

S-100 Immunostain as an Ancillary Aid in the Diagnosis of Tuberculoid Leprosy

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Abstract

To study the patterns of nerve involvement in tuberculoid leprosy using S-100 immunostain and to compare its sensitivity with that of hematoxylin and eosin stain. The paraffin sections of twenty five cases of leprosy including five of tuberculoid (TT) and 20 of borderline tuberculoid (BT) type were subjected to both H&E and S-100 immunostaining. The patterns of nerve involvement on S-100 immunostaining were graded as infiltrated (A), fragmented (B), absent (C) and intact (D). On H&E staining only 2/5 (40%) TT cases and 9/20 (45%) of BT cases revealed neural inflammation. On S-100 staining of TT cases, 3/5 (60%) showed pattern B of nerve involvement followed by pattern A and C in 1/5 (20%) and 1/5 (20%) cases respectively. Out of the 20 cases of BT, S-100 demonstrated B pattern of nerve involvement in 14 (70%), A in 5 (25%) and C in 1 (5%). S-100 Stain is an efficient ancillary aid in histopathological diagnosis of tuberculoid pole of leprosy and is superior to H&E stain in identifying the neural inflammation in granulomas. The fragmented pattern of nerve involvement was the most common pattern seen in both TT (60%) and BT (70%) cases.

Key Words

Tuberculoid leprosy, S-100 immunostain, Granuloma, Nerve Elements

Introduction

Leprosy is a chronic disease caused by *Mycobacterium leprae* and affects predominantly the peripheral nervous system and skin.(1) Histopathology is the key to diagnosis of leprosy. Histopathological diagnosis usually is not difficult in the lepromatous spectrum because of abundance of bacilli in the lesions.(2) This is often not possible in the tuberculoid leprosy because of rarity of bacilli in the lesions. Active destruction of nerve by granulomatous inflammation is an equally reliable finding to confirm the diagnosis of tuberculoid leprosy. Demonstration of residual nerve elements in acid-fast bacilli negative sections increase the certainty level of

diagnosis.(3) On routine hematoxylin and eosin (H&E) staining, the nerve fibres do not stand out well from the background and often are confused with epithelioid cells, fragments of arrector pilli muscles and small vessels.(4) (5) This has led to the use of S-100 stain as a sensitive and reliable marker of nerve damage.(6) S-100 is an acidic calcium binding protein and called S-100 because of its solubility in 100% ammonium sulphate.(7) It is an immunocytochemical marker of Schwann cells of peripheral nerves.(8) Four pattern of nerve damage are demonstrable on S-100 immunostaining, namely A. Infiltrated B. Fragmented C. Absent D. Intact.(6)(9) The

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patterns A,B and C have sensitivity , specificity, positive and negative predictive values of 100% in diagnosing tuberculoid leprosy (tuberculoid and borderline tuberculoid).(9)

AIM

This study was carried out for the purpose of comparing ability of H&E with S-100 stain in demonstration of nerve damage in cases of tuberculoid leprosy (both tuberculoid and borderline tuberculoid) and to study various pattern of nerve damage on S-100 immunoperoxidase staining.

Material and Methods

Twenty five cases of leprosy including 5 tuberculoid (TT) and 20 borderline tuberculoid (BT) (as defined by Ridley and Jopling) were included in study. Skin biopsies from these cases were stained by hematoxylin & eosin stain (H&E). Immunocytochemistry was done by labelled streptavidin -biotin technique using antibody to S-100 protein (Rabbit polyclonal antibody RTU-S-100) after heat antigen retrieval. Both H&E and S-100 stained slides were studied for the patterns of nerve involvement. They were categorized as: (A) infiltrated, (B) fragmented, (C) absent and (D) intact. Sensitivities of H&E and S-100 stains in identifying nerve involvement were calculated

Results

There were 5 cases of tuberculoid and 20 borderline tuberculoid leprosy. Of the total 25 cases, H&E identified nerves in 11(Fig-1), while S-100 identified nerves in 23 cases (Table-1). S-100 proved to be more sensitive than H&E with overall sensitivity being 0.92 versus 0.42. Fibillar structures staining positively for S-100 protein were identified as nerve twigs. Different pattern of nerve involvement in histopathologic sections of TT and BT cases with S-100 stain were noted as:-

(A) Infiltrated -Dark staining fibillar structure in a wavy pattern associated with inflammatory cells (Fig-2).

(B) Fragmented- Small dark staining fibillary structures inside a granuloma (Fig-3).

(C) Absent - No dark staining fibillary structure inside or outside the granuloma

(D) Intact- Dark staining fibillar structure with smooth outline (Fig-4)

In both TT and BT leprosy on immunoperoxidase S-100 staining, fragmented pattern of nerve involvement was the most commonly observed pattern followed by infiltrated (table-2). Intact pattern of nerve involvement was not observed in either type of leprosy cases.

Discussion

Leprosy is a neurotropic disease caused by *Mycobacterium leprae*.(2) In the lepromatous end of the spectrum, a definitive diagnosis is possible owing to abundance of acid fast bacilli, which may be detected even on H&E sections with a bluish hue.(10) Demonstrable evidence of nerve involvement by the granulomatous infiltrate has been considered an important feature of tuberculoid leprosy, its significance being same as the finding of acid-fast bacilli in a nerve.(11) It has been advocated that the finding of nerve twigs within a granuloma should suggest a diagnosis of leprosy.(12) The difficulty in recognizing small nerve twigs on routine H&E sections has led to use of the S-100 stain as an aid to the histological diagnosis of tuberculoid leprosy. Fleury and Bacchi were the first to use S-100 stain in biopsies of clinically suspected cases of tuberculoid leprosy with no histological or bacteriological evidence of same and affirmed its utility in demonstration of cutaneous nerve alteration in 8 out of 9 cases.(5) Rajalakshmi et al found S-100 to be nearly twice as sensitive as H&E (0.78 versus 0.42) in identification of nerves in adequate biopsies of BT leprosy cases.(13) The present study also reaffirms the superiority of S-100 over H&E in demonstration of nerves in skin biopsies of tuberculoid leprosy.

Immunohistochemical stain for S-100 protein was done in tuberculoid and borderline tuberculoid leprosy cases of present study to examine different patterns of nerve involvement which revealed the fragmented pattern of nerve damage as the most common pattern in both tuberculoid (60%) and borderline tuberculoid (70%) cases. Intact nerves were not observed in any of the cases. On S-100 staining of biopsies of tuberculoid leprosy (TT&BT), Job et al reported fragmented nerves in the granulomas in 70% cases.(12) Thomas et al also reported similar findings in their study with fragmented nerves in biopsies of 100% tuberculoid and 64.2% borderline tuberculoid cases.(6) Rajalakshmi et al

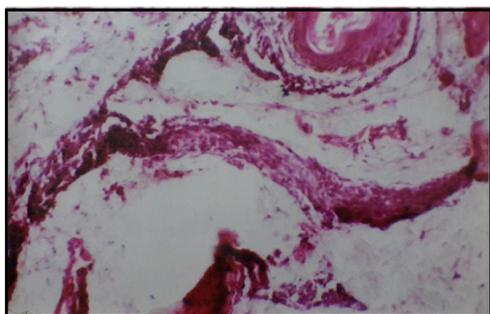
Table -1. Comparison of H&E Staining and S-100 Immunostaining in Identification of Nerve Involvement In Biopsies of Tuberculoid and Borderline Tuberculoid Leprosy Cases

Histopathological Type of Leprosy	H&E	H&E	H&E	H&E	H&E	S-100	Total Cases
	+VE	-VE	+VE	-VE	Sensitivity	Sensitivity	
	S-100 +VE	S-100 +VE	S-100 -VE	S-100 -VE			
Tuberculoid	2	2	0	1	0.40	0.80	5
Borderline Tuberculoid	9	10	0	1	0.45	0.95	20

Table -2 S-100 Staining Pattern of Nerve Involvement In Tuberculoid & Borderline Tuberculoid Leprosy Cases

S-100 Staining Pattern of Nerve	Histopathologic Type of Leprosy	
	TT (9)	BT (21)
Infiltrated	1 (20%)	5 (25%)
Fragmented	3 (60%)	14(70%)
Absent	1(20%)	1(5%)
Intact	0	0

Fig-1. Borderline Tuberculoid Leprosy Section Showing Neural Infiltration (H&E, 100X)



reported infiltration and/or fragmentation of nerves by inflammatory cells or granuloma by S-100 as only the significant decisive factor for tuberculoid leprosy.(13) In contrast, Anand et al observed absent pattern of staining of nerve twigs with S-100 stain in most of the skin biopsies of borderline tuberculoid leprosy cases.(14) Singh et al found complete absence of nerve twigs in adequate skin biopsies as a most reliable criterion for the diagnosis of tuberculoid leprosy.(15)

In conclusion, S-100 is superior to H&E in identifying intact or damaged nerves in skin biopsies. Infiltration with or without fragmentation of nerves is the only reliable indicator of leprosy. Absence of nerves in adequate biopsies is not exclusive to leprosy and can be seen in other granulomatous disorders so should not be regarded as decisive.(13) We therefore root for performing S-100 immunostain routinely in all biopsies with a clinical

Fig-2. Tuberculoid Leprosy section Showing Infiltrated Cutaneous Nerve at the Periphery of a Granuloma positive for S-100 protein (Avidin-Biotin Peroxidase Complex, 400X)

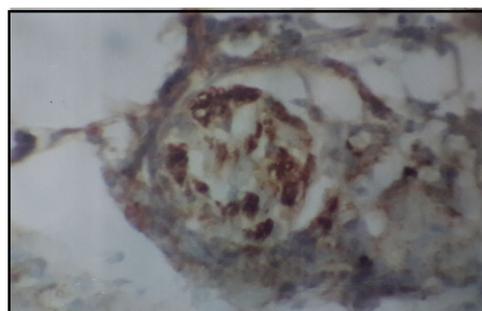


Fig-3. Tuberculoid Leprosy Section Showing Fragmented Remains of Cutaneous Nerve Inside a Granuloma Detected By S-100 Protein (Avidin-Biotin Peroxidase Complex, 400X)

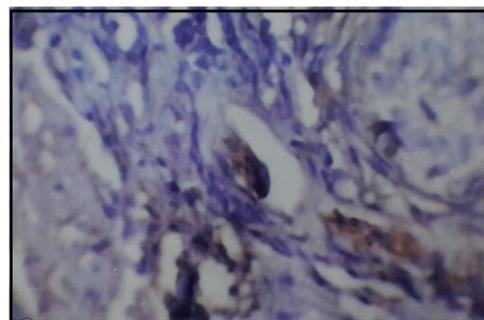
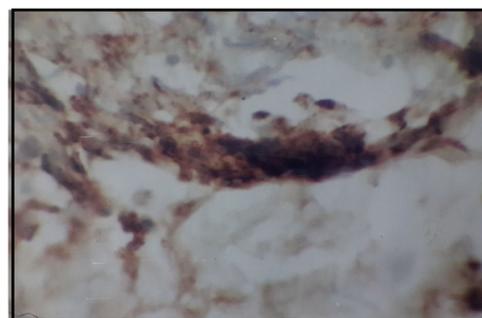


Fig-4. Borderline Tuberculoid Leprosy Section Showing Intact Cutaneous Nerve At The Periphery Of A Granuloma Positive For S-100 Protein (Avidin-Biotin Peroxidase Complex, 400X)



suspicion of tuberculoid (TT & BT) leprosy. It is a simple, practical, and extremely valuable adjunct that facilitates early diagnosis and treatment of leprosy cases and thus can help us in reducing morbidity, drug resistance and prevention of development of deformity and disability in the patients of leprosy.

Conclusion

A definitive diagnosis of leprosy can be made on the basis of finding active destruction of cutaneous nerves by granulomatous inflammation in a skin biopsy even in the absence of lepra bacilli. However, small nerve branches are difficult to recognize in H& E stained sections especially when there is appreciable inflammation. Staining for S-100 protein allows easy identification even of small nerve twigs and fragments of nerves in granulomas as positively staining, fibrillary structure. Immunoperoxidase staining for S-100 protein was used to delineate nerves in lesional skin biopsies of 25 cases of leprosy in tuberculoid spectrum and found to be useful in 23 cases as compared to 11 with H&E Stain. The majority of the cases showed fragmented pattern of nerve staining with S-100 stain. The significance of this study lies in the high sensitivity of S-100 to demonstrate nerve damage in tuberculoid leprosy, for an earlier diagnosis.

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