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S-100 Immunostain-an aid to diagnosis in tuberculoid leprosy

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Abstract
Introduction: Leprosy is one of chronic granulomatous disease that is caused by Mycobacterium leprae. This bacillus has a high affinity for skin and peripheral nerve cells. In multibacillary (lepromatous leprosy) cases, the demonstration of acid fast bacilli usually concludes the diagnosis, which is not the case with paucibacillary (tuberculoid leprosy) cases. The diagnosis of tuberculoid leprosy is often difficult as most of the cases do not show acid fastness with 5% H_2SO_4 and hematoxylin and eosin (H&E) stain also fails to demonstrate nerve involvement in granulomas. This study evaluates the utility of S-100 staining in identifying nerve fragmentation and differentiation of tuberculoid leprosy from other cutaneous granulomatous diseases.
Aim: To study various pattern of nerve involvement in leprosy by S-100 and to calculate and compare sensitivity of S-100 and H&E stain in nerve demonstration in tuberculoid cases and overall.
Methods: Thirty (30) adequate skin biopsies with a clinical and histopathological diagnosis of leprosy were taken. Out of these 30 cases, 12 cases had borderline tuberculoid leprosy, 7 had borderline lepromatous, 4 were of lepromatous leprosy. Tuberculoid, indeterminate and midborderline leprosy constituted 3, 3 and 1 case respectively. 10 cases of granulomatous dermatosis of skin other than leprosy (lupus vulgaris) were included as controls. All biopsies were subjected to H & E, Modified Ziehl-Neelsen and S-100 immunostaining and were observed for following nerve patterns: infiltrated (A), fragmented (B), absent (C), and intact (D) nerves. The sensitivity of S-100 and H&E stain in nerve demonstration in tuberculoid cases and overall was calculated and compared.
Results: **H&E stain*:*** Ten out of 30 (33.3%) cases demonstrated neural inflammation (inflammed). Among the 15 tuberculoid cases, only 6 (40%) cases showed neural inflammation, rest of the cases no nerve was demonstrable on H& E. No nerve was demonstrable in indeterminate cases. Contact group showed intact nerves in all cases.
S-100 Immunostain: On S-100 immunostaining, the most common pattern observed was nerve fragmentation (pattern B) seen in 15 (50%) cases, followed by absence of detectable nerves (pattern C) in 9 (30%) cases. Infiltrated pattern was seen in 6 (20%) cases. All the tuberculoid cases showed either of patterns of nerve involvement. The most common pattern observed was fragmented (pattern B) in 7/15 cases which was followed by absent pattern (pattern C) in 6/15 cases. Indeterminate cases had infiltrated pattern in all the cases. Lepromatous cases had fragmented pattern being commonest followed by absent pattern. All controls showed pattern D i.e. intact nerves. Intact nerve was not observed in any of the leprosy cases. The sensitivity of S-100 for demonstration of nerve in tuberculoid cases (BT and TT) was 100% and for H&E it was 40%.
Conclusion: S-100 aids in diagnosis in tuberculoid cases by nerve demonstration in the granulomas. It also helps in differentiating paucibacillary (BT and TT) leprosy from other granulomatous conditions.

Keywords: tuberculoid leprosy, immunohistochemistry, nerve

Introduction
Leprosy is a chronic infectious disease that is caused by the obligate intracellular parasite Mycobacterium leprae. This obligatory intracellular bacillus has a high affinity for skin and peripheral nerve cells. The disease can express itself in different clinicopathological forms depending on immune response of host [1].
A definitive diagnosis of mutibacillary leprosy (lepromatous (LL) and borderline lepromatous (BL) of Ridley-Jopling classification) is easily made because Mycobacterium leprae is easily demonstrable on modified Ziehl Neelsen stain (ZN), which is not the case with paucibacillary leprosy (tuberculoid (TT), borderline tuberculoid (BT) and indeterminate type of Ridley-Jopling classification). The ZN positivity rate for tuberculoid cases is low and it ranges from 0 to 36% [2-4]. So, granulomas of these cases may be misinterpreted as

granulomas of other infectious and non-infectious lesions, notably tuberculosis, sarcoidosis and fungal infections [5, 6]. It is cutaneous nerve involvement by the inflammatory reaction that permits the definitive diagnosis of tuberculoid cases and helps in differentiating these cases from other cutaneous granulomatous condition.

There is difficulty in recognizing small nerve twigs on routine hematoxylin and eosin (H&E) sections which is overcomed by use of S-100 stain [7]. S-100 is an acidic protein expressed on number of cells including dendritic cells and schwann cells. By staining Schwann cells, S-100 allows easy recognition of dermal nerve twigs and helps in identifying neural inflammation and destruction in tuberculoid cases. It also helps in excluding other granulomatous lesions of the skin which are histologically similar to tuberculoid cases (TT and BT) [2].

The objective of the present study was to demonstrate different patterns of cutaneous nerve involvement in different forms of leprosy and other granulomatous conditions using S-100 immunostain. The sensitivity of S-100 over H&E was also studied in tuberculoid cases (TT and BT).

Materials and Methods

Study population

The study was conducted at GMCH Chandigarh over a period of one year. Thirty (30) adequate skin biopsies with a clinical and histopathological diagnosis of leprosy were taken. Out of these 30 cases, 12 cases had borderline tuberculoid leprosy, 7 had borderline lepromatous, 4 were of lepromatous leprosy. Tuberculoid, indeterminate and midborderline leprosy constituted 3, 3 and 1 case respectively. 10 cases of granulomatous dermatosis of skin other than leprosy (lupus vulgaris) were included as controls. The sections containing subcutaneous fat and/or multiple (at least three) granulomas were considered adequate.

Histopathology and Immunohistochemistry

The skin biopsies from both cases and controls were immediately fixed in 10% formalin, processed and were embedded in paraffin blocks. Sections of 2-3µm thickness were cut and were subjected to haematoxylin and eosin (H&E) staining, modified Ziehl-Neelsen and S-100 immunostaining. H&E-stained and modified Ziehl-Neelsen sections were evaluated. Neural involvement was assessed.

Assessment of S-100 in nerve demonstration

The presence or absence of neural changes and pattern of nerve involvement was assessed in all the 40 cases (cases as well as controls) and interpreted as four patterns as stated by Thomas *et al* [8]

- Infiltrated: dark staining fibrillar structures in a wavy pattern associated with inflammatory cells.
- Fragmented: small, dark staining, fibrillar structures, usually multiple, inside a granuloma.
- Absent: no dark staining fibrillar structures in or outside the granuloma.
- Intact: dark staining, large fibrillar structures in a wavy pattern with no inflammatory cells inside.

Assessment of sensitivity of S-100 and H&E for nerve demonstration

The sensitivity of S-100 and H&E for demonstration of nerve was also calculated in tuberculoid (BT and TT) leprosy cases and was compared.

Results

Clinical characterstics

Out of 30 patients, 23 (76%) were males and 7 (24%) were females. The mean age of presentation was 33.7 years. The most common presentation was hypopigmented patch (53.3%). Other common complaints were erythematous patch, nodules and macules. The lesions in all the 30 cases were either hypoaesthetic or anaesthetic.

Histopathological findings

Histopathological analysis of tuberculoid cases showed characteristics epithelioid cell granulomas with peripheral cuffing by lymphocytes and langhans giant cells. Lepromatous forms showed sheets and granulomas of parasitized foamy histiocytes (Figure 1a). Globii also noted at places.

Ten out of 30 (33.3%) cases demonstrated neural inflammation (inflammed) (Figure 1b). No intact/inflamed/absent nerves were detected in rest 20/30 (66.7%) leprosy cases (Table 1). Among the 15 tuberculoid cases, only 6 (40%) cases showed neural inflammation, rest of the cases no nerve was demonstrable on H& E. No nerve was demonstrable in indeterminate cases. Contact group showed intact nerves in all cases.

Table 1: Pattern of nerve involvement on hematoxylin and eosin stain

Histological type (n=30)	Inflammed (n=10)	Absent (n=20)	Intact (n=0)
Indeterminate (n=3)	0	3 (100%)	0
Tuberculoid (n=3)	1 (33.3%)	2 (66.7)	0
Borderline Tuberculoid (n=12)	5 (41.6%)	7 (58.4%)	0
Midborderline (n=1)	0	1 (100%)	0
Borderline Lepromatous (n=7)	3 (42.8%)	4 (57.2%)	0
Lepromatous (n=4)	1 (25%)	3 (75%)	0

Table 2: Pattern of nerve involvement on S-100

Histological type (n=30)	**Infiltrated: Pattern A (n=6)**	**Fragmented: Pattern B (n=15)**	**Absent: Pattern C (n=9)**	**Intact: Pattern D (n=0)**
Indeterminate (n=3)	3	0	0	0
Tuberculoid (n=3)	0	1	2	0
Borderline Tuberculoid (n=12)	2	6	4	0
Mid Borderline (n=1)	1	0	0	0
Borderline Lepromatous (n=7)	0	6	1	0
Lepromatous (n=4)	0	2	2	0

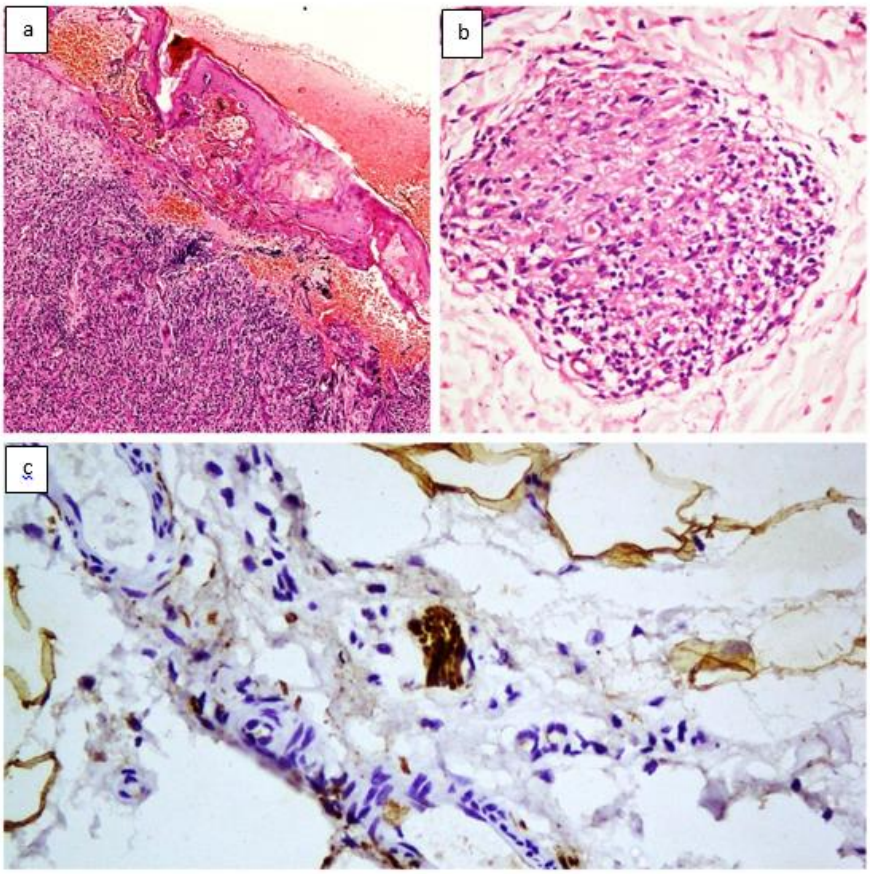

Fig 1a): Sheets of foamy histiocytes (H&E; X20) **Fig 1b):** Inflammed nerve (H&E; X40) **Fig 1c):** Intact nerve (S-100; X40)

Modified Ziehl Neelsen stain findings

All skin biopsies were examined for lepra bacilli with modified Ziehl Neelsen stain

Only 1 case (7%) of tuberculoid cases (BT and TT) showed lepra bacilli. Indeterminate cases were negative. All lepromatous cases showed Mycobacterium leprae. All the 10 controls were negative for lepra bacilli.

Immunohistochemical findings

Pattern of nerve involvement on S-100 immunostain

On S-100 immunostaining, the most common pattern observed was nerve fragmentation (pattern B) seen in 15 (50%) cases, followed by absence of detectable nerves (pattern C) in 9 (30%) cases. Infiltrated pattern was seen in 6 (20%) cases (Table 2). All the tuberculoid cases showed either of patterns of nerve involvement. The most common pattern observed was fragmented (pattern B) in 7/15 cases which was followed by absent pattern (pattern C) in 6/15 cases. Indeterminate cases had infiltrated pattern in all the cases. Lepromatous cases followed the same pattern of nerve involvement as observed in tuberculoid cases with fragmented pattern being commonest followed by absent pattern. All controls showed pattern D i.e. intact nerves. Intact nerve was not observed in any of the leprosy cases (Figure 1c).

Comparison of sensitivity of H&E and S-100 stains for nerve demonstration

The sensitivity of S-100 for demonstration of nerve in tuberculoid cases (BT and TT) was 100% and for H&E it was 40%.

Discussion

Leprosy is a chronic infectious disease of great epidemiological importance. The immune response of individual determines the manifestation of disease. No laboratory test alone is considered enough to diagnose leprosy. Clinical data usually conclude the diagnosis in symptomatic multibacillary cases. In paucibacillary cases histopathological examination complemented with immunohistochemistry often proves a valuable tool to confirm the diagnosis and subtyping of leprosy [9]. The diagnosis of paucibacillary leprosy is challenge to the histopathologist as these cases are generally lepra negative so can be confused with other granulomatous conditions. It is cutaneous nerve involvement that permits the definitive diagnosis of leprosy. Routine hematoxylin and eosin sections fails to demonstrate small nerve twigs. S-100 immunostain helps in such scenario. So this study was conducted to study role of S-100 in nerve demonstration in different forms of leprosy especially tuberculoid forms.

On S-100 immunostaining, all the 30 cases showed neural involvement in either of four patterns of nerve, as stated in the literature. The most common pattern was nerve fragmentation (pattern B) seen in 15 (50%) cases, followed by absence of detectable nerves (pattern C) in 9 (30%) cases and infiltrated pattern in 6 (20%) cases.

In BT cases, the most common pattern was fragmented nerves (50%) followed by absent nerves (33.3%). The finding was consistent with study by Gupta *et al* [10] but the percentage of cases showing nerve fragmentation and absent pattern was 73% and 21% respectively. Thomas *et al* [8] also showed similar results in their study with fragmented pattern being the most common followed by absent pattern but the

percentage of cases showing fragmented pattern (64.2%) was higher as compared to our study. In TT cases the most common pattern observed was pattern C (66%) followed by pattern B in 44% cases. This finding was consistent with study done by Singh *et al*. [11] However in study by Thomas *et al* [8] and Gupta *et al* [10], the most common pattern was fragmented pattern. The sensitivity of S-100 for demonstration of nerve in tuberculoid cases (BT and TT) was 100% and for H&E it was 40%.

In conclusion, S-100 adds to diagnostic accuracy in tuberculoid cases by nerve demonstration in the granulomas. It also helps in differentiating paucibacillary (BT and TT) leprosy from other granulomatous conditions.

Conflict of interest: There is no conflict of interest to this study.

Funding: Cost to this study is nil.

Ethical consideration: The study was conducted on 30 clinically and histologicaly diagnosed cases of leprosy. Patients were not subjected to any additional procedure for the purpose of study. The study was conducted on ethical guidelines for biomedical research on human subject as given in "Declaration of Helsinki" and by Central Ethics Committee on Human Research (CECHR) of ICMR, New Delhi.

Patient consent: Patients were informed beforehand that biopsy is a routinely established procedure with no additional risk. Confidentiality will be maintained.

Authorship and Contributorship: Amrita- data collection, analysis; **Dr Nitesh**-analysis **RPS punia**-interpretation of data, **Deepak basia**-helped in finalising draft.

Data availability statement: The supporting data is not shared

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