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Original Research Article

S-100 Immunostaining for Differentiating Leprosy from other Granulomatous Lesions of Skin

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Abstract

Introduction: Leprosy is a chronic granulomatous disease caused by Mycobacterium leprae, diagnosed based on clinical signs, histopathological findings and demonstration of acid fast bacilli on skin biopsy. But diagnosis becomes challenging in case of tuberculoid spectrum of leprosy where diagnosis is made on histological findings like nerve destruction and granuloma. The histological findings like granuloma may simulate other granulomatous dermatosis like sarcoidosis. In such cases immunostaining with S-100 helps in differentiating leprosy from other granulomatous dermatosis on the basis of nerve staining.

Materials and Method: This is a prospective descriptive study on 50 skin biopsy specimen diagnosed as leprosy and other cutaneous granulomatous dermatosis. Histological examination was done using H&E and fite faracco. Immunohistological examination was done after staining with S-100. Diagnostic efficacy of test in terms of sensitivity were calculated using histopathological features as gold standard. Correlation between pattern of neutritis by S-100 staining and agreement between histological diagnosis were analysed.

Result: Out of total 50 skin biopsies, 30 were diagnosed as leprosy and 20 cases were other granulomatous dermatosis. Maximum cases among leprosy were of tuberculoid leprosy (46.67%). Sensitivity of S-100 in nerve identification in tuberculoid leprosy was 100% whereas it was 85.71% with H&E. Fragmented (64.29%) and infiltrated (21.43%) pattern was most common in tuberculoid spectrum of leprosy whereas lepromatous spectra showed mostly fragmented pattern (80%). Intact nerves were seen in all the other cutaneous granulomatous lesions.

Conclusion: Therefore, S-100 is an effective auxillary tool in early diagnosis and differentiation of leprosy from other granulomatous dermatosis.

Keywords: Granulomatous dermatosis; Leprosy; Nerve staining; S-100 immunostaining.

Introduction

Leprosy is a chronic disease caused by Mycobacterium leprae and affects predominantly peripheral nervous system and skin. Diagnosis of leprosy is based on clinical signs, characteristic histopathological findings and demonstration of acid fast bacilli on skin biopsy.[1]

The diagnosis of lepromatous leprosy is relatively easy with demonstration of bacilli.[2] In tuberculoid and indeterminate form; detection rate of mycobacterium leprae is however low.[3] In such cases histological evidence of active nerve destruction by granulomatous inflammation is accepted as diagnostic of tuberculoid leprosy and nerve damage and sensory impairment remain key factors in pathogenesis of leprosy.[4]

However granulomas and inflammatory cells often totally blot out the nerve structure and identification of nerve remnants in H&E sections become difficult.[5] Also morphological similarities with other granulomatous dermatosis like lupus vulgaris, sarcoidosis, scrofuloderma, granuloma annulare add to the problem.

Therefore, recently immunostaining with S-100 has been used for demonstration of neural changes in leprosy. S-100 is an acidic calcium binding protein, an immunocytochemical marker of Schwann cells of peripheral nerves.[6]

The present study is undertaken to examine different patterns of cutaneous nerve involvement in leprosy with S-100 immunostaining and differentiate leprosy from other cutaneous granulomatous lesion based on S-100 staining.

Material and Methods

A prospective descriptive study was carried out in department of Pathology, including 50 skin biopsy over a period of 1 year. Ethical approval was taken. 30 cases diagnosed clinicohistologically as leprosy and 20 cases of cutaneous granulomatous lesions like lupus vulgaris, sarcoidosis, scrofuloderma, granuloma annulare etc. were studied. Tissue sections from all the cases were stained with H&E, Fite faraco and S-100 immunostain. Skin biopsy from representative lesion were examined histologically using H&E, and fite faraco staining for Mycobacterium leprae. These 50 skin biopsy specimen were subjected to S-100 immunoperoxidase stain and stained slides were read without any reference to histopathological data. Histopathological features and S-100 staining pattern were then correlated. Diagnostic efficacy of test in terms of sensitivity were calculated using histopathological features as gold standard. Correlation between pattern of neutritis by S-100 staining and agreement between histological diagnosis were also analysed.

Result

Out of total 50 cases included in this study, frequency distribution of various spectra of leprosy amongst total 30 cases of leprosy and the rest 20 cases of other cutaneous granulomatous lesion is shown in Table 1.

Table 1: Frequency distribution of leprosy and other granulomatous skin lesions.

Sr. No.	Types of lesion	Frequency
1	Lepromatous leprosy	5(16.67%)
2	Borderline lepromatous leprosy	2(6.67%)
3	Borderline tuberculoid leprosy	7(23.33%)
4	Tuberculoid leprosy	14(46.67%)
5	Indeterminate leprosy	2(6.67%)
6	Other cutaneous granulomatous lesion	20

Among the 30 cases of leprosy, 5 (16.67%) were diagnosed as lepromatous leprosy based on clinical and histopathological findings such as epidermal thinning, presence of grenz zone and foam cells. 2 (6.67%) cases were of borderline lepromatous leprosy. 14 (46.67%) cases were diagnosed as tuberculoid leprosy as were showing well formed epitheloid granulomas with lymphocytic infiltrate. 2 (6.67%) cases were of indeterminate leprosy as were showing perivascular and periadnexal lymphoplasmacytic infiltrate.

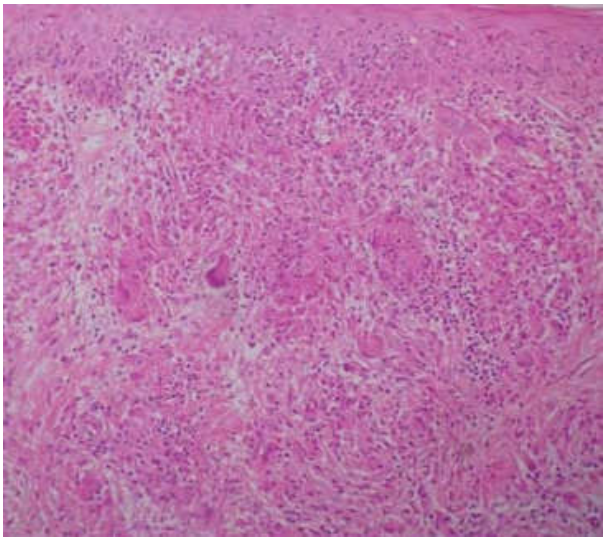

Fig. 1: Epitheloid granulomas in dermis, extending into adjacent skin adnexae and nerve bundles, in tuberculoid leprosy, H&E, 40X.

All 50 cases were examined for bacilli on fite faraco stain. Lepromatous leprosy cases were positive for bacilli on fite faraco stain, showing high bacillary index ranging from 3 to 5 whereas tuberculoid, indeterminate and other cutaneous granulomatous lesions were negative for bacilli on fite faraco and showed bacillary index of 0.

On H&E stain, nerves were demonstrable in 17 cases of leprosy and in all the 20 control cases. However, in 2 cases of tuberculoid leposy there was extensive granuloma formation due to strong immune response of the host causing nerve destruction, thus there was no demonstrable nerve on both H&E and S-100 stain. Table 2 shows correlation between H&E and S-100 stain in the

Table 2: Nerve identification by H&E and S-100 staining and their sensitivity.

Type of Leprosy	Total Case	H&E and S-100 Positive	H&E Negative and S-100 Positive	H&E Positive and S-100 Negative	H&E and S-100 Negative	Sensitivity of H&E	Sensitivity of S-100
Lepromatous leprosy	5	2	3	0	0	62.50%	100%
Borderline lepromatous leprosy	2	1	1	0	0	66.67%	100%
Borderline tuberculoid leprosy	7	3	4	0	0	63.64%	100%
Tuberculoid leprosy	14	10	2	0	2	85.71%	100%
Indeterminate leprosy	2	1	1	0	0	66.67%	100%

identification of nerve to differentiate various spectra of leprosy and further differentiate leprosy from other cutaneous granulomatous lesions like lupus vulgaris, sarcoidosis, scrofuloderma etc. Also sensitivity of S-100 in identification of nerve is shown in varius granulomatous skin lesions.

On S-100 staining, 4 different patterns of nerve damage were observed (based on study by Gupta et al)[3] – infiltrated, fragmented, intact and absent.

1. *Infiltrated:* Dark staining, fibrillar structures in a wavy pattern associated with inflammatory cells.
2. *Fragmented:* Small, dark staining structures within granuloma, identified as nerve fragments because of their fibrillar and wavy appearance though no intact nerve could be visualized.

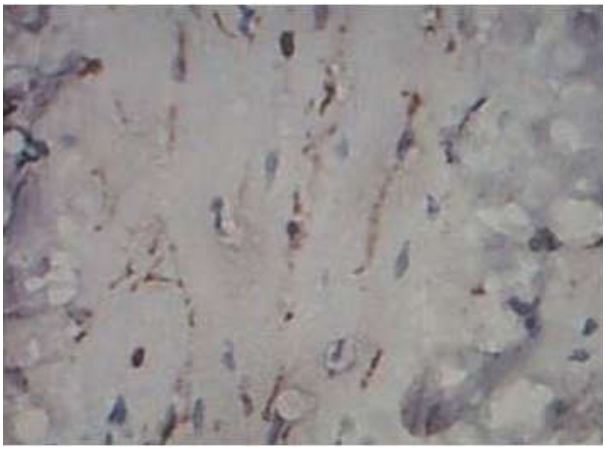

Fig. 2: Fragmented pattern of nerve change, detected by S-100 immunostain,40X.

3. *Absent:* No dark staining fibrillar structureswithin or outside the granuloma in an adequate biopsy containing subcutaneous fat and/or multiple granuloma. This pattern is observed due to destruction of nerve beyond recognition by the granuloma.
4. *Intact:* Dark staining, large fibrillar structures in a wavy pattern with no inflammatory cells inside.

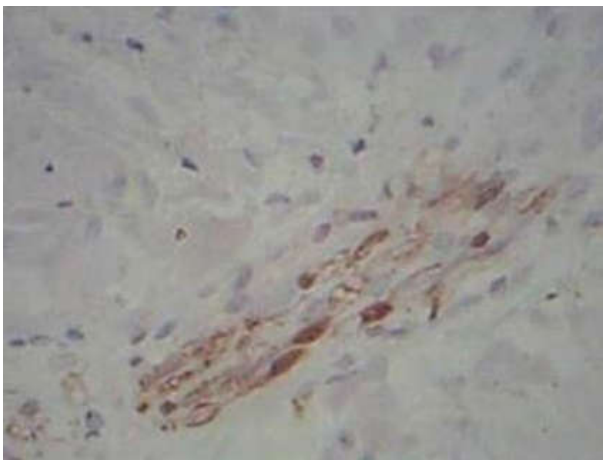

Fig. 3: Intact pattern of nerve change, detected by S-100 immunostain, 40X.

Table 3 shows staining pattern of nerve by S-100 staining in various spectra of leprosy and other cutaneous granulomatous lesion. Fragmented and infiltrated pattern was most common in tuberculoid spectrum of leprosy whereas lepromatous spectra showed mostly fragmented pattern. Intact nerves were seen in all the other cutaneous granulomatous lesions.

Table 3: Pattern of nerve involvement on S-100 staining in leprosy and other cutaneous granulomatous lesion.

Sr. No.	Type of Leprosy	Infiltrated	Fragmented	Absent	Intact	Total
1	Lepromatous leprosy	1(20.0%)	4(80.0%)	–	–	5
2	Borderline lepromatous leprosy	0	2(100%)	–	–	2
3	Borderline tuberculoid leprosy	3(42.86%)	4(57.14%)	–	–	7
4	Tuberculoid leprosy	3(21.43%)	9(64.29%)	2(14.29%)	–	14
5	Indeterminate leprosy	1(50.0%)	1(50.0%)	–	–	2
6	Other cutaneous granulomatous lesion	0	0	0	20	20

Discussion

A diagnosis of leprosy brings anxiety in minds of people not only because of the known deformities associated with it but also the fear of being categorised as a social outcast.[7] Thus, an utmost caution and care need to be considered in diagnosing leprosy. But making correct diagnosis is quite challenging for the histopathologist as skin biopsy in leprosy shows granulomatous reaction and destruction of neurovascular bundles which needs to be differentiated from other cutaneous granulomas due to difference in treatment. Thus it is important to identify the nerve involvement by granuloma in leprosy.[8]

Nerve remnants within granuloma are however confused with epitheloid cells, fragments of smooth muscle or endothelial cells.[5]

S-100 is an immunohistochemical marker for Schwann cells, melanocytes, langerhans cells, chondrocytes and myoepithelial cells.[4]

Gupta et al reported 4 patterns of nerve damage demonstrable on S-100 immunostaining, namely- infiltrated, fragmented, absent and intact.[3]

In the present study, H&E staining alone could detect neural inflammation or destruction in 17 cases, but when S-100 stain was applied the nerve fragments were readily identified in 28 cases.

Fleuri and Bacchi in their study found that 8 out of 9 biopsies with a clinical diagnosis of tuberculoid leprosy but with no histological or bacteriological evidence of the same, showed cutaneous nerve alteration by S-100 staining.[5]

Diagnostic value of S-100 in detecting nerve components was superior over H&E stain as sensitivity of S-100 is higher than H&E stain in any spectra of leprosy and also in differentiating leprosy from other cutaneous granulomatous lesion.

The fragmented pattern of nerve damage due to dermal neuritis is the most common pattern seen in 20 leprosy cases and was more often seen in tuberculoid spectra of leprosy.

Intact pattern of nerve staining was not observed in any of the leprosy cases as host immunity comes into play by mounting a nerve destructive inflammatory response forming granuloma.

However, intact nerve pattern of S-100 staining was observed in all the other granulomatous lesion of skin except leprosy, either inside or outside the granuloma and thus S-100 staining differentiate leprosy from other granulomatous lesion of skin.

Conclusion

S-100 immunostain is a useful auxillary aid due to its high sensitivity in early diagnosis of leprosy, thus preventing morbidity and also in differentiating other granulomatous lesion of skin by pattern of nerve staining seen in leprosy.

Conflict of Interest: The authors declare that they have no conflict of interest.

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