were reported globally, corresponding to a new case detection rate of 22.7 per million population. The number of new cases detected globally was 5% higher than in 2022. Brazil, India, and Indonesia continued to report more than 10,000 new cases, together accounting for 79.3% of new cases detected globally (Saunderson 2023).

The challenges faced in the fight to eliminate leprosy are numerous. This disease, common among the less affluent, often occurs in overcrowded and impoverished areas, which accelerates its spread among individuals. The resulting deformities, worsened by ignorance, contribute to a widespread social stigma that causes people to hide the disease. This stigma often delays seeking treatment. Because of the very slow growth rate of the bacteria, leprosy has a long incubation period and cannot be cultivated in a laboratory. These factors have contributed to delays and gaps in understanding the disease. Additionally, the low sensitivity of traditional diagnostic methods, such as bacillary counts from skin smears (slit skin smear) and histology, makes detection challenging in resource-limited settings. The identification of cases without skin lesions, such as pure neural leprosy (PNL), also remains a public health issue often going unnoticed. Histology or molecular biological assays require extensive expertise and are mostly confined to reference laboratories. Since the bacillus remains non-cultivable despite decades of *in vitro* cultivation attempts, the only available method to assess drug susceptibility phenotypically is testing in animal models, like mouse footpad (MFP) testing. However, MFP studies for phenotypic sensitivity testing are cumbersome, difficult to interpret, and have low sensitivity (Sharma & Singh 2022).

To eradicate leprosy, screening the population for the disease is a first step. If infected individuals go undiagnosed, they spread the disease to healthy people, making global leprosy control programs ineffective. As a result, the target date for leprosy eradication in endemic countries has been delayed by almost two decades. During the COVID-19 pandemic, many private sector laboratories have been equipped with Real Time Polymerase Chain Reaction (RT-PCR) facilities, which can be used for extensive screening of suspected leprosy patients. Developing an indigenous in-house PCR assay would be of great utility in this direction. The goal of this study was to design, develop, and validate a real-time PCR assay for accurate leprosy detection in a time-bound manner. Although several PCR-based assays, including RLEP-targeted conventional PCR, nested PCR, and real-time PCR assays, have previously been described for the diagnosis of leprosy, most remain confined to research or reference laboratories because of issues related to cost, technical complexity, lack of assay standardization, and limited availability in resource-constrained endemic settings. In addition, considerable variability exists among published assays with respect to DNA extraction methods, primer design, amplification platforms, and diagnostic performance, particularly in paucibacillary disease. Following the COVID-19 pandemic, RT-PCR infrastructure became widely available across many tertiary-care and private laboratories in India, creating an opportunity to adapt these existing molecular facilities for leprosy diagnosis using affordable and locally standardized protocols. Therefore, the present study did not aim to introduce a novel molecular target, but rather to standardize and evaluate an indigenous in-house RLEP-based RT-

PCR assay using locally available reagents and infrastructure, and to assess its diagnostic utility in comparison with conventional methods such as slit-skin smear and histopathology in clinically suspected Hansen's disease cases.

This study was carried out with the objectives of clinically categorizing different types of leprosy, and to correlate them with histopathology, standardizing a RT-PCR protocol for the detection of *M. leprae* and comparing the efficacy of RT-PCR with conventional methods for the detection of *M leprae*.

Material and Methods

This prospective cross-sectional study with a sample size of 33 was conducted at a tertiary care centre in Navi Mumbai from January 2023 to June 2024. The sample size of 33 was based on the number of clinically suspected Hansen's disease cases presenting to the tertiary care dermatology outpatient department during the study period (January 2023–June 2024). As leprosy is a declining yet focal disease, consecutive sampling was employed. This study was designed as a pilot comparative diagnostic study to evaluate the performance of RT-PCR against conventional diagnostic methods rather than to establish population-level estimates.

This study included clinically suspected Hansen's disease cases with cutaneous lesions, both treatment-naïve and post-treatment patients and those providing written informed consent. Cases with inadequate biopsy material, poor DNA yield or quality on extraction and those unwilling to participate were excluded. The study was approved by the Institutional Ethics Committee of MGM Medical College, Navi Mumbai (Approval No. DHR-EC/2023/03/16, approved on 03 April 2022). The study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment.

Slit skin smear scrapings were sent to the microbiology laboratory, biopsy samples for histopathology were sent to the histopathology laboratory, and samples for RT-PCR were sent to the Central Research Laboratory, MGM Medical College and Hospital, MGM Institute of Health Sciences, Kamothe, Navi Mumbai.

Procedure for slit skin smears:

After aseptic precautions, tissue samples were taken from the right ear lobe, forehead, chin, and buttocks as recommended by WHO. The skin fold was pinched tightly with the thumb and index finger until it blanched. Using a scalpel, a cut approximately 5mm long and 2mm deep was made, and the material was placed on a clean slide to prepare a smear about 8-10mm in diameter. Ziehl-Neelsen staining was performed, and the bacterial and morphological indices were calculated. The Morphological Index was assessed in smear-positive cases by examining Ziehl–Neelsen–stained smears under oil immersion. A minimum of 100–200 bacilli were evaluated, and the proportion of uniformly stained, solid bacilli was expressed as a percentage of the total bacilli counted, indicating bacillary viability.

Tissue biopsy for histopathology and RT-PCR:

Two tissue biopsies were taken from the cutaneous lesion of suspected Hansen's disease with aseptic precautions. One specimen was utilized for histopathological diagnosis and the Fite-Faraco stain. The other specimen was used for RT-PCR analysis.

Procedure for histopathological analysis:

Following adequate fixation, all skin biopsies were routinely processed, with tissue sections cut to a thickness of 5 micrometers. Each section was stained using Hematoxylin and Eosin (H&E) as well as Fite-Faraco stain (FF) following standard protocols. The classification of the disease was based on clinical findings

and histopathological analysis, and the results of H&E and Fite-Faraco stains. The disease categories included tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), lepromatous leprosy (LL), indeterminate (IL), lepra reaction 1, lepra reaction 2, and histoid leprosy (HL) (Ridley & Jopling 1966, IAL 1982).

The sections stained using the Fite-Faraco method were examined for organisms under a 100X objective lens, and the findings were documented. The bacteriological index (BI) was calculated using an oil immersion field, and according to Ridley's logarithmic scale, it was graded from 0 to 6+, reflecting the average number of bacilli observed in a microscopic field at 100X magnification.

Procedure for RT-PCR analysis:

The second skin biopsy material was carefully transported in containers labelled in advance containing Normal saline, and simultaneously shifted in a pre-cooled ice box at 4°C to the MGM Central Research Laboratory, MGM Medical college and Hospital, Kamothe, Navi Mumbai, where they were stored at -80°C after snap-freezing. The materials were processed in batches of 12, as feasible, and subjected to genomic DNA isolation followed by a process of PCR analysis, as needed. All samples and materials were processed and handled strictly according to the standardized procedures. Moreover, master mix preparation, addition of control samples, and template samples were conducted in physically separate areas to avoid potential cross-contaminations.

Quality assurance for the standardized assay was ensured through the incorporation of adequate control samples during the reaction process. A positive control DNA was employed for reaction confirmation, a non-bacterial DNA-based negative control was used for ensuring assay specificity, and a no template control was incorporated by adding nuclease-free water to a reaction mixture for monitoring potential contaminants

Standardization of DNA Isolation Protocol:

The DNA from collected biopsy samples was analysed using the Qiagen Mini DNA Extraction kit as per the manufacturer's instructions. The isolated DNA samples were quantified by using a Nanodrop Spectrophotometer for concentration and purity analysis.

Standardization of RT-PCR assay:

The RT-PCR assay for detecting *Mycobacterium leprae* was standardized using well-established primers targeting the highly specific and conserved RLEP genomic region of M. leprae according to the standardized primer set from the literature. The PCR reaction was optimized using Takara's TB Green Real-Time PCR Master Mix (Cat No. RR820A) containing Syber Green as a reporter dye. Each 20 µL reaction mixture consists of 10 µL of TB Green Master Mix, 1 µL each of forward and reverse primers specific to the RLEP region, 1 µL of ROX dye as a passive reference, 2 µL of PCR-grade molecular water, and 5 µL of isolated DNA sample. The primer sequences are depicted in Table 1. These primers are taken from the literature (Chaitanya et al 2017), are specific for RLEP region and these were validated by using bioinformatics software for the specificity.

Thermal cycling was performed using the HiMedia Insta Q48 Real-Time PCR system with the following conditions: initial denaturation at 95°C for 10 minutes, followed by 40 amplification cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 45 seconds, and final extension at 72°C for 10 minutes. Amplification results were interpreted based on fluorescence

curve analysis, and the presence of *M. leprae* was determined by evaluating the standard cycle threshold (Ct) values. The obtained Ct value in between 15 to 35 cycles was considered as a positive and the samples failing to satisfy the depicted Ct value were labelled as the negative.

Statistical Analysis: The diagnostic sensitivity and specificity of the RT-PCR were assessed by comparing its results with those obtained from the conventional method used for diagnosis of leprosy. The statistical analysis was done by using SciPy and Scikit learning package of Python programing. Diagnostic performance of slit skin smear (SSS) and RT-PCR was evaluated using histopathology as the reference standard. Data were analyzed using 2×2 contingency tables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated along with 95% confidence intervals (CI).

Receiver Operating Characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of SSS and RT-PCR. Comparison between diagnostic methods was performed using the Chi-square test, and a p value <0.05 was considered statistically significant.

Results

A total of 33 volunteers (24 males, 9 females) were recruited for this study at the outpatient department of Dermatology. Of these 33 cases, 26 were of borderline tuberculoid, 2 were of borderline lepromatous, 4 were of lepromatous leprosy, and 1 case was of indeterminate leprosy. Out of these, 2 patients were also diagnosed with type 2 lepra reaction, and 1 case with type 1 lepra reaction (Table 2).

Out of 33 cases, 6 were positive on slit skin smears for AFB, the spectrum being 2 cases of lepromatous leprosy (4+ BI), 2 cases of borderline lepromatous (2+ BI), and 2 cases of borderline tuberculoid leprosy (1+ BI). Sensitivity of SSS was found to be 18% and specificity 100%.

Histopathology yielded positive results in 22 cases and negative results in 11 cases. Among these 11 cases, one case of indeterminate leprosy was falsely negative. The calculated sensitivity and specificity of histopathology were 95.6% and 100% respectively.

Table 3 shows comparison between results of slit skin smear with histopathology. Only 6 out of 33 clinically suspected cases were positive on slit-skin smear, while 27 cases were negative despite histopathological positivity. No false-positive slit-skin smear results were observed, indicating high specificity but markedly low sensitivity of slit-skin smear in detecting leprosy, particularly in paucibacillary and borderline forms. RT-PCR was positive in 20 out of 33 cases, with 3 false negatives identified. It could also diagnose one case of indeterminate leprosy that was negative on histopathology. However, it also produced false negatives in two cases where treatment had been completed, and one case of BT Hansen's disease. The calculated sensitivity and specificity of RT-PCR were 86% and 90% respectively.

Histopathology was considered the reference (gold standard) for diagnosis in this study. False-positive results were defined as cases that tested

Table 1 : Primer sequences for RT-PCR.

Name of Primer	Primer Sequence 5' to 3 '	Amplicon Size
RLEP forward	TGCATGTCATGGCCTTGAGG	129 bp
RLEP reverse	CACCGATACCAGCGGCAGAA	

Table 2 : Demographic and clinical profile of study participants (n = 33).

Patient ID	Age (years)	Sex	Duration of symptoms (months)	Lesion / biopsy site	Clinical diagnosis	Lepra reaction
1	28	M	4	Forearm	Borderline Tuberculoid	Type 1
2	35	F	6	Upper arm	Borderline Tuberculoid	None
3	42	M	8	Thigh	Borderline Tuberculoid	None
4	31	M	5	Forearm	Borderline Tuberculoid	None
5	46	M	12	Leg	Borderline Tuberculoid	None
6	39	F	7	Upper arm	Borderline Tuberculoid	None
7	51	M	18	Thigh	Borderline Tuberculoid	None
8	27	M	3	Forearm	Borderline Tuberculoid	None
9	44	M	10	Leg	Borderline Tuberculoid	None
10	36	F	6	Upper arm	Borderline Tuberculoid	None
11	29	M	4	Forearm	Borderline Tuberculoid	None
12	48	M	14	Thigh	Borderline Tuberculoid	None
13	55	M	20	Leg	Borderline Tuberculoid	None
14	33	F	5	Upper arm	Borderline Tuberculoid	None
15	41	M	9	Forearm	Borderline Tuberculoid	None
16	26	M	3	Forearm	Borderline Tuberculoid	None
17	52	M	16	Thigh	Borderline Tuberculoid	None
18	38	F	7	Upper arm	Borderline Tuberculoid	None
19	47	M	11	Leg	Borderline Tuberculoid	None
20	34	M	6	Forearm	Borderline Tuberculoid	None
21	29	F	4	Upper arm	Borderline Tuberculoid	None
22	56	M	24	Thigh	Borderline Tuberculoid	None
23	40	M	8	Forearm	Borderline Tuberculoid	None
24	37	F	6	Upper arm	Borderline Tuberculoid	None
25	45	M	10	Leg	Borderline Tuberculoid	None
26	32	M	5	Forearm	Borderline Tuberculoid	None
27	49	M	18	Back	Borderline Lepromatous	None
28	54	F	22	Trunk	Borderline Lepromatous	None
29	50	M	30	Back	Lepromatous Leprosy	Type 2
30	58	M	36	Trunk	Lepromatous Leprosy	Type 2
31	46	M	20	Back	Lepromatous Leprosy	None
32	61	M	40	Trunk	Lepromatous Leprosy	None
33	23	F	2	Forearm	Indeterminate Leprosy	None

Table 3: Comparison of slit skin smear results with histopathology.

Slit Skin Smear	Histopathology Positive	Histopathology Negative	Total
Positive	6	0	6
Negative	27	0	27
Total	33	0	33

Table 4: Comparison of RT-PCR with histopathology.

RT-PCR result	Histopathology Positive	Histopathology Negative	Total
Positive	19	1	20
Negative	3	10	13
Total	22	11	33

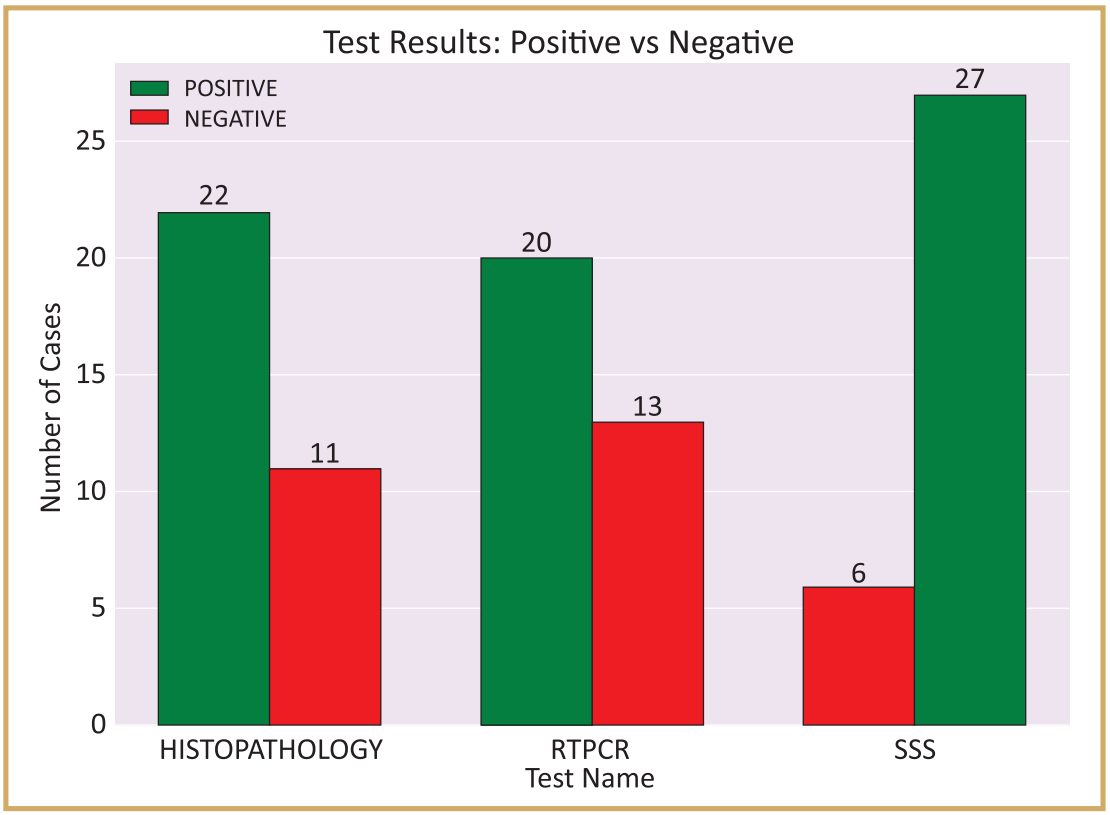

Fig. 1 : Comparative outcomes of histopathology, slit skin smear positivity, and RT-PCR assay; * 1 – denotes the positive case finding and *0 – Denotes the number of Negative cases detected.

Table 5: Diagnostic performance of investigative modalities evaluated.

Diagnostic method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Slit Skin Smear	18.2	100	100	28.6
Histopathology	95.6	100	100	90.9
RT-PCR	86.4	90.9	95.0	76.9

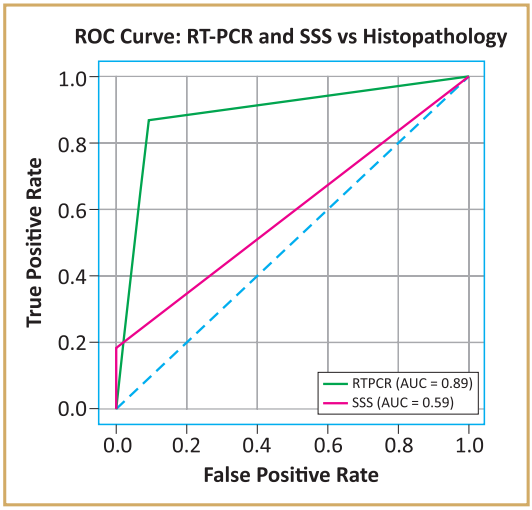

Fig. 2 : ROC analysis for assessing the diagnostic performance of RT-PCR and Slit Skin Smear in comparison to the histopathology assay.

positive by RT-PCR or slit skin smear but were negative on histopathological examination. False-negative results were defined as cases that tested negative by RT-PCR or slit skin smear despite a positive histopathological diagnosis (Table 4).

Table 5 depicts the comparison of overall performance of all the diagnostic methods used.

The comparative outcomes of each assay conducted for the diagnosis of leprosy are shown in Fig. 1.

The lower positivity rate of SSS, despite positive findings on histopathology and RT-PCR, highlights its lower sensitivity, especially in conditions with a low bacterial load, like paucibacillary leprosy. Considering histopathology as the gold standard method, further analysis using the Receiver Operating Characteristic (ROC) curve (Fig. 2) to evaluate the sensitivity and specificity of SSS and RT-PCR was performed. RT-PCR achieved an Area Under the Curve (AUC) of 0.89, indicating the diagnostic accuracy of the test. In contrast, SSS had an AUC of 0.59, reflecting moderate diagnostic performance. The lower sensitivity and specificity of SSS compared to RT-PCR are particularly evident in its failure to detect paucibacillary leprosy cases, where bacterial presence is minimal. RT-PCR, on the other hand, exhibits superior sensitivity, capable of detecting even small amounts of *Mycobacterium leprae* DNA, ensuring early and accurate diagnosis of suspected cases.

Discussion

Accurate and early diagnosis of leprosy remains a challenge in endemic countries such as India, despite the widespread availability of multidrug therapy. Delayed diagnosis contributes to continued transmission, irreversible nerve damage, and persistent social stigma. Conventional diagnostic modalities such as slit-skin smear (SSS) and histopathology continue to form the backbone of leprosy diagnosis; however, both have inherent limitations, particularly in cases with low bacillary load, early disease, or atypical clinical presentation (Saunderson 2023, Singh & Sharma 2022).

Underdiagnosis of leprosy has been attributed to the wide clinical spectrum of the disease, subtle early manifestations, and limited sensitivity of

conventional diagnostic tools. Reviews assessing newer diagnostic strategies emphasize that reliance on clinical examination and slit-skin smear alone often leads to missed diagnoses, particularly in paucibacillary disease (Gamma et al 2020). From a laboratory perspective, Malhotra and Husain have emphasized that no single diagnostic modality is sufficient across the entire leprosy spectrum and that integration of clinical, histopathological, and molecular methods improves diagnostic accuracy, especially in early and low-bacillary states (Malhotra & Husain 2022).

In the present study, slit-skin smear demonstrated very low sensitivity (18%) despite a specificity of 100%. This finding is consistent with previous reports demonstrating poor performance of SSS in borderline and paucibacillary forms of leprosy due to low bacillary density. While SSS remains useful for bacterial load estimation and treatment classification, its limited sensitivity restricts its role as a standalone diagnostic test (Banerjee et al 2010).

Histopathology showed the highest diagnostic accuracy in this study, with a sensitivity of 95.6% and specificity of 100%, reaffirming its role as the diagnostic gold standard. However, one case of indeterminate leprosy was falsely negative on histopathology, highlighting known limitations related to lesion selection, sampling error, and subtle or evolving histological changes in early disease. Similar limitations of histopathology in early and paucibacillary leprosy have been documented previously (Chan & Smoller 2018).

Advances in molecular diagnostics aim to overcome these limitations. Molecular assays targeting repetitive genomic elements such as RLEP, including isothermal amplification-based platforms, have shown promise as adjunctive diagnostic tools, particularly in endemic and resource-limited settings (Saar et al 2021).

In the present study, RT-PCR demonstrated higher sensitivity than slit-skin smear but did not outperform histopathology. RT-PCR successfully detected *Mycobacterium leprae* DNA in cases that were negative on SSS and in one case that was inconclusive on histopathology, underscoring its value in diagnostically challenging situations. False-negative RT-PCR results were observed in treated cases and in one borderline tuberculoid case, likely due to reduced bacterial DNA load following therapy, DNA degradation, or sampling variability. Similar observations have been reported in earlier molecular studies (Yan et al 2014, Mohanty et al 2020). Several studies from India and other endemic regions have consistently reported higher sensitivity of PCR-based assays compared to slit-skin smear, particularly in paucibacillary disease, while histopathology continues to demonstrate high specificity with variable sensitivity depending on disease spectrum and lesion sampling (Martinez et al 2014, Mohanty et al 2020).

Comparative evaluations of PCR techniques indicate that although nested PCR may offer higher analytical sensitivity, real-time PCR provides advantages such as improved specificity, reduced contamination risk, and quantitative assessment, making it more suitable for routine laboratory use (Tatipally et al 2018). PCR-based diagnostics are particularly valuable in diagnostically challenging entities such as pure neuritic leprosy, where the absence of skin lesions limits the utility of slit-skin smear and histopathology (Razdan & Sadhu 2024).Systematic reviews have further highlighted the potential role of PCR as a near point-of-care diagnostic tool for early detection of leprosy in endemic settings (Huang et al 2023).

Beyond individual diagnosis, molecular assays have also demonstrated utility in detecting subclinical infection among household contacts, aiding early case detection and potentially

reducing transmission (Banerjee et al 2010). Despite these advantages, RT-PCR has practical limitations, including higher cost, requirement for specialized infrastructure, and lack of uniform standardization across laboratories. Social vulnerability, delayed health-seeking behaviour, and health-system barriers further contribute to late diagnosis of leprosy in endemic regions (Jesus et al 2023, NTD 2022) Additionally, PCR detects bacterial DNA and may not reliably distinguish active infection from residual DNA following treatment, complicating interpretation in post-treatment cases (Martinez et al 2014).

Overall, the findings of this pilot study support a combined diagnostic approach integrating RT-PCR with conventional methods to improve diagnostic accuracy, particularly in endemic settings and in cases with low bacillary load or inconclusive routine investigations. Compared to the previously described molecular assays for leprosy, the present RT-PCR assay demonstrated diagnostic performance comparable to published RLEP-based real-time PCR studies, particularly in detecting paucibacillary and borderline cases with low bacillary load. Unlike several earlier PCR assays that were developed primarily in specialized research settings, the current assay was standardized using routinely available RT-PCR infrastructure and commercially accessible reagents, making it more feasible for implementation in tertiary-care laboratories in endemic regions. While the assay does not introduce a novel molecular target, it supports the practical adaptation of established RLEP-based RT-PCR methodology into locally applicable diagnostic workflows.

Larger multicentric studies with standardized molecular validation are required to further define the role of RT-PCR in national leprosy control programs (Tatipally et al 2018, WHO 2018, Chaitnaya et al 2017).

Conclusion

In this pilot study, slit skin smear demonstrated very low sensitivity despite high specificity, limiting its utility as a standalone diagnostic test, particularly in paucibacillary disease. Histopathology showed the highest diagnostic accuracy and remains the gold standard for leprosy diagnosis. RT-PCR exhibited higher sensitivity than slit skin smear and was able to detect *Mycobacterium leprae* in cases with low bacillary load or inconclusive conventional investigations, though its performance was inferior to histopathology.

These findings highlight the practical role of RT-PCR as a complementary diagnostic tool rather than a replacement for established methods. In endemic settings, RT-PCR can enhance diagnostic confidence in clinically suspected cases with negative slit skin smears or equivocal histopathological findings, facilitating earlier diagnosis and appropriate classification.

This study is limited by its small sample size and single-centre design, which may restrict generalizability of the findings. Molecular validation parameters such as assay efficiency and limit of detection were not independently evaluated. In addition, the disease spectrum was skewed toward borderline forms, and pure neuritic leprosy cases were rare. Larger multicentric studies with standardized molecular validation are required to further substantiate these findings.

Given the constraints of cost, infrastructure, and standardization, routine use of RT-PCR may not be feasible in all settings. However, its targeted application in selected cases—such as paucibacillary disease and diagnostically challenging presentations—can strengthen existing diagnostic algorithms. Larger multicentric studies with standardized molecular validation

are required to further define its role in leprosy control programs.

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