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Utility of S-100 Immunostaining in the Demonstration of Nerve Changes in Borderline Leprosy

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Abstract
Hansen's disease (leprosy) primarily affects the skin and peripheral nerves. Diagnosing borderline forms-Borderline Tuberculoid (BT) and Borderline Borderline (BB)-is often challenging due to overlapping clinical and histopathological features, particularly in paucibacillary cases with minimal bacillary load. Early and accurate detection of nerve involvement is critical to prevent long-term complications. S-100 immunostaining, a marker for Schwann cells, may enhance visualization of nerve structures in skin biopsies. Aim of the study was to assess the utility and diagnostic accuracy of S-100 immunostaining in detecting nerve involvement in borderline leprosy cases and to compare its sensitivity with routine Hematoxylin and Eosin (H and E) staining. This observational study included 30 biopsy-proven borderline leprosy cases (18 BT and 12 BB) over a period of one year. Skin punch biopsies were processed and stained with H and E, Fite-Faraco (for Bacillary Index) and S-100 immunostaining. Nerve involvement was categorized into infiltrated, fragmented, intact, or absent patterns. Sensitivity of H and E and S-100 staining was calculated for both subtypes. S-100 staining showed infiltrated nerve patterns in 94% of BT and 91% of BB cases. Fragmented nerves were observed in 8% of BB cases and intact nerves in 5% of BT cases. S-100 immunostaining had a sensitivity of 100% in detecting nerve involvement in both BT and BB cases, while H and E showed 100% sensitivity in BT and 83.3% in BB cases. Two BB cases with negative H&E results were positive on S-100 staining. S-100 immunostaining significantly improves detection of nerve involvement in borderline leprosy, especially in diagnostically challenging BB cases. It is a valuable adjunct to routine histopathology and should be considered in all suspected leprosy biopsies to enhance diagnostic accuracy and support early treatment.

INTRODUCTION

Leprosy, caused by Mycobacterium leprae, remains a significant public health concern in several endemic countries, including India. It primarily affects the skin and peripheral nerves, with nerve involvement being a hallmark feature of the disease[1]. Borderline leprosy, which lies at the immunological spectrum's midpoint, presents diagnostic challenges due to its unstable and variable immune response, often leading to ambiguous clinical and histopathological findings[2]. In this context, accurate detection of early nerve involvement is crucial for diagnosis, classification and timely treatment to prevent irreversible nerve damage and disability. Conventional hematoxylin and eosin (H and E) staining may not sufficiently demonstrate early nerve changes or the degree of nerve involvement in borderline cases[3]. Immunohistochemistry (IHC), particularly S-100 protein staining, has emerged as a valuable technique in this regard. S-100 protein is a marker of Schwann cells, which are the primary targets of M. leprae in peripheral nerves. It allows for specific visualization of nerve tissue and helps detect subtle morphological alterations such as nerve fragmentation, inflammation and fibrosis, which may not be clearly seen in routine staining[4]. Several studies have highlighted the utility of S-100 immunostaining in enhancing the visibility of nerve bundles and detecting granulomatous inflammation around them in leprosy lesions[5,6]. Moreover, S-100 staining has proven useful in monitoring the progression and regression of nerve damage, particularly in reactional states and post-treatment biopsies[7]. However, the data specifically focusing on borderline leprosy remain limited. The research gap lies in the paucity of systematic studies evaluating the effectiveness of S-100 immunostaining in differentiating nerve involvement patterns across the borderline spectrum (i.e., BT, BB and BL) and correlating them with clinical and histological classifications. Although prior studies have established the general usefulness of S-100 in leprosy[8], few have addressed how it contributes to subclassifying borderline cases or identifying early neural damage when clinical signs are subtle or equivocal. The aim of the present study is to assess the diagnostic utility of S-100 immunostaining in demonstrating peripheral nerve changes in borderline leprosy patients and to evaluate its potential role in improving diagnostic precision, especially in cases with unclear histological features on H and E staining. By correlating S-100 staining patterns with clinical subtype and histological findings, the study seeks to bridge the gap in current knowledge and propose a more sensitive approach to evaluating nerve involvement in leprosy.

MATERIALS AND METHODS

Study Design and Setting: This was a one-year observational study conducted in the Departments of Pathology and Dermatology. The aim was to evaluate peripheral nerve changes in clinically diagnosed borderline leprosy using S-100 immunohistochemistry.

Sample Selection: A total of 30 patients with biopsy-confirmed borderline leprosy were included. Only cases classified as Borderline Tuberculoid (BT) and Borderline Borderline (BB) were considered. Cases diagnosed as Tuberculoid (TT), Lepromatous (LL), or Indeterminate leprosy were excluded to ensure uniformity within the borderline spectrum.

Biopsy Procedure and Histopathological Processing: Skin punch biopsies (5 mm) were collected from clinically active lesions and sent for histopathological analysis with detailed clinical notes. Specimens were fixed in 10% neutral buffered formalin, processed routinely and paraffin-embedded. Sections were cut at 4-5 microns thickness and stained with Hematoxylin and Eosin (H and E) for routine evaluation.

Fite-Faraco Staining and Bacillary Index Assessment: Fite-Faraco staining was carried out to demonstrate Mycobacterium leprae and determine the Bacillary Index (BI). Based on the BI, cases were categorized as paucibacillary or multibacillary. BI values were also used to support histopathological classification within the borderline group.

Immunohistochemistry for S-100 Protein: For immunohistochemical evaluation, deparaffinized sections were incubated with monoclonal rabbit anti-human S-100 primary antibody (PathnSitu) for one hour. Endogenous peroxidase activity was blocked using a peroxidase-blocking solution for 5-10 minutes. The sections were then rinsed and incubated with a secondary antibody for 30 minutes, followed by the application of a chromogen substrate. Harris hematoxylin was used as a counterstain.

Microscopic Evaluation: Immunostained slides were assessed under a light microscope for nerve-specific features. The S-100 stain was used to enhance visualization of Schwann cells and peripheral nerve structures. Each case was evaluated for the presence of nerve fragmentation, perineural and intraneural inflammation, loss of nerve architecture and fibrosis. The extent and pattern of nerve involvement were recorded and compared between BT and BB subtypes.

Statistical Analysis: All data were compiled and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Categorical variables such as nerve involvement,

bacillary load and type of leprosy were expressed as frequencies and percentages. Continuous variables were presented as mean±standard deviation. Comparisons between BT and BB groups were made using the Chi-square test or Fisher's exact test for categorical data. A p-value of <0.05 was considered statistically significant. The correlation between Bacillary Index and degree of nerve involvement was also assessed using Pearson or Spearman correlation coefficients, based on data distribution.

RESULTS AND DISCUSSIONS

Table 1. Distribution of Clinical Features in Borderline Leprosy Cases (N=30)

Clinical features	Borderline Tuberculoid (n=18)	Borderline Borderline (n=12)
Hypopigemented patches	18 (60 %)	12(40 %)
Erythematous patches	-	-
Loss of sensation	18(60 %)	7(26.6%)
Tingling/numbness	13(46.6%)	7 (26.6%)
Thickened nerves	18(60%)	6 (20%)

This (Table 1) presents the distribution of key clinical features among the 30 patients diagnosed with borderline leprosy, classified into Borderline Tuberculoid (BT) and Borderline Borderline (BB) subtypes. Hypopigmented patches were observed in all cases (100%), with a higher proportion in the BT group (60%) compared to BB (40%). Loss of sensation and thickened peripheral nerves were significantly more frequent in BT cases (60% each), reflecting the prominent neural involvement characteristic of this subtype. Tingling or numbness was also more commonly reported in BT (46.6%) than in BB (26.6%). Interestingly, no cases in either group presented with erythematous patches.

Table 2: Pattern of Nerve Involvement on S-100 Immunostaining in Borderline Leprosy

Type of disease	Infiltrated	Fragmented	Absent	Intact	Total
Borderline tuberculoid	17	0	0	1	18
Borderline borderline	11	1	0	0	12

This (Table 2) summarizes the nerve staining patterns observed using S-100 immunohistochemistry in the 30 borderline leprosy cases. In the Borderline Tuberculoid (BT) group (n=18), S-100 staining revealed nerve infiltration in 17 cases, with only one case showing intact nerves. There were no instances of nerve fragmentation or absence. In the Borderline Borderline (BB) group (n=12), 11 cases showed infiltrated nerves, while one case displayed fragmented nerve bundles. None of the cases in either group demonstrated complete absence of nerves. These findings suggest that S-100 staining effectively highlights peripheral nerve involvement, particularly infiltration, in borderline leprosy. The presence of fragmented nerves in a BB case may indicate more advanced or fluctuating disease activity typical of the immunologically unstable BB spectrum.

Table 3: Diagnostic Accuracy of Nerve Identification by H and E and S-100 Immunostaining in Borderline Leprosy (N=30)

Type of leprosy	Total cases	H and E and S100+	H and E and S100+	H and E + and S100	Sensitivity of -H and E	Sensitivity of S100
BORDERLINE TUBERCULOID	18(60%)	18(60%)	0	0	100%	100%
BORDERLINE BORDERLINE	12(40%)	10(33.3%)	2(6.6%)	0	83.3%	100%

This (Table 3) compares the effectiveness of routine Hematoxylin and Eosin (H and E) staining with S-100 immunohistochemistry in identifying nerve involvement in borderline leprosy cases. In the Borderline Tuberculoid (BT) group (n=18), both H and E and S-100 staining detected nerve involvement in all cases, yielding a sensitivity of 100% for both techniques. In the Borderline Borderline (BB) group (n=12), S-100 staining detected nerve involvement in all 12 cases, whereas H and E identified nerves in only 10 cases. Two cases that were negative on H and E showed nerve involvement on S-100, indicating its higher sensitivity in detecting subtle or early nerve changes. The sensitivity of H and E in the BB group was 83.3%, while S-100 achieved 100%.

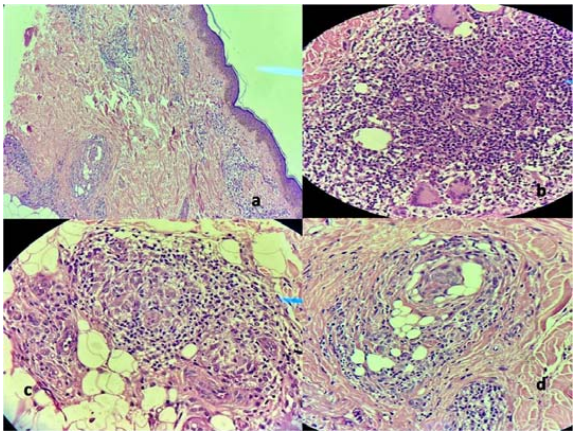

Fig. 1: A) BT Type of Hansen's Disease with Atrophic Epidermis (H and E, x100) (B) Well Defined Granulomas with Multinucleate Giant Cells (H and E, x400) (C,D) Perineural Granulomas (H and E, x400)

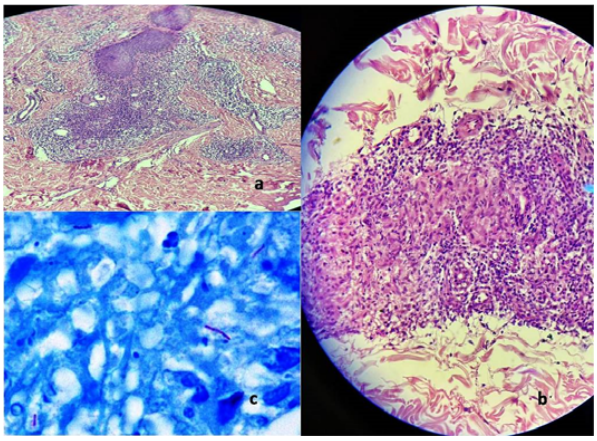

Fig. 2: A,B) BB type Hansen's Disease with Lymphocyte Rich Granulomas (H and E, x100,400)(c) BI-3 in Fite-Feraco Stain in Oil Immersion

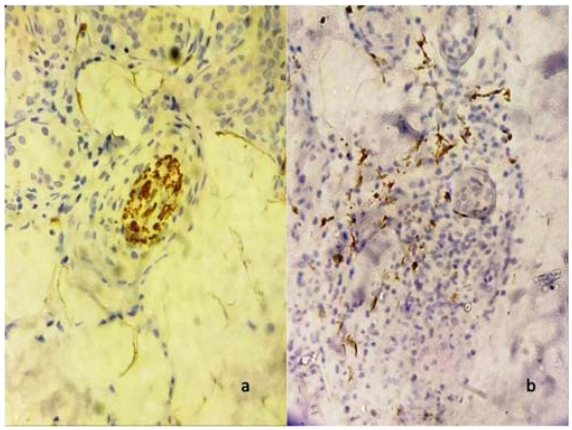

Fig. 3: A) S-100 Immunostaining-Infiltrating Pattern (x400) (B) Fragmented Pattern (x400)

The present study provides a focused evaluation of peripheral nerve involvement in clinically diagnosed borderline leprosy cases using S-100 immunohistochemistry, alongside traditional H and E and Fite-Faraco staining. Among the 30 patients analyzed, distinct differences were observed between Borderline Tuberculoid (BT) and Borderline Borderline (BB) subtypes, both clinically and histopathologically. Clinically, hypopigmented patches, sensory loss and nerve thickening were more frequently observed in the BT group. This pattern is consistent with previous studies, which have shown that Borderline Tuberculoid (BT) leprosy typically demonstrates more pronounced neural involvement and well-formed granulomas due to a stronger cell-mediated immune response[12,13]. In contrast, the Borderline Borderline (BB) group tends to exhibit less consistent neural symptoms, reflecting the immunological instability and shifting immune response characteristic of this intermediate form of leprosy[14,15]. Histopathologically, S-100 immunostaining markedly enhanced the identification of peripheral nerves. In the BT group, all cases showed clear nerve infiltration with no evidence of fragmentation or complete nerve absence, indicating active but organized nerve inflammation. In the BB group, although infiltration was also predominant, one case demonstrated fragmented nerve bundles, suggesting either more advanced or fluctuating neuropathic changes, consistent with the unstable immunological profile seen in BB leprosy. These findings are in line with earlier studies that reported S-100 as a sensitive marker for Schwann cells, enabling improved visualization of peripheral nerve involvement across the leprosy spectrum, especially in borderline forms where nerve changes may be subtle or evolving[16,17]. The occurrence of nerve fragmentation in BB cases also supports observations by Kaur *et al.* (2000), who noted varying degrees of nerve degeneration corresponding to the immunological instability in borderline leprosy[18]. When comparing diagnostic modalities, S-100 immunostaining exhibited superior sensitivity. While H and E detected nerves in all BT cases, it missed two cases in the BB group that were identified by S-100. This underscores the added diagnostic value of immunohistochemistry, particularly in early or ambiguous cases where routine H and E may fail to delineate nerve architecture due to overlapping inflammation or subtle neuropathy. These findings are consistent with prior studies. For instance, Mahajan *et al.* (2017) and Moorthy *et al.* (2004) demonstrated that S-100 enhances nerve detection and better outlines Schwann cells and peripheral nerve fibers, thus improving diagnostic accuracy in leprosy[19,16]. Furthermore, the absence of completely degenerated or "absent" nerves in either group may reflect the relatively early stage of disease in the studied population or prompt clinical diagnosis and biopsy. Previous research by Kaur *et al.* (2000) and Job et al. (2008) also emphasized that early detection of nerve involvement using S-100 can be instrumental in timely therapeutic intervention, reducing the risk of permanent disability. The findings also emphasize the importance of incorporating S-100 immunohistochemistry into routine diagnostic workflows in dermatopathology, especially in regions where leprosy remains endemic. Not only does it aid in confirming clinical diagnosis, but it also provides objective evidence of nerve damage, which is critical for disease classification, prognosis and treatment planning.

CONCLUSION

This study reinforces the utility of S-100 immunohistochemistry in detecting peripheral nerve involvement in borderline leprosy. S-100 staining showed 100% sensitivity in both BT and BB subtypes, outperforming H and E in the BB group. The technique proved valuable in highlighting nerve infiltration and subtle nerve damage, particularly in BB leprosy where routine histology may miss early neuropathic changes. These findings support existing literature advocating the use of S-100 as a reliable marker for neural tissue in leprosy. Integrating S-100 immunostaining in routine evaluation can enhance diagnostic precision, guide appropriate treatment and contribute to better patient outcomes in leprosy management.

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