Study of S100 Immunostaining in Demonstrating Neural Granulomas in Paucibacillary Leprosy

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Abstract

Context: Neural granulomas are hallmark of leprosy. Challenges faced in diagnosing paucibacillary leprosy include: (i) Difficult visualization of nerve twigs on hematoxylin and eosin (H and E) sections due to their small size and (ii) Paucity of organisms on acid–fast bacilli stain. Aims: (1) This study aimed to test the role of S100 immunostain in demonstrating neural granulomas in skin biopsies of paucibacillary leprosy, (2) to compare morphology of S100 staining of nerves inside granulomas among clinicohistologically defined different types of leprosy, and (3) to test whether the pattern of S100 immunostaining can distinguish nerve fragmentation/destruction from a normal intact nerve in skin biopsy. Materials and Methods: Sixty four diagnosed cases of leprosy were included in this study. Five skin biopsies with no significant pathology (for studying intact nerve) and nine nonleprosy cutaneous granulomas were also studied. Results: (i) In demonstrating neural granuloma, sensitivity of H and E was 48.27% and that of S100 was 100%, (ii) Morphology of nerve fragments on S100 stain for cases of leprosy was fragmented and infiltrated in 37, intact and infiltrated in 19, reduced, fragmented, and infiltrated in seven, and absent in one, (iii) There was a significant difference (P<0.001) in the pattern of staining of S100 on intact nerve and nerves involved by granuloma in leprosy, and (iv) The probability to differentiate between leprosy and nonleprosy granuloma was statistically significant (P<0.001). Conclusion: S100 immunostaining showed to be an effective adjuvant to histopathology in diagnosing paucibacillary leprosy and differentiating it from nonleprosy cutaneous granuloma.

Key Words: Granuloma, paucibacillary leprosy, S100 immunostaining

Introduction

Leprosy (Hansen’s disease [HD]) is a chronic infectious disease caused by Mycobacterium leprae, which presents itself in different clinicopathological forms. Skin granulomas pose a variety of differential diagnostic possibilities to histopathologists which include paucibacillary leprosy and nonleprosy granulomatous inflammation such as tuberculosis, sarcoidosis, and fungal infections. The presence of neural granulomas is a diagnostic hallmark for leprosy. However, nerve twigs within granuloma are often difficult to visualize on hematoxylin and eosin-stained sections (H and E) either due to their small size or due to extensive destruction. This problem is compounded by the paucity of acid–fast bacilli (AFB) in the tuberculoid spectrum.

S100 is an immunohistochemical marker of Schwann cells. A few reports in literature attempt to address the role of S100 in diagnosis of leprosy. This study aims at testing the role of S100 in demonstrating neural granulomas in various clinicopathological entities in leprosy. An attempt was also made to test whether S100 could distinguish nerve fragmentation/destruction from normal intact nerve and whether it could help in differentiating leprosy from nonleprosy granuloma based on pattern of staining.

Materials and Methods

This is a descriptive study conducted in our department from August 2013 to December 2014, after...
obtaining prior clearance from the Institutional Ethics Committee.

Sixty-four clinicohistologically diagnosed cases of tuberculoid (HD-TT), borderline tuberculoid (HD-BT), mid-borderline (HD-BB), borderline lepromatous (HD-BL), indeterminate (HD-I) leprosy, and nine cases of cutaneous nonleprosy granuloma were studied. Five skin biopsies with no significant pathology were also included for studying S100 staining pattern on normal intact nerve. The following cases were excluded from the study: cases which were in any of the lepra reactions, cases on medication for leprosy, and cases of relapse after inadequate treatment.

All skin biopsies were adequately fixed in formalin and were processed to prepare paraffin-embedded blocks. H and E and AFB staining were done on all skin biopsies of leprosy. Initial histological evaluation was done in the context of clinical findings available from case records.

Immunohistochemistry for S100 protein (antibody dilution determined by pre-titration) was done on paraffin-embedded tissue sections. For each staining batch, appropriate positive and negative controls were kept.

Initially, a brief clinical analysis of cases of HD-TT, HD-BT, HD-BB, HD-BL and HD-I was done. This was followed by evaluation of histopathological findings. After initial interpretation of slides, data obtained were entered in an excel sheet and statistical analysis was done.

A total of 58 cases of paucibacillary leprosy were studied [Table 1 and Figure 1]. Visualization of the nerves within the granuloma was studied and compared for H and E and S100 stained sections [Figure 2 and Table 2]. While interpreting S100 positivity, morphology was used to distinguish nerve fragments from other cells such as macrophages, sweat gland ducts, and Langerhans cells, which also show S100 positivity. The presence of nuclear and cytoplasmic staining of nerve twigs was taken as the criterion for the visualization of nerves on S100 immunostaining.

Sensitivity of the results obtained was calculated for both methods and was 100% with S100 but 48.78% with H & E. Clinicohistological diagnosis was taken as gold standard.

The patterns of S100 staining of nerves inside skin granulomas among clinicohistologically defined HD-TT, HD-BT, HD-BB, HD-BL, and HD-I were compared [Table 3]. Five patterns with defined criteria were set to categorize the morphology of nerve fragments [Figure 3]: (1) Absent – Here, no nuclear or cytoplasmic staining for nerve fragments inside granuloma was seen on S100 immunostaining, (2) Intact – Continuous and closely stained nerve fragments were observed, (3) Intact and infiltrated – Continuous and closely stained nerve fragments were observed along with intermixed inflammatory cells which were seen surrounding them, (4) Fragmented and infiltrated – The staining pattern was discontinuous with small nerve twigs separated and infiltrated by dense inflammatory infiltrate, and (5) Reduced, fragmented and infiltrated – Very few nerve twigs were seen showing nuclear and cytoplasmic positivity scattered in a background of dense inflammation.

To test whether the pattern of S100 immunostaining could distinguish nerve fragmentation/destruction in leprosy from a normal intact nerve, sections from biopsies of skin showing normal histology were stained for S100.
Shenoy and Nair: S100 in demonstrating neural granulomas in leprosy

Table 3: Morphology of nerve fragments observed on S100 immunostaining

| Diagnosis | Absent | Fragmented and infiltrated | Intact and infiltrated | Reduced, fragmented, and infiltrated |
|-----------|--------|-----------------------------|------------------------|--------------------------------------|
| HD-TT     | 0      | 2                           | 1                      | 3                                    |
| HD-BT     | 0      | 31                          | 10                     | 3                                    |
| HD-BB     | 0      | 1                           | 2                      | 0                                    |
| HD-BL     | 1      | 1                           | 0                      | 1                                    |
| HD-I      | 0      | 2                           | 6                      | 0                                    |
| Total     | 1      | 37                          | 19                     | 7                                    |

HD: Hansen’s disease, TT: Tuberculoid, BT: Borderline tuberculoid, BB: Mid-borderline, BL: Borderline lepromatous, I: Indeterminate

The applicability of S100 immunostaining in differentiating leprosy from other cutaneous nonleprosy granuloma was studied. Sections from skin biopsies of cases of lupus vulgaris, tuberculosis verrucosa cutis (TBVC), chromoblastomycosis, and sarcoidosis were obtained [Figure 4]. These were stained for S100. Visualization of nerves and pattern of staining within the granulomas was analyzed using the criteria defined above. Results obtained were compared with that observed for cases of leprosy. Significance of the difference was calculated by using Chi-square test [Table 5].

Results

Of the 58 cases of paucibacillary leprosy studied, fragmented nerves within the granuloma were visualized only in 28 cases on H and E sections but could be easily identified using S100 immunostaining in all the cases. Sensitivity for both the methods was compared and S100 staining showed more sensitivity than H and E alone [Table 2] in demonstration of neural granuloma.

The most common patterns observed were reduced, fragmented, and infiltrated for HD-TT, fragmented and infiltrated for HD-BT, and intact and infiltrated for HD-I and HD-BB. A variable pattern was observed for HD-BL [Table 3].

No statistical analysis of these observations was done because some of the variable counts were below 5.

Nerves in biopsies of skin with no significant pathology showed an intact staining pattern [Figure 3b] on S100 when compared to fragmented and infiltrated, intact and infiltrated, absent and reduced, fragmented and infiltrated patterns in cases of leprosy [Figure 3a and c-f]. This difference in the patterns observed was significant ($P<0.001$) [Table 4].

Cases of lupus vulgaris, tuberculosis verrucosa cutis, chromoblastomycosis, and sarcoidosis were studied [Figure 4]. Comparison of patterns of staining of nerves on S100 within granuloma in cases of leprosy and nonleprosy dermatoses is shown in Table 5. The probability to differentiate between leprosy and nonleprosy granuloma based on pattern of staining of nerve within granuloma on S100 immunostaining was statistically significant ($P<0.001$) [Table 5].

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 categorized as a social outcast. Thus, an utmost caution and care need to be considered in diagnosing leprosy.

However, giving a correct diagnosis also brings in many challenges to the histopathologist.

Skin biopsies in leprosy show granulomatous reaction with destruction of neurovascular bundles. It is important to differentiate it from other cutaneous granulomas due to difference in treatment. Thus, it is important to identify the nerve involvement by granulomas in leprosy.

S100 is a sensitive and reliable marker of nerve damage. Fleury and Bacchi in their study were able to visualize peripheral nerves in tuberculoid granuloma by staining for S100. Job et al. studied twenty skin biopsies which were reported as granulomatous inflammation of skin in which a definite diagnosis of tuberculoid leprosy could not be made. Using S100, they were able to identify portions of peripheral nerves in granuloma in 14 out of these 20 skin biopsies. In the present study, out of the 58 cases of paucibacillary leprosy studied, fragmented nerves within the granuloma were visualized only in 28 cases on H and E sections. However, fragmented nerve twigs within the granuloma could be easily identified using S100 immunostaining in all the cases. The sensitivity of histopathological study using H and E alone for visualization of nerve twigs within the granuloma was only 48.27% when compared to 100% with the use of S100. These figures clearly justify that S100 may be used as an adjuvant aid to histopathology in identifying neural granulomas in leprosy.

Ismail EA demonstrated increased nerve bundles by S100 immunostain in leprosy than in nonleprosy granulomas. Nerve bundles were much better visualized using S100 than H and E stain.

In a similar study, Thomas et al. categorized nerve involvement within granuloma as infiltrated, fragmented, absent, and intact based on the pattern of S100 staining of nerves. They calculated the correlation between pattern of nerve involvement by H and E and S100 staining. The results obtained were sensitivity 100%, specificity 100%, positive predictive value 100%, and negative predictive value 100%. However, no test statistically can show 100% sensitivity and 100% specificity simultaneously.

Similar results were reported by Gupta et al. and Mohanraj and Srinivasan. In the above two studies, there was no uniformity in the pattern definition set. Absent and intact patterns were considered for nerve fragments inside and outside the granuloma, whereas fragmented and infiltrated patterns were restricted to nerve fragments seen within the granulomas. Moreover, the destruction of nerves by inflammation is significant within the granulomas and not outside.

In the present study, the observations were restricted to nerve fragments seen only within the granuloma while comparing patterns in HD-TT, HD-BT, HD-I, HD-BB, and HD-BL. Furthermore, it was observed that nerves within the granuloma, whether fragmented or intact, were invariably infiltrated with inflammatory cells. Thus, the patterns observed were classified as absent, intact, intact and infiltrated, fragmented and infiltrated, and reduced, fragmented, and infiltrated. Difference in staining patterns of nerve fragments by S100 provides a clue about the spectrum to which a case of leprosy belongs.

Thomas et al. compared the morphology of nerves based on pattern of S100 staining in cases of leprosy and nonleprosy granuloma. They observed that the patterns...
observed in leprosy were infiltrated, fragmented, or absent. Only intact nerve fibers were seen in controls comprising nonleprosy cutaneous granuloma. But again, there is no uniformity in the pattern definition set. In the present study, the observations were restricted to nerve fragments seen only within the granuloma. In nonleprosy cases, eight cases showed no involvement of the nerve (absent pattern) by granuloma and one case showed intact nerve within granuloma when compared to other patterns in leprosy. The probability to differentiate between leprosy and nonleprosy granuloma based on the pattern of staining nerve within granuloma on S100 immunostaining was statistically significant (P<0.001).

Conclusion

1. S100 protein on immunoperoxidase stain can demonstrate the destroyed and fragmented nerve fragments within the granulomas which are not seen easily on routine H and E-stained sections
2. Sensitivity of S100 immunostain in visualization of nerve fragments in paucibacillary leprosy is superior to the sensitivity of histopathology using H and E alone. Thus, supplementing routine H and E sections with immunostaining by S100 has an advantage in demonstrating neural granulomas over using histopathology alone
3. The difference in the staining patterns of nerve fragments by S100 may provide a clue about the spectrum to which a case of leprosy belongs
4. S100 immunostaining can distinguish nerve fragmentation/destruction in leprosy from a normal intact nerve in a skin biopsy
5. It can help in distinguishing leprosy from nonleprosy cutaneous granuloma based on the pattern of staining nerve within granuloma.

Thus, S100 immunostaining of nerves might prove to be an effective adjuvant to histopathology in diagnosing paucibacillary leprosy and differentiating them from nonlepromatous cutaneous granulomatous dermatoses.

Financial support and sponsorship
We would like to acknowledge the financial assistance of INR. 15,000/- from the “Science Education division, Kerala State Council for Science, Technology and Environment (KSCSTE).”

Conflicts of interest
There are no conflicts of interest.

What is new?
S100 immunostaining is an adjuvant to H and E in diagnosing leprosy and differentiating it from nonleprosy granuloma.

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