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Original Article

Role of S-100 Immunostaining in Differentiation of Borderline Leprosy from Other Granulomatous Diseases of Skin

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Leprosy is a chronic granulomatous disease affecting skin, peripheral nerves and other tissues. On histopathology leprosy mimics other infectious and non-infectious lesions like tuberculosis, sarcoidosis and fungal infections, which are also common in our country. In tuberculoid and indeterminate forms, where Acid Fast Bacilli cannot be demonstrated, the diagnosis becomes more difficult. *Mycobacterium leprae* is the only bacterium which has the ability to infiltrate peripheral nerves leading to Schwann cell disintegration. On routine Hematoxylin and Eosin stains (H&E), the nerve fibers may not be easily identifiable in some cases , hence S-100 immunostaining is used to highlight the nerve elements and to demonstrate and compare the nerve changes in spectrum of leprosy. With widespread use of multi-drug treatment, there has been changes in the profile of disease. The aim of the present study was to observe different patterns of cutaneous nerve involvement in leprosy and to correlate these with the clinical and histopathological findings in currently referred cases for histopathological opinion. The study was conducted in the Department of Pathology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, over a period of 12 months (July 2016 - July 2017) Subjects were recruited from patients presenting in Dermatology OPD. A total 35 consecutive cases with clinical suspicion / diagnosis of leprosy were included in the study. Biopsies were processed and stained by H&E, Fite-Faraco as well as S100 immunostaining. It was observed that on S-100 immunostaining, 43.7% cases showed granulomas infiltrating the dermal nerves whereas these changes could not be demonstrated in 16.6% cases of Borderline leprosy on H&E staining alone. Thus S-100 staining appears to serve as an important tool to diagnose leprosy from other granulomatous diseases of skin even in current scenario of leprosy.

Key words : Skin biopsy, S-100 Immunostaining, Borderline Leprosy, Granulomatous diseases, Nerves

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacteria leprae.* It affects almost any tissues or organs but has special affinity for skin, eyes, testes, the peripheral nerves and mucosa of the upper respiratory tract. "Hansen's disease" is

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name is given after physician Gerhard Armauer Hansen who discovered leprosy bacillus to be cause of this disease (Suzuki et al 2012). Leprosy is also called as "Kustharoga" (McMenamin 2011). With the implementation of MDT, India has succeeded in bringing down the prevalence rate of leprosy from 57.8/10,000 in 1983 to less than 1/10,000 in Dec 2005 and even further down to 0.66/10,000 in 2016 (NLEP 2016) As of 2016, 14 countries contain 95% of the globally reported leprosy cases. Of these, India has the largest number of cases (59%), followed by Brazil (14%) and Indonesia (8%) (NLEP 2016).

The Ridley-Jopling classification categorizes leprosy into 6 subtypes. TT-Tubercular polar, BT-Borderline Tubercular, TI - Tubercular Indefinite, BB - Mid Borderline, LI - Lepromatous Indefinite, BL - Borderline Lepromatous, LL - Lepromatous Polar. (Ridley & Jopling 1966). Indian Leprologists use a slightly different classification which also recognizes neuritic leprosy as distinct variety (IAL 1982). WHO has come out with a broad classification of paucibacillary (PB) and multi-bacillary (MB) types for treatment purposes which is based on clinical extent of disease as determined by number of lesions and nerves involved.

With wide use of multi-drug treatment (MDT), there have been changes in the profile of cases which may be reported more in early stages. Further, with integration of leprosy eradication and control programmes into general health services, more cases specially with atypical presentations are being referred for histopathological confirmation. Histological diagnosis of some of Borderline and Indeterminate leprosy may be difficult because leprosy granuloma cannot be distinguished from other granulomas that are seen in Leishmaniasis, Sarcoidosis and Tuberculosis. Moreover, the acid fast bacilli (AFB) in leprosy granulomas in paucibacillary cases may be scanty and usually fragmented (Khan 1998). On routine hematoxylin and eosin, the nerve fibers do not stand out well from the background in some cases. Therefore, there is need to use different techniques for molecular/immunological confirmation such as plastic embedding, osmium-hematoxylin staining on paraffin embedded sections or antibody to S100 protein have been used in order to make the nerve identifiable. (Singh et al 1994). Immuno-staining for S100 has been used for more than three decades to confirm the nerve involvement in histopathological specimens in leprosy (Fleuri & Bacchi 1987, Singh et al 1994, Khan 1998, Thomas et al 1999, Ismail 2007, Mohanti & Srinivas 2014, Tirumalee et al 2014). Four patterns of nerve damage are demonstrable on S-100 in leprosy, namely 1.) Infiltrated (nerve continuous however surrounded by inflammatory cells) 2.) Fragmented (discontinuous nerve fragments separated by dense inflammatory infiltrate) 3.) Absent (no nuclear or cytoplasmic staining for nerve fragments inside granuloma seen) 4.) Intact continuous and closely stained nerve fragments. (Gupta et al 2006). This approach of immunostaining for identifying patterns of nerve involvement will be of significance, especially in Tuberculoid and Indeterminate forms, where it may not be not possible to demonstrate *Mycobacterium leprae* bacilli using Fite-Faraco stain. It will be important to have fresh experience of use of this well known method in currently reported cases from different settings so that it may be adopted for application in currently reported cases from such cases. The present study was undertaken to study the clinical and histopathological findings in leprosy and to compare the different patterns of cutaneous nerve involvement (fragmented, intact, infiltrated and absent/not demonstrable) using both H&E as well as S-100 immunostaining so that this

procedure may be used for enhancing the histopathological diagnosis in leprosy in such difficult to diagnose cases.

Materials and Methods

The study was conducted in the Department of Pathology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, over a period of 12 months (July 2016-July 2017) after obtaining clearance from Institutional Ethics Committee. Subjects were recruited from patients presenting in Dermatology OPD. All 35 consecutive new cases with clinical suspicion/diagnosis of leprosy were included in the study. Ten cases of non lepromatous skin diseases like Tuberculosis, chromoblastomycosis, leishmaniasis and foreign body granulomatous disease were also studied. Paraffin block from non-lesional skin was taken as negative control while a previously diagnosed case of BT Hansens with perineural granuloma was taken as positive control for S-100 immunostaining.

All relevant clinical details related to history, physical examination and investigation was recorded. All biopsies were adequate i.e included dermis and part of subcutaneous fat. Sections were stained for Hematoxylin and Eosin (H&E) and Modified Ziehl Nelson or Fite-Faraco stain. S100 immunostaining was done on all the cases (a monoclonal mouse antibody manufactured by Biogenex). (Khan 1998, Thomas et al 1999, Ismail 2007, Tirumalae et al 2014).

Non-parametric test (χ^2 test) were used as the test of significance at $p<0.05$. All the statistical analysis was done in SPSS version 20.

Results

Of the 35 cases included in the study, males accounted for 24 cases with male : female ratio of 2.1:1. Ages ranged from 11 to 80 years with a mean age of 40 years. Out of 35 cases of leprosy, BT was most common type (n=23; 65.7%) followed by BL type (n=7, 20%). Most common sign was hypopigmented macule (n=21; 60%) followed by erythematous plaque (n=12, 34.28%)

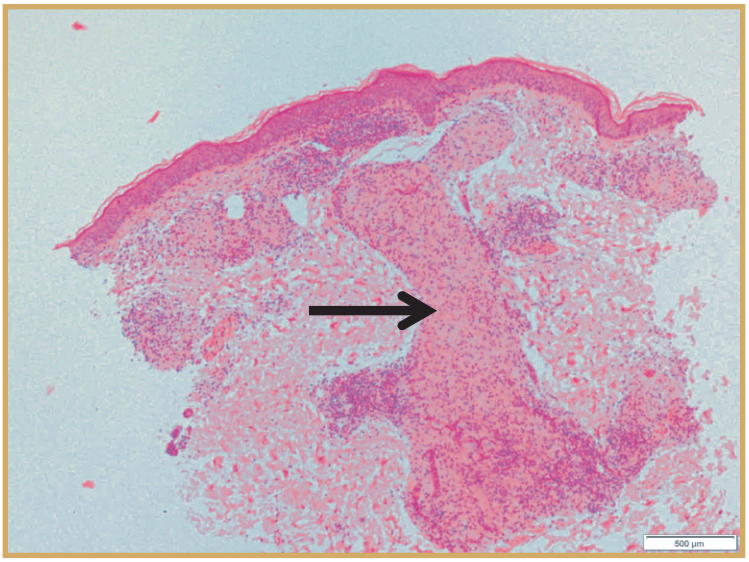

Fig. 1 : Vertically oriented nerve in BT Hansen's (H&E staining, 4x10X)

Table 1 : Nerve changes in spectrum of Leprosy on H & E on basis of location, orientation, morphology and nerve destruction

Nerve change	BT (n=23)	BB (n=1)	BL (n=7)	LL (n=3)	Inderminate (n=1)
Location					
Within granuloma	18	1	7	2	1
Non specific	5	0	0	1	0
Orientation					
Vertical	8	1	6	2	0
Non specific	15	0	1	1	1
Morphology					
Rounded	6	0	2	0	0
Sharp	7	0	2	1	0
Mixed	10	1	1	2	1
Nerve Destruction					
Yes	18	1	5	2	0
No	5	0	2	1	1

Table 2 : Comparison of nerve changes on H&E and S-100 staining (n=35)

	Nerve change on HE		Nerve Change on S-100	
	n=35	%	n=35	%
Infiltrated	14	40	15	42.85
Infiltrated & Fragmented	2	5.71	14	40
Infiltrated & Destroyed	1	2.85	3	8.57
Intact	7	20	1	2.85
Not seen	8	22.85	1	2.85
Perineural inflammation	3	8.57	1	2.85

Chi square = 20.98 p = 0.0008

and with loss of sensation (n=17; 48.57%). Fite-Faraco stain was was positive for AFB in 15 (42.85%) were positive.

Most common site of involvement was hand (17.1%) followed by face (14.2%) and trunk (11.4%). Atrophic epidermis was the most common epidermal changes seen in 15/23 cases of BT Hansen's and 4/7 cases of BL Hansen's disease. Well formed epitheloid granulomas were seen in 10/23 cases of BT, however, they were inconspicuous in 5/7 cases of BL Hansen's. Almost all granulomas were periadnexal with destruction of sweat glands, hair follicle and nerve bundles. (Fig. 1). Bacilli could be easily demonstrated in BL and LL Hansen's whereas no AFB could be demonstrated in 18/23 cases of BT Hansen's.

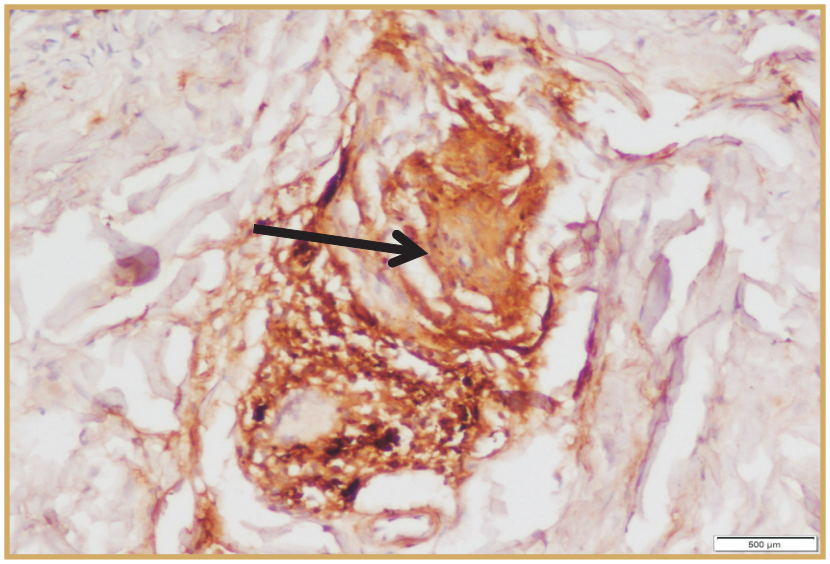

Fig. 2 : Nerve surrounded and partly destroyed by granulomas in BT Hansen's (S-100 staining, 4x40X)

Table 3 : Distribution of cases on basis of dendritic cells scoring on S-100 immunohistochemistry (n=35)

S100	BT (n=23)	BB (n=1)	BL (n=7)	LL (n=3)	Indeterminate (n=1)
3+	0	0	0	0	0
2+	10	1	3	2	0
1+	13	0	4	1	1
0	0	0	0	0	0

Nerves were entrapped and partly destroyed by the granuloma in 18/23 cases of BT Hansen whereas no nerve involvement was seen in the remaining 5 cases. (Table 1,2) Moderate Dendritic cells positivity was seen in 10/23 cases of BT Hansen's where as 13/23 cases of Bl showed weak dendritic cells positivity (Table 3). This showed there was reduction in number of Langerhans cells from Tuberculoid to lepromatous spectrum of leprosy.

On H&E, 14 cases of leprosy showed infiltrated nerve i.e surrounded and destroyed by granuloma, 8 cases show absent and 7 cases show intact nerve but in granulomatous disease 5 cases show intact nerve, 2 cases showed no demonstrable nerve whereas on S100, 15 cases of leprosy show infiltrated nerve by granuloma, the nerves were surrounded and destroyed by granuloma (Fig. 2)

Out of 10 cases of non-lepromatous granulomatous diseases, 6/10 cases show nerve changes in both H&E and S100 while 3/10 cases did not show any change on HE but demonstrable changes were seen on S100 staining. These granulomatous diseases were finally diagnosed as Tuberculosis (n=6), foreign body giant cell reaction (n=2), Chromoblastomycosis (n=1), Leishmaniasis (n=1). The nerves were entrapped within the granulomas however no nerve destruction was seen. This may indicate that the nerves were entrapped secondary to inflammatory reaction in all these cases.

Discussion

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, a slowly multiplying pathogen exhibiting varying severity. Granulomatous skin lesions often present as a diagnostic challenge to dermatopathologists due to various modes of presentation and identical histological picture produced by several other causes like Tuberculosis, Dermatomycoses, NTM disease due to atypical mycobacteria, Sarcoidosis, Leishmaniasis, foreign body etc. A definite diagnosis of leprosy is based on demonstration of either acid fast bacilli or nerve elements within the granulomas.

The present study included 35 patients ranging from 11-80 years. There was male predominance with male:female ratio of 2.1:1. ThiS was comparable with the findings of other studies (Thakkar & Patel 2014), (Moorthy et al 2001). In the present study BT was the most common clinico-histological type (n=23;65.7%) of leprosy which is similar to the findings of Khan (1998) and Shirazi et al (2015).

In our study the most common clinical sign was hypopigmented macule (n=21; 60%). This study was similar to a study by Suneetha et al (1998) followed by loss of sensation (n=17; 48.57%) and thickened or tender nerve (n=10; 28.57%). Similar observations were made by Kaur et al (1999). The most common site of involvement in the present study was hand and arms followed by 4 (11.42%) cases each of foot and trunk this was similar to the study by Kumar et al (2004) whereas Zafar et al (2008) in their study saw most common site of involvement in the head and neck region and in the upper extremity (22.43%). Histopathologically, atrophic epidermis was most common epidermal changes seen in 15/23 cases of BT Hansen in our study which is in concordance with the findings of Suneetha et al (1998).

In present study out of 35 cases, 15(42.85%) cases were positive for Fite stain and 20(57.14%) were negative. Well formed epitheloid granulomas were seen in 10 (4.3%) cases of BT however they were inconspicuous in 5 (71.4%) cases of BL Hansen. AFB could be easily visualised in BL and LL Hansen where as no AFB could be demonstrated in 18 (78.2%) cases of BT Hansen and 1 case of indeterminate leprosy. This study was in concordance with other studies by Shirazi et al (2015) and Abulafia & Vignale (1999) In present study nerves were entrapped within granuloma in 18/23 cases of BT Hansen and 29/35 of all cases of Hansen, in concordance with study reported by Singh et al (1994 Nerve bundles were much better highlighted by Ismail (2007) using S-100 immunostain as compared to H&E staining. Thomas et al (1999) compared granulomas of leprosy with those of non-leprosy cases and found intact nerves in all cases of non-Hansens granulomatous conditions.

In the present study, out of 35 cases, BT Hansens (8/23; 34.7%) showed infiltrated nerve on H&E and (n=6/23;26%) show intact nerve whereas in BL and LL Hansens the nerve was absent indicating that it was replaced by lepra cells but on S100 nerve was not seen in only 1 case of BT. In present study in control cases (granulomatous) moderate dendritic cell positivity was seen in

5/10 cases of control (granulomatous disease) while 3 cases show mild positivity. On H&E, 5/10 cases of non Hansens granulomatous diseases (control) showed intact nerve while in 2/10 cases nerves were not visualized where as only 1/10 case showed infiltrated and fragmented nerve, whereas there was reduction in number of Langerhans cells from Tuberculoid to lepromatous spectrum of leprosy. This was in discordance with the study done by Mohanraj et al (2014) who showed increased cytoplasmic and membranous staining of dendritic cells in the lepromatous spectrum.

Similar study was conducted by Tirumalae et al (2014) where they have highlighted the role of S-100 staining in demonstrating nerve elements in Hansens disease and distinguishing it from other granulomatous dermatoses. However, they highlighted the drawback of their study was false positive staining by Langerhans cell thereby mimicking nerve cells. In our study we considered these S-100 positive cells as nerve cells when they were present along the contour of nerves or in small clusters. Fleuri and Bacchi (1987) in their study found that eight out of nine cases of clinically suspected Tuberculoid leprosy cases showed cutaneous nerve alteration by S-100 immunostain although histopathology and bacteriological studies were inconclusive.

This indicates that S-100 serves as a useful immunomarker in leprosy, particularly in TT and BT type. It not only highlights the nerve staining patterns but also demonstrates the changes in dendritic cells. S-100 thus contributes significantly in providing an earlier diagnosis thus reducing morbidity and drug resistance and thereby achieving higher elimination rates.

Borderline Tuberculoid (BT) was the most common type of leprosy in our study. On S-100 immunostaining, 43.7% cases showed granulomas infiltrating the dermal nerves whereas these changes could not be demonstrated in 16.6% cases of Borderline leprosy on H&E staining alone. Thus we can conclude that S-100, by highlighting various nerve patterns serves as an important tool to differentiate borderline leprosy from other granulomatous diseases of skin. S-100 immunostaining is useful in leprosy diagnosis not only by nerve staining patterns, but it may also help in further characterizing the cell types across the spectrum of leprosy by highlighting the changes in dendritic cells. The significance of this study lies in the application of S-100 to demonstrate nerve damage along with studying the modifications in dermal dendritic cells for an earlier diagnosis Another important facet of this study is to arrive at an accurate diagnosis of Tuberculoid spectrum of leprosy, especially the borderline and indeterminate forms and to differentiate from other granulomatous diseases.

References

1. Abulafia J, Vignale RA (1999). Leprosy: pathogenesis updated. *Int J Dermatol*. **38**: 321-34.
2. Fleuri RN, Bacchi CE (1987). S-100 protein and immunoperoxidase technique as an aid in the histopathologic diagnosis of leprosy. *Int J Lepr*. **55**: 338-44.
3. Gupta SK, Nigam S, Mandal AK, Kumar V (2006). S-100 as a useful auxiliary diagnostic aid in tuberculoid leprosy. *J Cutan Pathol.* **33**: 482-6.
4. Indian Association of Leprologists (1982). Clinical histopathological and immunological features of the five type classification approved by the Indian association of leprologists. *Lepr India.* **54**: 22-32.
5. Ismail EA (2007). Comparison of leprosy and non-leprosy granulomas by using S-100 protein. *J Med Res Inst*. **28**: 181-4.
6. Kaur I, Das A, Sharma VK (1999). Macular lesions in leprosy: a clinical, bacteriological and histopathological study. *J Dermatol*. **26**: 569-76.
7. Khan AR (1998). S100 protein in the diagnosis of tuberculoid/borderline tuberculoid leprosy. *Annals Saudi Med*. **18**: 4-6.

8. Kumar B, Kaur I, Dogra S, Kumaran MS (2004). Pure neuritic leprosy in India: an appraisal. *Int J Lepr Other Mycobact Dis*. **72**: 284-90.
9. McMenamin D (2011). A region-by-region history with first person accounts. In: Leprosy and stigma in the South Pacific. Jefferson NC (Ed), : McFarland pp 20-23.
10. Mohanraj A, Srinivasan S (2014). Role of S-100 immunostaining in demonstration of nerve changes and quantification of dendritic cells in leprosy. *J Clin Diagn Res.* **8**: 38-40.
11. Moorthy BN, Kumar P, Chatura KR et al (2001). Histopathological correlation of skin biopsies in leprosy. *Indian J Dermatol Venereol Leprol.* **67**: 299-301.
12. National Leprosy Eradication Programme-Progress report for the year 2015-16 Central Leprosy Division.; Direct Gen Health Serv. Available from: http://www.nlep.nic.in/data (accessed on 2017 Oct 5).
13. Ridley DS, Jopling WH (1966). Classification of leprosy according to immunity: A five group system. *Int J Lepr Other Mycobact Dis.* **34**: 255-73.
14. Singh N, Arora VK, Ramam M et al (1994). An evaluation of S-100 stain in the histological diagnosis of Tuberculoid leprosy and other granulomatous dermatoses. *Int J Lepr Other Mycobact Dis*. **62**: 263-7.
15. Shirazi N, Jindal R, Ahmad S et al (2015). Clinico-pathological correlation of leprosy: A 4 years retrospective study from a tertiary referral center in north India. *Int J Med Res Health Sci.* **4**: 350-354.
16. Suneetha S, Arundhati S, Kurian Net al (1998). Histopathological studies in primary neuritic leprosy: Change in the apparently normal skin. *Lepr Rev* . **69**: 351-7.
17. Suzuki K, Akama T, Kawashima A et al (2012). Current status of leprosy: epidemiology, basic science and clinical perspectives. *J Dermatol.* **39**: 121-9.
18. Thakkar S, Patel SV (2014). Clinical profile of leprosy patient: A prospective study. *Indian J Dermatol*. **59**: 158-62.
19. Thomas MM, Jacob M, Chandi SM et al (1999). Role of S-100 staining in differentiating leprosy from other granulomatous diseases of the skin. *Int J Lepr Other Mycobact Dis*. **67**: 1-5.
20. Tirumalae R, Stany AI, Shanubhogue S, Yeliur IK (2014). S-100 immunostaining in the distinction of Borderline Tuberculoid leprosy from other cutaneous granulomas. *Indian J Dermatol*. **59**: 421-.
21. Zafar MNU, Sadiq S, Menon MA (2008). Morphological study of different granulomatous lesions of the skin. *J Pak Assoc Dermatol*. **18**: 21-8.

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