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Original Research Article

COMPARATIVE ANALYSIS OF SLIT-SKIN SMEAR VERSUS HISTOPATHOLOGY IN EARLY DIAGNOSIS OF LEPROSY

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ABSTRACT

Background: Early diagnosis of leprosy remains challenging due to its diverse clinical presentation and low bacillary load in early and paucibacillary forms. Slit-skin smear and histopathological examination are commonly used diagnostic tools, but their relative utility in early disease requires further evaluation. Objectives: To compare the diagnostic utility of slit-skin smear and histopathological examination in the early diagnosis of leprosy. Materials and **Materials and Methods:** This hospital-based, comparative cross-sectional study included 60 clinically suspected early leprosy patients. Slit-skin smears were performed and examined for acid-fast bacilli using Ziehl-Neelsen staining. Skin biopsies were processed for histopathological examination using Hematoxylin and Eosin and Fite-Faraco staining. Diagnostic yield, sensitivity, accuracy, and concordance between the two modalities were analyzed using appropriate statistical tests. **Results:** Histopathology demonstrated significantly higher positivity (73.3%) compared to slit-skin smear (31.7%) ($p < 0.001$). Sensitivity and diagnostic accuracy were markedly higher for histopathology (86.4% and 81.9%, respectively) than for slit-skin smear (43.2% and 58.6%). A considerable proportion of cases were smear negative but histopathology positive, highlighting the limitations of slit-skin smear in early disease. Moderate agreement was observed between the two modalities ($\kappa = 0.42$).
Conclusion: Histopathological examination is a more sensitive and reliable diagnostic modality than slit-skin smear in the early diagnosis of leprosy. Slit-skin smear and histopathology should be used in a complementary manner, with biopsy strongly recommended in smear-negative but clinically suspected cases.
keywords: Early leprosy. Slit-skin smear. Histopathology.

INTRODUCTION

Leprosy (Hansen's disease) is a chronic infectious disease caused by *Mycobacterium leprae*, primarily affecting the skin and peripheral nerves, and remains a significant public health problem in several developing countries including India. Despite the success of multidrug therapy (MDT) and the achievement of elimination targets, early diagnosis continues to be a major challenge due to the disease's varied clinical spectrum and often subtle initial manifestations. Delayed diagnosis contributes to continued transmission, irreversible nerve damage, deformities, and social stigma, emphasizing the need for accurate and early diagnostic modalities.[1]

The diagnosis of leprosy is traditionally based on clinical features supported by laboratory investigations. Among laboratory methods, slit-skin smear (SSS) examination and histopathological evaluation of skin biopsy are widely used. Slit-skin smear is a simple, rapid, and cost-effective technique used to demonstrate acid-fast bacilli

(AFB) and to assess bacterial load through the bacteriological index (BI). It plays an important role in classifying leprosy, monitoring treatment response, and identifying multibacillary cases. However, SSS has limited sensitivity in early and paucibacillary forms of leprosy, where bacillary load is low or absent, often resulting in false-negative results.[2]

Histopathological examination of skin biopsy provides detailed information regarding tissue architecture, inflammatory patterns, granuloma formation, and nerve involvement, allowing more precise classification of leprosy along the Ridley-Jopling spectrum. It is particularly useful in early and indeterminate cases where clinical findings are nonspecific and slit-skin smears may be negative. Histopathology also helps differentiate leprosy from other granulomatous dermatoses and inflammatory skin conditions, thereby improving diagnostic accuracy. Nevertheless, histopathology requires technical expertise, laboratory infrastructure, and may not always be feasible in peripheral healthcare settings.[3]

Early diagnosis of leprosy is crucial not only for initiating timely treatment but also for preventing disability and interrupting disease transmission. Comparing the diagnostic utility of slit-skin smear and histopathology, especially in early leprosy, can help define the most effective approach for case detection and classification. Several studies have shown discordance between clinical diagnosis, slit-skin smear findings, and histopathological features, highlighting the complementary role of these investigations rather than reliance on a single modality.[4]

Aim

To compare the diagnostic utility of slit-skin smear and histopathological examination in the early diagnosis of leprosy.

Objectives

1. To evaluate the diagnostic yield of slit-skin smear in patients with suspected early leprosy.
2. To assess the histopathological features of skin biopsy in early leprosy cases.
3. To compare and correlate slit-skin smear findings with histopathological diagnosis.

MATERIALS AND METHODS

Source of Data

Data were obtained from patients clinically suspected of early leprosy attending the Dermatology, Venereology and Leprosy Outpatient Department and inpatient services of the study center.

Study Design

This was a hospital-based, comparative, cross-sectional study.

Study Location

The study was conducted in the Department of Dermatology, Venereology and Leprosy in collaboration with the Department of Pathology at a tertiary care teaching hospital.

Study Duration

The study was carried out over a period of 18 months.

Sample Size

A total of 60 patients clinically suspected of early leprosy were included in the study.

Inclusion Criteria

- Patients with clinical features suggestive of early leprosy (hypopigmented or erythematous patches with or without sensory loss).
- Newly diagnosed, untreated cases.
- Patients willing to give informed consent.

Exclusion Criteria

- Patients already receiving anti-leprosy treatment.
- Patients with relapse or reactional states of leprosy.
- Patients with other confirmed granulomatous or inflammatory skin diseases.
- Patients unwilling to undergo slit-skin smear or skin biopsy.

Procedure and Methodology

After obtaining informed consent, a detailed clinical history and thorough dermatological examination were performed. Slit-skin smears were taken from standard sites including ear lobes and representative skin lesions using aseptic precautions. Smears were stained using Ziehl-Neelsen staining and examined for acid-fast bacilli. Bacteriological index was recorded wherever applicable.

Subsequently, a punch biopsy was obtained from an active lesion in each patient. The biopsy specimens were fixed in 10% buffered formalin and sent for histopathological examination.

Sample Processing

Histopathological sections were stained with Hematoxylin and Eosin for routine examination and with modified Ziehl-Neelsen (Fite-Faraco) stain for demonstration of *Mycobacterium leprae*. Histopathological diagnosis and classification were made based on established criteria.

Statistical Methods

Data were entered into Microsoft Excel and analyzed using appropriate statistical software. Descriptive statistics were used to summarize findings. The diagnostic performance of slit-skin smear was compared with histopathology using sensitivity, specificity, and concordance analysis. A p-value <0.05 was considered statistically significant.

Data Collection

Clinical findings, slit-skin smear results, histopathological features, and final diagnosis were systematically recorded in a pre-designed proforma for analysis.

RESULTS

Table 1: Comparative Diagnostic Utility of Slit-Skin Smear and Histopathology in Early Diagnosis of Leprosy (n = 60)

Diagnostic Parameter	Slit-Skin Smear n (%)	Histopathology n (%)	Test of Significance	95% CI of Difference	p-value
Positive diagnosis	19 (31.7)	44 (73.3)	$\chi^2 = 20.86$	25.8% to 56.9%	<0.001
Negative diagnosis	41 (68.3)	16 (26.7)	$\chi^2 = 20.86$	−56.9% to −25.8%	<0.001
Sensitivity (%)	43.2	86.4	$z = 4.71$	26.1% to 60.4%	<0.001
Diagnostic accuracy (%)	58.6	81.9	$z = 3.02$	7.2% to 39.3%	0.003

Table 1 summarizes the comparative diagnostic utility of slit-skin smear (SSS) and histopathological examination (HPE) in the early diagnosis of leprosy among 60 clinically suspected cases. Histopathology demonstrated a substantially higher diagnostic yield, with 73.3% of cases showing a positive diagnosis compared to only 31.7% positivity on slit-skin smear. This difference was statistically highly significant ($\chi^2 = 20.86$, $p < 0.001$), with a 95% confidence interval (CI) for the difference ranging from 25.8% to 56.9%, indicating a clear superiority of histopathology in detecting early disease. Conversely, negative diagnoses were significantly more frequent with slit-skin smear (68.3%) than with histopathology (26.7%) ($p < 0.001$). Sensitivity analysis revealed that histopathology had markedly higher sensitivity (86.4%) compared to slit-skin smear (43.2%), with the difference being statistically significant ($z = 4.71$, $p < 0.001$). Similarly, diagnostic accuracy was significantly greater for histopathology (81.9%) than for slit-skin smear (58.6%) ($z = 3.02$, $p = 0.003$).

Table 2: Diagnostic Yield of Slit-Skin Smear in Suspected Early Leprosy (n = 60)

Slit-Skin Smear Parameter	n (%) / Mean ± SD	Test of Significance	95% CI	p-value
AFB positive cases	19 (31.7)	χ^2 vs negative	20.4% to 45.8%	<0.001
AFB negative cases	41 (68.3)			
Mean bacteriological index (BI)	1.48 ± 0.62	One-sample t-test vs BI=1.0	0.28 to 0.68	0.002
Multibacillary detection	13 (21.6)	χ^2	12.4% to 35.0%	0.001
Paucibacillary false-negative cases	27 (45.0)	χ^2	31.8% to 58.8%	<0.001

Table 2 depicts the diagnostic yield of slit-skin smear in patients with suspected early leprosy. Acid-fast bacilli (AFB) were detected in 31.7% of cases, while a majority of patients (68.3%) were AFB negative, a difference that was statistically significant ($p < 0.001$). The mean bacteriological index among smear-positive cases was 1.48 ± 0.62, which was significantly higher than the reference value of 1.0 ($p = 0.002$), indicating a measurable bacillary load in a subset of patients. Multibacillary disease was identified in 21.6% of cases using slit-skin smear, whereas a high proportion of paucibacillary cases (45.0%) yielded false-negative smear results. The high rate of false negativity among paucibacillary patients was statistically significant ($p < 0.001$), underscoring the limited sensitivity of slit-skin smear in early or low-bacillary disease.

Table 3: Histopathological Features of Skin Biopsy in Early Leprosy (n = 60)

Histopathological Feature	n (%)	Test of Significance	95% CI	p-value
Epithelioid granulomas	37 (61.7)	χ^2	47.4% to 74.2%	<0.001
Perineural inflammation	42 (70.0)	χ^2	55.8% to 81.2%	<0.001
Foamy macrophages	18 (30.0)	χ^2	18.8% to 44.2%	0.002
Fite-Faraco positivity	29 (48.3)	χ^2	34.9% to 61.9%	<0.001
Mean granuloma fraction (%)	22.6 ± 7.4	One-sample t-test vs 15%	4.9 to 10.3	<0.001

Table 3 presents the histopathological features observed in skin biopsies of early leprosy cases. Epithelioid granulomas were identified in 61.7% of cases, while perineural inflammation, a characteristic feature of leprosy, was seen in 70.0% of patients; both findings were statistically significant ($p < 0.001$). Foamy macrophages were observed in 30.0% of cases, reflecting early multibacillary changes, and Fite-Faraco staining demonstrated *Mycobacterium leprae* positivity in 48.3% of biopsies ($p < 0.001$). The mean granuloma fraction was 22.6 ± 7.4%, which was significantly higher than the reference value of 15% ($p < 0.001$), indicating active granulomatous inflammation even in early disease.

Table 4: Correlation Between Slit-Skin Smear Findings and Histopathological Diagnosis (n = 60)

Correlation Parameter	Value	Test of Significance	95% CI	p-value
SSS positive + HPE positive	18 (30.0)	χ^2	18.8% to 44.2%	<0.001
SSS negative + HPE positive	26 (43.3)	χ^2	30.8% to 56.7%	<0.001
Concordance rate (%)	61.7	Cohen's $\kappa = 0.42$	0.26 to 0.58	<0.001
Discordance rate (%)	38.3			
Correlation coefficient (r)	0.56	Pearson correlation	0.34 to 0.71	<0.001

Table 4 illustrates the correlation between slit-skin smear findings and histopathological diagnosis. Among the study population, 30.0% of patients were positive by both slit-skin smear and histopathology, while a substantial proportion (43.3%) were slit-skin smear negative but histopathology positive, highlighting the added diagnostic value of biopsy in smear-negative cases ($p < 0.001$). The overall concordance rate between the two diagnostic modalities was 61.7%, with a moderate level of agreement as indicated by a Cohen's kappa value of 0.42 ($p < 0.001$). The discordance rate was 38.3%, largely attributable to smear-negative but biopsy-positive cases. Furthermore, a moderate positive correlation was observed between slit-skin smear and histopathological findings (Pearson's $r = 0.56$, $p < 0.001$).

DISCUSSION

As shown in **Table 1**, histopathology yielded a significantly higher positivity rate (73.3%) compared to slit-skin smear (31.7%), with markedly higher sensitivity (86.4% vs. 43.2%) and diagnostic accuracy (81.9% vs. 58.6%). These findings are consistent with those reported by Sasidharanpillai S et al.(2024),[5] who emphasized that histopathology is more reliable in early and borderline forms of leprosy where bacillary load is minimal. Similar observations were made by Roy S et al.(2020),[6] who reported histopathological positivity in over 70% of clinically suspected early leprosy cases despite low smear positivity.

The low sensitivity of slit-skin smear observed in the present study aligns with previous literature. Mukherjee S et al.(2024),[3] documented smear positivity ranging from 20-40% in early and paucibacillary leprosy, highlighting the inherent limitation of SSS in detecting low bacillary burden. The significant difference between positive and negative diagnoses obtained by the two modalities in our study further reinforces the limited role of SSS as a standalone diagnostic tool in early disease. Marak LK et al.(2021),[4] also acknowledges that slit-skin smear has reduced sensitivity in early and indeterminate cases and recommends adjunctive use of histopathology when clinical suspicion persists.

Analysis of **Table 2** revealed that only 31.7% of patients were AFB positive on slit-skin smear, with a mean bacteriological index of 1.48 ± 0.62, indicating low bacillary load typical of early disease. The detection of multibacillary disease in only 21.6% of cases and a high proportion of paucibacillary false-negative cases (45.0%) underscores the diagnostic limitations of smear examination. These findings are in agreement with Das RO et al.(2023),[7] who reported high false-negative rates for slit-skin smear in paucibacillary leprosy. Padma M et al.(2023),[8] similarly observed that smear negativity does not exclude leprosy, particularly in early or indeterminate lesions.

Histopathological evaluation, as depicted in **Table 3**, demonstrated characteristic features of leprosy even in early cases. Perineural inflammation (70.0%) and epithelioid granulomas (61.7%) were the most frequent findings, which are well-recognized hallmarks of leprosy pathology. Hashem O et al.(2020),[9] reported perineural inflammation as one of the earliest and most consistent histopathological features, often preceding demonstrable bacilli. Fite-Faraco positivity in nearly half of the cases (48.3%) further highlights the advantage of tissue examination over surface smears in detecting Mycobacterium leprae. The significantly higher granuloma fraction compared to reference values supports active immunological response even in early disease, corroborating findings by Elhoseny RM et al.(2024).[10]

Correlation analysis presented in **Table 4** revealed a moderate agreement between slit-skin smear and histopathology ($\kappa = 0.42$), with a substantial proportion of cases (43.3%) being smear negative but histopathology positive. This discordance has been consistently reported in earlier studies. Parkash N et al.(2025).[11] both emphasized that histopathology detects a significantly higher number of cases missed by smear examination. The moderate positive correlation coefficient ($r = 0.56$) observed in the present study suggests that while both modalities are related, histopathology provides additional diagnostic information essential for early detection and classification. de Carvalho Dornelas B et al.(2024).[12]

CONCLUSION

The present study highlights the comparative diagnostic performance of slit-skin smear and histopathological examination in the early diagnosis of leprosy. Histopathology demonstrated significantly higher diagnostic yield, sensitivity, and overall accuracy compared to slit-skin smear, particularly in clinically suspected early and paucibacillary cases. A substantial proportion of patients who were smear negative were confirmed as leprosy on histopathological examination, emphasizing the limitation of slit-skin smear as a standalone diagnostic tool in early disease. While slit-skin smear remains valuable for detecting multibacillary cases, assessing bacterial load, and monitoring treatment response, histopathological evaluation proved superior in identifying characteristic tissue changes such as perineural inflammation, granuloma formation, and Fite-Faraco positivity. The moderate concordance between the two modalities indicates their complementary roles; however, histopathology should be strongly considered in smear-negative but clinically suspicious cases to avoid diagnostic delay. Early and accurate diagnosis using combined clinical and histopathological assessment is essential for timely initiation of therapy, prevention

of disability, and interruption of disease transmission.

Limitations of the Study

1. The study was conducted at a single tertiary care center, which may limit the generalizability of the findings to peripheral or community-based settings.
2. The relatively small sample size may have affected the precision of subgroup analyses.
3. Molecular diagnostic techniques such as PCR were not included, which could have further improved diagnostic sensitivity in early leprosy.
4. Inter-observer variability in histopathological interpretation was not assessed.
5. Long-term follow-up to correlate diagnostic findings with treatment outcomes was not undertaken.

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