

Role of S-100 stain for demonstration of different patterns of nerve changes in spectrum of leprosy – A prospective study



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ABSTRACT

Background: A confirmatory diagnosis of tuberculoid (TL) and borderline TL (BTL) leprosy is dependent on the demonstration of nerve involvement within granuloma or acid-fast bacilli. It is very difficult to identify nerve elements in hematoxylin and eosin (H&E) stains. S-100 stain in this study is used to highlight nerve in TL leprosy so as to reach a definitive diagnosis of TL leprosy. **Aim and Objectives:** The aim of the study was to demonstrate nerve involvement and various changes of nerves in different forms of leprosy and use of S-100/or confirmation of TL leprosy/BTL leprosy. **Materials and Methods:** Skin biopsies of a total of 53 cases of leprosy were studied. The study is conducted in the department of pathology, HIMS, Safedabad, Barabanki U.P. The present study included all the new leprosy patients visiting the outpatient department of Skin and V.D, department, HIMS, Safedabad, Barabanki, U.P. Study period was 2015–2016 (1 year). All biopsies were studied in H&E stain. Biopsies were further subjected to S-100 stain so as to make a confirmatory diagnosis of TL leprosy/BTL and were then classified according to Ridley-Jopling classification only histological parameters of classification were used in this study. **Results:** In the present study, it was observed that the majority of patients were between the ages of 31 and 40 years (39.5%), males were mostly affected (10.5%), and family history was positive (8.4%). Hypopigmented skin lesion was the most common clinical lesion (31.2%). TT was the most common clinical diagnosis (25.4%). IL was the most common histopathological diagnosis (26.1%). The nerve changes were demonstrated in TL/BTL leprosy using S-100 immunostain. It was observed that the most of the cases of TL/BTL demonstrated fragments of nerves that were infiltrated by lymphocytes. This was the most common pattern seen among the patients of TL/BTL leprosy. Complete nerve was not identified in any of the cases. **Conclusions:** S-100 immunostain simplified detection of nerve twigs in TL/BTL leprosy. It was also seen that the most of the fragmented nerve elements were present within the granulomas. The TL spectrum of leprosy significantly affected nerves and using S-100 helped in differentiating it from other forms of leprosy.

Key words: Borderline tuberculoid leprosy; Granuloma; S-100; Tuberculoid leprosy

INTRODUCTION

Leprosy or Hansen's disease is a chronic inflammatory disease that affects the nerves. Diagnosis of leprosy is based on clinical signs and there is a correlation with the histopathological investigation.¹ Nerve changes in

leprosy are caused due to damage caused by the acid-fast lepra bacilli known as *Mycobacterium leprae*. This disease affects nerves and in some cases also affects other organs. National leprosy program 2014–2015 report shows a sharp decline in the prevalence rate of Hansen disease in India.²

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In the tuberculoid (TL) spectrum of leprosy, detection of *Lepra* bacilli is very low.³ Histopathological examination of biopsy from affected skin is significant in confirmation of leprosy, nerves are affected in TL leprosy as bacteria enter nerves through blood vessels and target Schwann cells.⁴

In patients with leprosy, there is an abnormality of nerve conduction, due to demyelination of nerves. On routine hematoxylin and eosin (H&E) stain, it is difficult to demonstrate nerve within granulomas, but using S-100 immunostain, a marker of Schwann cell made it easy to demonstrate nerve twigs and damaged nerves in TL leprosy.⁵

Many studies have shown that the S-100 stain can be used as an adjunct marker for the confirmatory diagnosis of leprosy.

Aims and objectives

1. To demonstrate nerve involvement and various changes of nerves in different forms of leprosy.
2. Use of S-100/or confirmation of tuberculoid leprosy/ borderline tuberculoid leprosy.

MATERIALS AND METHODS

The study is a hospital-based cross-sectional study conducted in the Department of Pathology, HIMS, Safedabad. The present study included all the new leprosy patients visiting the outpatient department of Skin and V.D, department, HIMS, Safedabad, Barabanki U.P. India. The study period was 2015–2016 over a period of 1 year. Skin biopsies of all patients diagnosed as TL/borderline TL (BTL)/lepomatous/borderline lepomatous/indeterminate leprosy are included in the study. Immunological parameters in the Ridley-Jopling classification were excluded from the study. Only histological parameters of classification were used for the study. IEC/IRB No: HIMS/IRB/2015/752.

Cases with TL/BTL leprosy/lepomatous/borderline lepomatous/indeterminate leprosy were included in the study. Patients who had skin lesions such as hypopigmented patches, anesthetic patches, and pure nerve thickening were included in the study. All partially treated cases and immunological parameters of Ridley-Jopling classification were excluded from the study.

Skin punch biopsies of representative skin lesions were received and these were subjected to H&E stain for histopathological examination. Fite - Faraco stain was used to demonstrate *M. leprae* which was an acid-fast bacilli.

In addition, those cases which were diagnosed as TL/BTL/lepomatous/borderline lepomatous/indeterminate leprosy on histopathological examination were subjected to S-100 immunostain (Dako) to assess the pattern of nerve involvement and damage caused to the nerve due acid-fast bacilli. The clinical diagnoses, histopathological features, and S-100 staining patterns were correlated. Hematoxylin-Eosin slides and S- 100 stained slides were examined independently so as to study, the nerve involvement sensitivity of both the stains was calculated. Data were calculated using SPSS software.

RESULTS

The study was conducted with the objective to study the spectrum of leprosy with histopathological confirmation using S-100 immunostain. This study included 53 cases. The 53 cases studied showed various forms of leprosy 14 (25.4%) cases showed TL leprosy and 9 (17%) showed BTL leprosy.

Table 1 showed that TL leprosy was the most common clinical diagnosis seen in patients; out of 53 cases, 14 (26.4%) were diagnosed as TL leprosy and borderline leprosy was diagnosed in 9 (17.0%) cases.

Table 2 shows the distribution of patients according to histopathological diagnosis of leprosy. IL was the most common histopathological diagnosis 14 (26.1 %), TT-4 (7.5%), LL-11 (20.8%), BB-7 (13.2%), and BL-5(9.4%).

Table 1: Distribution of patients according to clinical diagnosis of leprosy

Clinical diagnosis	Number (n=53)	Percentage
TT	14	25.4
BT	9	17.0
BB	6	11.3
BL	5	9.4
IL	7	13.2
LL	12	22.6

TT: Tuberculoid leprosy, BT: Borderline tuberculoid leprosy, BB: Borderline borderline, BL: Borderline lepomatous, LL: Lepomatous leprosy

Table 2: Distribution of patients according to histopathological diagnosis of leprosy

Histopathological diagnosis	Number (n=53)	Percentage
TT	4	7.5
BT	12	22.6
BB	7	13.2
BL	5	9.4
IL	14	26.1
LL	11	20.8

TT: Tuberculoid leprosy, BT: Borderline Tuberculoid Leprosy, BB: Borderline Borderline, BL: Borderline Lepomatous LL: Lepomatous Leprosy

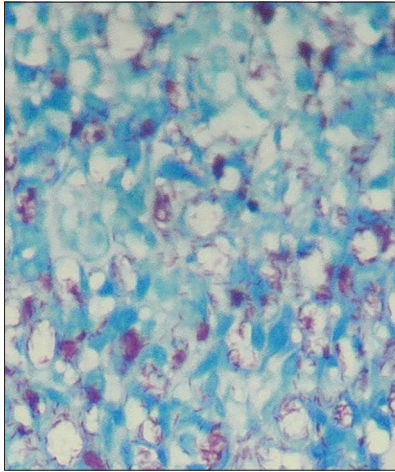


Figure 1: Photomicrograph showing acid-fast bacilli in Fite-Faraco staining (Fite-Faraco x1000)

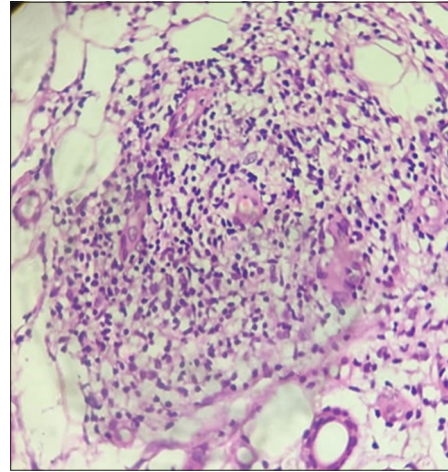


Figure 3: Tuberculoid leprosy; Photomicrograph showing well-defined epithelioid cell granuloma (H&E x40) and Langhans giant cell (H&E x40)

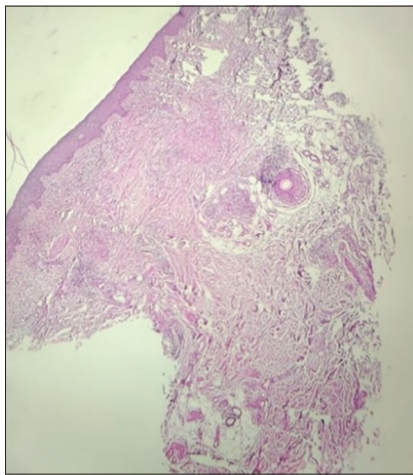


Figure 2: Photomicrograph showing thinned out epidermis (H&E)

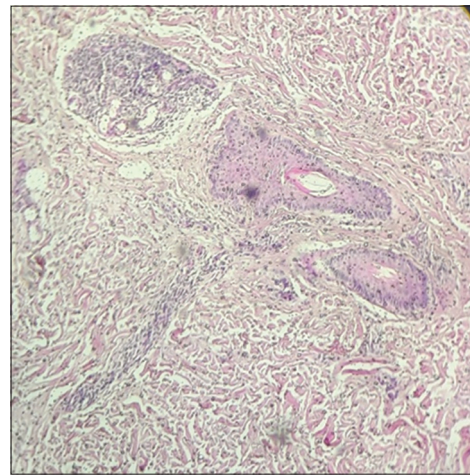


Figure 4: Photomicrograph showing perineural lymphocytic infiltration

DISCUSSION

Leprosy is an infectious disease caused by *M. leprae*, it affects skin and nerves. This disease is common in tropical and subtropical countries. The most affected countries are India, Africa, and parts of South-east Asia.^{6,7}

Diagnosis of leprosy is made on clinical presentation and histopathological findings. Mostly H&E stain is used for the diagnosis of leprosy. Bacterial index (BI) is calculated using Fite-Faraco stain (Figure 1). S-100 immunostain was used to identify nerve damage, as routine, H&E stain was not very useful for identification of nerve involvement in TL leprosy and indeterminate leprosy. Thus, the definitive diagnosis of nerve damage was made on the basis of findings in the S-100 stain which served as an adjuvant to the histopathological examination in H&E.⁸

The demographic profile showed that most patients were between the age group of <20 and 40 years. Most of the

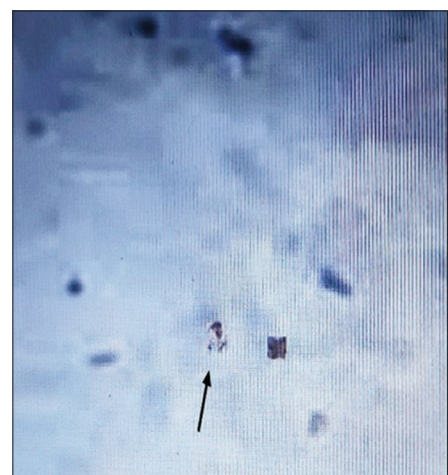


Figure 5: Photomicrograph demonstrating fragmented nerve involvement (S-100 IHC x400) (Shown with a black arrow)

patients who presented with symptoms were males between the age group of 20 and 41 years. Male patients who suffered

Table 3: Comparison of H and E staining and S-100 immunostain in the identification of nerves

Types of leprosy	Number of cases	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 and E (%)	Sensitivity of s-100 (%)
LL	11	4	7	-	-	61.4	100
BL	5	1	4	-	-	54.2	100
BT	12	10	2	-	-	82.2	100
TL	4	2	1	-	1	76.1	100
IL	14	8	6	-	-	67.4	100

Table 4: Patterns of involvement of nerve in various forms of leprosy

Leprosy	Infiltrated (%)	Fragmented (%)	Intact	Absent	Total
LL	5 (45.2)	6 (54.8)	-	-	11
BL	4 (75.4)	1 (24.6)	-	-	05
BT	5 (45.8)	7 (54.2)	-	-	12
TL	3 (65.0)	-	-	1 (35.0)	04
Indeterminate leprosy	9 (59.5)	5 (40.5)	-	-	14

from leprosy presented with clinical presentation of TL leprosy, the male to female ratio of the presentation was 0.8:1.^{9,10}

In our study, the most common clinical presentation was hypopigmented patches followed by loss of sensation and erythematous patches (Figure 2). Tiwari et al.,¹¹ Khamankar et al.,¹² Giridhar et al.,¹³ Tirumalae et al.,¹⁴ reported that hypopigmented patches were the most common presentation in TL leprosy (Figure 3).¹¹⁻¹⁴

It was seen that BI 3 or more had significant histopathological findings (Figure 4). In TL leprosy, bacilli could not be detected, diagnosis in such cases was made on the basis of histopathological findings that as the presence of epithelioid cell granuloma, giant cells, and periappendageal lymphocytes. Nerve elements within the granuloma could not be detected in routine H&E stains (Table 3).¹⁵

S-100 is immunostain (Figure 5) and is a marker of Schwann cell, myoepithelial cell, and chondrocytes. Many studies have suggested that S-100 can be used effectively as an adjuvant in finding out different patterns of nerve involvement in leprosy. Positive staining of nerve twigs or fragments suggests nerve damage in leprosy. Gupta et al.,¹⁶ demonstrated nerve damage using S-100 immunostain, there were four patterns of nerve damage reported by them that are infiltrated, fragmented, absent, and intact.^{15,16}

In the study, neural involvement was detected in 11 cases. S-100 stain was applied and 25 cases demonstrated nerve fragments. In one case of TL leprosy, nerve fragments could not be identified but on basis of clinical presentation and histopathological findings which demonstrated numerous granulomas, they were labeled as TL leprosy. Fleuri and Bacchi,¹⁷ in their study also stated that nine cases of TL leprosy diagnosis were made clinically as the histopathological diagnosis could not be made the H&E stain did not show

any nerve damage but with the use of immunostain S-100 their cutaneous nerve showed few changes.¹⁷

In the present study, use of S-100 proved to be a useful tool for the diagnosis of TL leprosy in comparison to H&E stain. The study restates the advantage of S-100 over H&E stain.

In the present study, nerve fragmentation was the most dominant pattern observed in all forms of leprosy in cases of TL leprosy; few cases showed infiltrated patterns and absence of nerve involvement (Table 4). Thomas et al.,³ in their study also observed fragmented patterns as the most common pattern and the absence of nerve damage as the other most frequent pattern. Singh et al.,¹ in their study noticed the complete absence of nerve as a most reliable pattern for diagnosis of TL leprosy. Tirumalae et al.,¹⁴ in their study demonstrated intact and normal nerves. In the study, skin biopsy of nine controls showed that intact nerves a few of them showed infiltrated patterns and fragmentation using S-100 immunostain. Tirumalae et al.,¹⁴ encountered some difficulties in their study as Langerhans cells were stained with S-100 and were often confused with nerves within granulomas.¹⁶

Limitations of the study

The sample size was small. Another study with a large sample size and longer duration may further help in establishing the efficacy of S-100 as an adjunct marker in leprosy.

CONCLUSIONS

S-100 immunostain proved to be convenient supplementary aid in the diagnosis of the TL spectrum of leprosy. S-100 along with a histopathological diagnosis of TL leprosy

proved to be a useful tool in the diagnosis of leprosy. Along with the diagnosis of leprosy, S-100 helped in studying different patterns of nerve involvement in leprosy.

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DG- Concept and design of the study, literature search, data acquisition, interpreted the result, reviewing of the literature, manuscript preparation, and prepared the first draft of study and JI, PJ, SS, RD, and SS- Concept, literature search, data analysis, manuscript editing, and manuscript review.

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