A Comparative Study- The Role of Skin and Nerve Biopsy in Hansen's Disease

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Abstract:

Aim and objectives:

To study the role of skin and nerve biopsy in hansen's disease in following aspects;

Diagnosis of leprosy, Detection of multi and paucibacillary leprosy,Classification of leprosy,Role in treated cases, Correlation between skin and nerve biopsy

Background:

Hansen's disease (Leprosy) is a community disease, caused by Mycobacterium Leprae. The diagnosis of leprosy is made by demonstration of the acid fastness of the lepra bacilli in biopsies of lesions.

Materials and methods:

22 Concurrent skin and nerve biopsy samples, 29 skin biopsy samples and 1 nerve biopsy sample were received by the Department of Pathology, Kottayam Medical College, Kerala, were fixed in formalin processed automatically, sectioned, stained by routine Hematoxyllin and Eosin method and specially stained by Fite Farracco stain for Lepra Bacilli. Light microscopically diagnosed as per the criteria of classification of Leprosy. Bacteriological Index also done for each biopsies.

Results:

Chi square test was applied which showed an association between the histological features of skin and nerve tissue sections (P<0.001). This confirms that there is no significant difference between histological features of skin and nerve lesions in Hansen's disease.

Keywords: Hansen's Disease, Leprosy, Skin Biopsy, Nerve Biopsy

INTRODUCTION:

LEPROSY, also known as HANSEN'S disease is an ancient disease, which has lost its significance recently due to better medications which are available. However, this skin deforming disease is still a serious health threat in many under developed countries because of poverty and the inability of the masses to afford effective anti-leprosy medication.[1] The cause for leprosy is Mycobacterium Leprae, an acid and alcohol fast bacillus similar to the one that causes tuberculosis. The pathological changes of leprosy are strikingly different from other infections because of the unique features of Mycobacterium Leprae. This is the only bacteria that prefer an immunologically protected environment i.e the most coolest part of the body(skin and peripheral nerves).

Diagnosis of leprosy is based on different clinical parameters which involves detailed examination of skin lesions and peripheral nerves. A reliable diagnosis hinges around a good histopathological diagnosis and demonstration of bacilli in histopathological sections. Modified Fite's procedure has proved most valuable in demonstrating lepra bacilli in tissue sections. Histopathology provides confirmatory information for suspect cases which can be missed in clinical practice or epidemiological studies and helps in exact typing. Histology also gives indication of progression and regression of disease under treatment.

Ridley and Jopling were the first to suggest a subdivision of leprosy on an immunological basis into five types[7]; Tuberculoid (TT), Borderline Tuberculoid (BT), Midborderline (BB), Borderline Lepromatous (BL) & Lepromatous (LL).

This study will also compare the results of skin and nerve biopsies and assess the role of both in classification of disease, so that we can have an accurate diagnosis and can reduce the drug resistance and relapse cases.

Keywords: Hansen's disease, skin biosy, nerve biopsy, leprosy.

MATERIALS AND METHODS:

The samples were selected randomly and they belonged to different clinical spectrum of leprosy. 22 Concurrent skin and nerve biopsy samples, 29 skin biopsy samples and 1 nerve biopsy sample were received by the Department of Pathology, Kottayam Medical College, Kerala. Clinical details of the patients from whom the biopsies were taken were collected from the TRF(test requisition form).The specimens were fixed in 10% formalin, were either hand processed or processed in histokinette along with other routine specimens. Paraffin blocks were made and sections of 4 – 5 microns thickness were cut. The slides were stained by routine H & E stain and Fite -Faraco (AFB)[10] stain for lepra bacilli. Histopathological criteria[62] used for classification of leprosy.

OBSERVATION AND RESULTS:

The period of study extended for 1 year from November 2009 to October 2010. Total numbers of biopsies received were 52, which comprised of 29 skin biopsies, 22 concurrent skin and nerve biopsies and one nerve biopsy without skin biopsy. The age of the patient ranges from 4 to 77 years with mean age being 40.15 years. Out of the 52 patients 33 were males (63.5%) and the rest (36.5%) were females. The following table and chart explains the age group and gender of the patients. (Table 1)
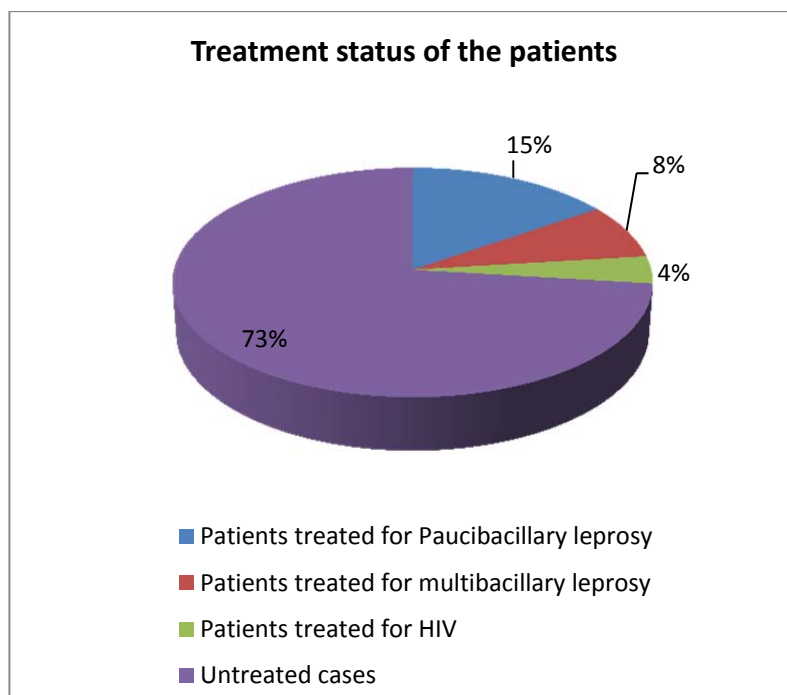
Treatment status of the patients

15%
8%
4%
73%

- Patients treated for Paucibacillary leprosy
- Patients treated for multibacillary leprosy
- Patients treated for HIV
- Untreated cases

Chart 2 : Treatment Status of the patients examined

Table 1: Age and gender distribution of patients examined

Age Range (Yrs)	Male patients	Female patients	Total number of patients	Percentage
1 - 10	1	0	1	2
11 - 20	1	3	4	8
21 - 30	10	1	11	21
31 - 40	5	6	11	21
41 - 50	10	3	13	25
51 - 60	3	5	8	15
61 - 70	2	0	2	4
71 - 80	1	1	2	4
Total number of patients			52	100

Table 2: Relation between histopathological and clinical spectrum of Skin Lesions

Clinical information	TT	BT	BB	BL	LL	Indeterminate
Hypopigmented Lesions	1	17	3	4	3	5
Erythematous Lesions	2	5	-	3	3	-
Nodular Lesions	3	1	-	-	1	-

Table3: Nerves selected for biopsy

Nerve	Number of Patients
Radial cutaneous nerve	14
Ulnar cutaneous nerve	6
Superficial peroneal nerve	3

Table 4: Histopathological Spectrum of Skin Lesions

Histopathological spectrum	HD- IND	TT	BT	BB	BL	LL	NON SPECIFIC
Number of cases	5	6	22	3	7	7	1
Percentage	9%	12%	43%	6%	14%	14%	2%

Table 5: Epidermal Changes in the Skin Biopsies in the study

Epidermal Changes	TT	BT	BB	BL	LL	Indeterminate	Total	Percentage
Atrophy	3	12	1	6	5	1	28	56
Focal Atrophy	2	1	-	-	1	-	4	8
Mild Acanthosis	-	1	-	-	-	1	2	5
Follicular Plugging	-	-	-	-	-	1	1	2
unremarkable	1	8	2	1	-	3	15	29

Table 6: Histopathological Spectrum of Nerve Lesions

Histopathological Spectrum	HD - IND	TT	BT	BB	BL	LL	NON SPECIFIC
Number of cases	0	4	7	0	5	6	1
Percentage	0	17%	30%	0	22%	27%	4%

Table 7: Relation between Paucibacillary and Multibacillary cases in skin and nerve biopsies studied

Skin	PB	PB	MB	Total
Nerve	PB	MB	MB	
No. of Cases	12	1	9	22

NERVE LESIONS	SKIN LESIONS						Total
	TT	BT	BB	BL	LL	Non Specific	
TT	4	-	-	-	-	-	4
BT	-	6	1	-	-	-	7
BB	-	-	-	-	-	-	0
BL	-	-	-	5	-	-	5
LL	-	-	-	2	4	-	6
Non Specific	-	-	-	-	-	-	0
Total	4	6	1	7	4	0	22

Table 8: Correlation between histopathological spectrum of skin and nerve biopsies studied

Of the 52 patients studied, 38 patients were untreated, 8 patients completed treatment for paucibacillary leprosy, 2 patients were incompletely treated with multibacillary leprosy treatment, 2 patients completed their treatment for multibacillary leprosy and 2 patients were treated with Anti-Retroviral drugs. (Chart-2).

Out of the 51 patients from whom the skin biopsies were taken, 36 patients had hypopigmented skin lesions, 11 patients had erythematous skin lesions and 4 had nodular lesions (Table-2).

All the 23 nerves biopsied were thickened nerves. Only purely sensory nerves were biopsied. Motor nerves or major nerve trunks were never biopsied. The details of nerves selected and the number of each biopsied are given below: (Table 3).

A total of 51 skin biopsies were studied and the classification of leprosy was done as per Ridley and Jopling[7] recommendations.

In 5 cases a diagnosis of Indeterminate leprosy was made when there was selective lymphocytic infiltration around the dermal nerve with or without involvement of other dermal appendages . In none of these cases AFB could be demonstrated inspite of examining serial sections (Table-4)

In 6 cases the diagnosis of TT spectrum of leprosy was made when the granulomas were compact and composed of epithelioid cells surrounded by a dense cuff of lymphocytes (Fig-1) with or without Langhan's giant cells (Fig-2) or a large nerve bundle and erosion of epidermis. No AFB could be demonstrated in such cases . (Table-4).

The diagnosis of BT spectrum of leprosy was made in 22 cases when the granuloma was less compact and epithelioid cells were admixed with lymphocytes. The cuffing of granuloma by lymphocytes was less pronounced (Fig-3). 1 case of BT was in Type 1 reaction which was diagnosed by edema in granuloma and dilated lymphatic channels.

BB spectrum of leprosy was diagnosed when the granulomas were composed of sheets of epithelioid cells

with few lymphocytes. This type of picture was seen in 3 patients (Table-4).

BL leprosy was diagnosed in 7 cases when granuloma was composed of macrophages admixed with a considerable number of lymphocytes . Out of this Bacterial Index (BI) of 4 cases were 1-4+ . 2 cases didn't show AFB even in serial sections (Table-4)

In 3 cases a diagnosis of LL spectrum was made when there were collections of foamy macrophages, which were seen infiltrating the dermal appendages(Fig-4). The grenz zone was present in all cases. Bacterial Index was 5-6+ (Fig - 5) (Table-4).

A total of 22 nerve biopsies were studied and classification of leprosy was done as per Ridley's classification [42]and Chandi SM et al[58].

A diagnosis of TT leprosy neuritis was diagnosed in 4 patients when a compact epithelioid granuloma was present in nerve with a dense cuff of lymphocytes with or without Langhan's giant cells(Fig -6). No AFB could be demonstrated in such cases (Table-6).

BT neuritis was diagnosed in 7 cases when the granuloma was less compact and admixed with lymphocytes. The lymphocytic cuffing was less pronounced . One of the BT neuritis case had completed the treatment for paucibacillary leprosy, which was diagnosed by skin biopsy (Table-6)

In 5 cases BL leprosy neuritis was diagnosed, when the nerve parenchyma was infiltrated by macrophages and lymphocytes; Bacterial index in 2 of those cases were 3+ (Table-6).

Diagnosis of LL leprosy neuritis was made in 6 cases when there were collection of foamy macrophages with AFB staining showing clumps of AFB bacilli. Bacterial Index in such cases were 3-4+ (Fig-7). In all cases there was disruption of perineurim with spilling of inflammatory cells into the adjacent fat (Table-6).

In this study, out of the 51 cases of the skin biopsies diagnosed as Leprosy 38 cases were paucibacillary (PB) i.e., BI=0 and 13 cases were Multibacillary (MB) i.e., BI ≥1+

Out of 22 cases of the nerve biopsies diagnosed as Leprosy, 14 cases were PB and 8 cases were MB (Table-7).

In our study 10 cases were treated completely and two patients had a history of incomplete treatment. Out of 10 completely treated cases, 2 of them were in LL spectrum, in which the skin biopsy showed evidence of treatment response, i.e., 1 case got upgraded to BT spectrum and other got upgraded to Indeterminate Leprosy. 6 of them were in BT spectrum, in which one showed complete treatment response, and the remaining 4 had no improvement. Out of 2 cases treated for TT one showed treatment response with Type1 reaction and other had no response to treatment.

Out of the 2 incompletely treated cases, 1 case of BL got upgraded to BT Leprosy and other case which had treatment for LL didn't show any evidence of improvement.

In the present study 2 cases which were HIV positive who are undergoing Anti Retroviral drug treatment where diagnosed as BT and TT respectively.

Concurrent skin and nerve biopsies were taken from 22 patients, clinically suspected to be having leprosy.(Table-8). Out of 22 cases Correlation between skin and nerve biopsies was obtained in 19 cases and disparity obtained in 3 cases. (Chi square test was done, P value of < 0.001).
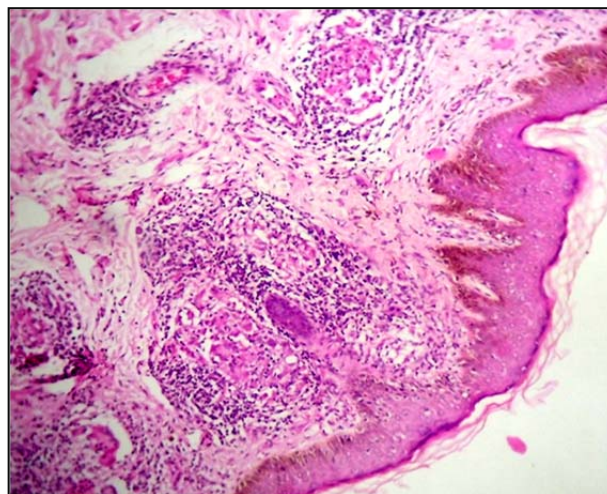

FIG -1 TUBERCULOID LEPROSY OF THE SKIN-SHOWING WELL DEFINED GRANULOMA
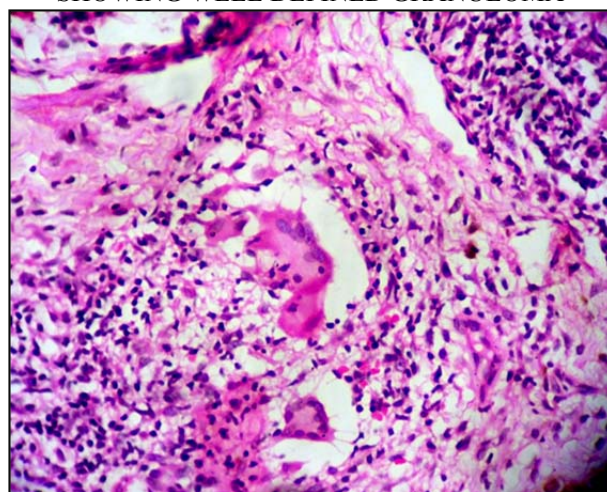

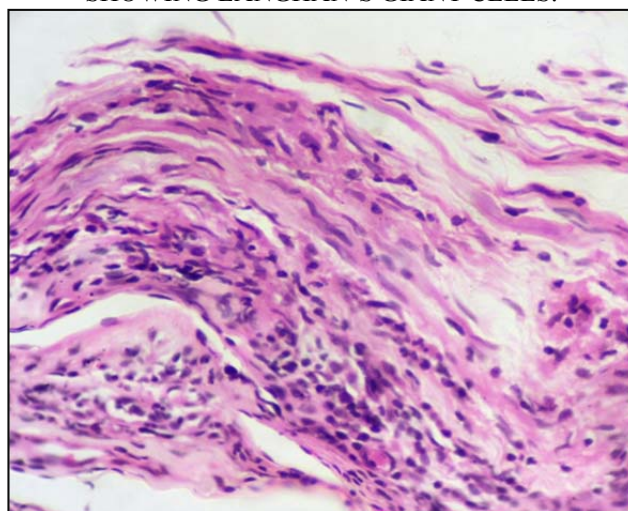FIG 2 TUBERCULOID LEPROSY- GRANULOMA SHOWING LANGHAN'S GIANT CELLS.

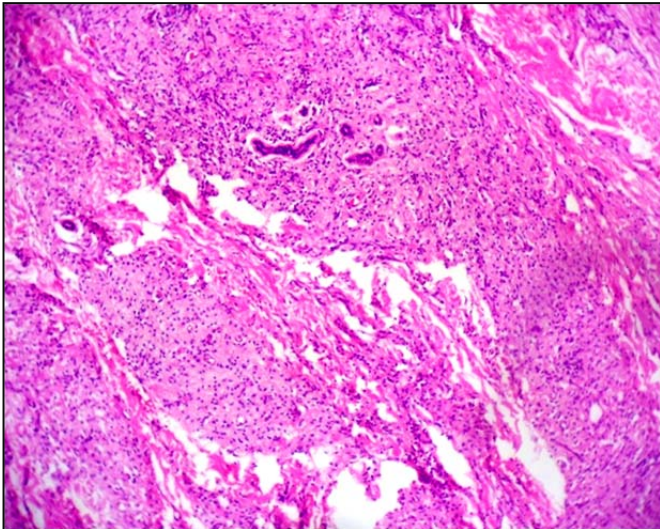FIG 3 BORDERLINE TUBERCULOID- NERVE FIBRES WITH DENSE LYMPHOCYTES

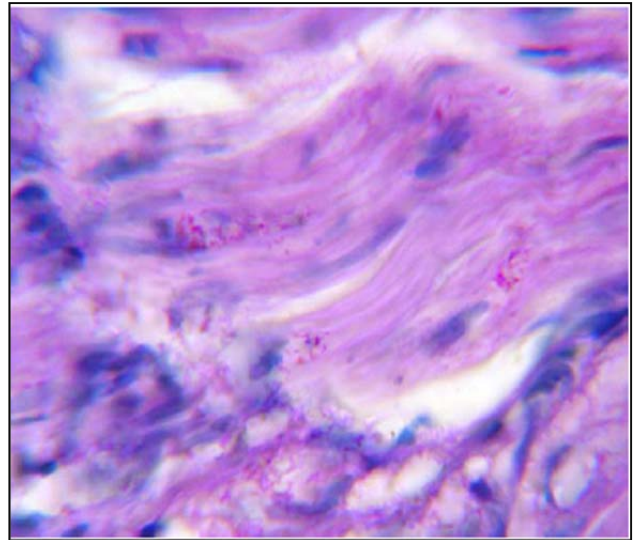FIG 4 LEPROMATOUS LEPROSY – LOBULES OF FOAM CELLS

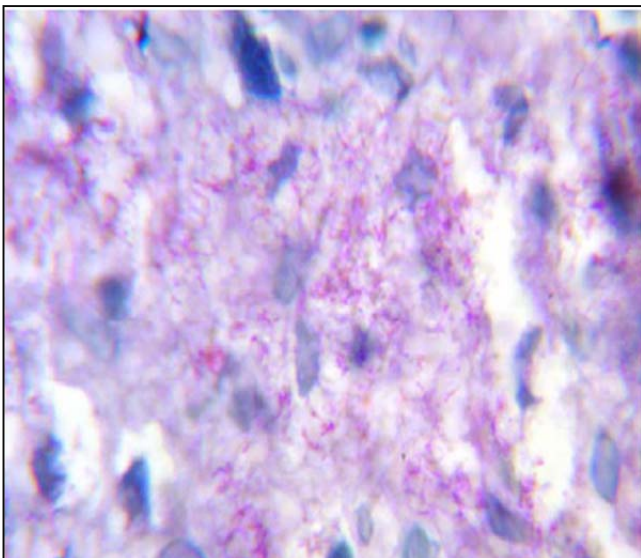

FIG 7 POSITIVE LEPRA BACILLI IN LL NERVE LESION.
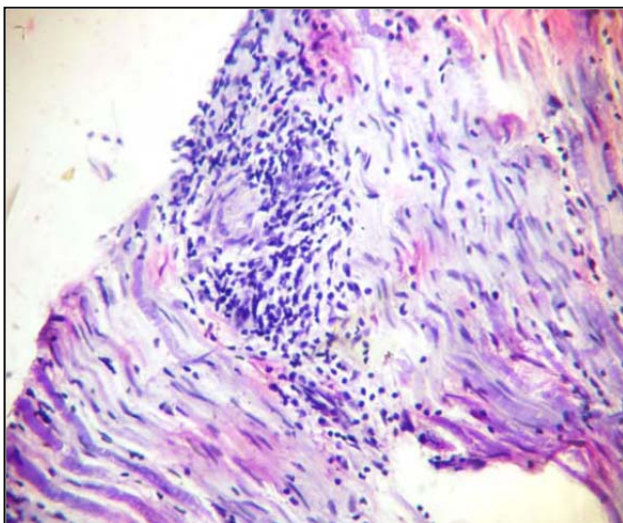

FIG 5 POSITIVE LEPRA BACILLI IN LL SKIN LESION

FIG 6 TUBERCULOID LEPROSY OF NERVE-GRANULOMA WITH LANGHAN'S GIANT CELL

DISCUSSION:

The present study is comparable to studies by Khan et al [51] where the total number of cases were 52 and concurrent skin and nerve biopsies taken in 22 patients which is comparable to the studies by Mukherjee et al[43] and Ashok Kumar et al[50].

The age distribution of the present study is comparable with that of the studies by Ridley & Jopling. .In the current study males (63.5%) are more commonly affected than female, this distribution correlating with other studies. Most of the skin lesions are hypopigmented (71%) comparable with the studies by Mukherjee et al[43] and Ashok Kumar et al[50].In the studies by Srinivasan et al[40] and Mukherjee et al[43] only thickened nerves were biopsied. The current study also followed the same method. The present study agrees with the claims of Job and Chacko [10] regarding their modifications of the Fite Faraco staining.

In the 51 skin biopsies studied, in 32 cases the epidermis was observed to have atrophy, which is 64 % of the total biopsies studied. Most of the cases (12 of 13, i.e. 92%) in Lepromatous spectrum show this change, which correlates with Ridley and Jopling study[7].

In 6 cases diagnosed as Lepromatous Leprosy, all showed narrow Grenz zone of normal collagen under the epidermis. Skin biopsies with compact and multiple granulomas along with neurovascular bundles and peripheral lymphocytic cuffing were diagnosed as tuberculoid spectrum, but the Langhans giant cells were found only in 50% of the cases diagnosed as Tuberculoid Leprosy (TT). There was one case diagnosed as Borderline Tuberculoid (BT) which had findings of Langhans giant cells, well formed granuloma and a few foamy macrophages as per Job and Britton et al[25]. This is not in correlation with Ridley and Jopling study[11].In the present study it is noticed that involvement of appendages i.e., dermal nerve twigs, hair follicles and erector pilli muscles were present in 88% of the cases studied. This is comparable with the studies by Ridley and Jopling[11].As per Jobs and Britton et al[16]. The degenerated bacilli accumulate in macrophages – the so called Lepra Cells or Virchow cells – which then have foamy or

vacuolated cytoplasm resembling xanthoma cells; these are described in Lepromatous spectrum of Leprosy (LL & BL). In the current study all of the LL cases and 2 of BL cases showed foam cells which are in line with Jobs and Britton et al [25]In our study acid fast bacilli stain showed highest Bacterial Index (BI) 4-5+ for Lepromatous Leprosy (LL) and 5 of 7 cases for Borderline Lepromatous (BL) showed 4+ BI. This is in correlation with the past studies.

In our study, disruption of Schwann cells was present in 90% of the cases, except for two cases in Borderline Tuberculoid (BT) spectrum. Well formed granuloma with lymphocytic cuffing were found in 6 cases (50%) of the Tuberculoid spectrum (BT&TT), which is in correlation with the studies by Ridley DS [42], Mukherjee et al [43].Lymphocytic infiltration in the perineurium & endoneurium were noticed in 31% of the cases out of which multiple aggregates of lymphocytes were noted in all cases of Tuberculoid Leprosy (TT) and Borderline Tuberculoid (BT). Moderate Lymphocytic infiltration was noticed in Borderline Lepromatous leprosy, which is in correlation with Srinivasan et al [40] and Ashok Kumar et al [50]. In the current study foam cells are noticed in all cases of Lepromatous Leprosy (LL) and Borderline Leprosy (BL). This is comparable with that of Mukherjee et al[43].In our study acid fast bacilli stain showed highest Bacterial Index (BI) 3-4+ for Lepromatous Leprosy (LL) and all cases for Borderline Lepromatous (BL) showed 3+ BI. As per Ridley DS[42] and Mukherjee Study[43] the highest BI for LL is 5-6+, from which our results show a slight variation.

As per present WHO classification, for treatment purpose leprosy is classified as paucibacillary (PB) when BI=0 and Multibacillary (MB) when BI≥1+. Nilsen et al [44] observed that in their study of 44 cases of leprosy, skin and nerve were paucibacillary in 33 cases; skin and nerve were multibacillary in 3 cases. In 8 cases skin was paucibacillary with a multi bacillary nerve. Ashok Kumar et al[50] studied 27 cases and observed that in 16 cases skin and nerve was PB; in 7 cases skin and nerve were MB. In 4 cases skin was PB and nerve was MB. (Table-9)

In the present study out of 22 cases, it was observed that in 12 cases the skin and nerve were PB, in 9 cases the skin and nerve were MB, in only 1 case the skin was PB and nerve was MB (Table-9).

Table 9: Comparison of value of skin and nerve biopsies in PB and MB cases in various studies

Author	Skin PB & Nerve PB	Skin PB & Nerve MB	Skin MB & Nerve MB	Skin MB & Nerve PB	Total Cases
Nilsen et al	33	8	3	0	44
Ashok Kumar et al	16	4	7	0	27
Present study	12	1	9	0	22

Table 10: Histopathological correlation between skin and nerve biopsies studied.

NERVE LESIONS	SKIN LESIONS						Total Nerve
	TT	BT	BB	BL	LL	Non Specific	
TT	4	-	-	-	-	-	4
BT	-	6	1	-	-	-	7
BB	-	-	-	-	-	-	0
BL	-	-	-	5	-	-	5
LL	-	-	-	2	4	-	6
Non Specific	-	-	-	-	-	-	0
Total skin	4	6	1	7	4	0	22

Table 11: Percentage of the cases showing discrepancy between skin and nerve spectrum.

Author	Total No. of cases	No. of cases showing disparity between skin & nerve classification	Percentage of the total
Srinivasan et al	36	21	58
Mukerjee et al	22	8	36
Ashok Kumar et al	27	15	56
Current study	22	3	14

In our study 10 cases were treated completely and two patients had a history of incomplete treatment. Out of 10 completely treated cases, 2 of them were in LL spectrum, in which the skin biopsy showed evidence of treatment response. 6 of them were in BT spectrum in which one showed complete treatment response, one got upgraded to indeterminate Leprosy and the remaining 4 had no improvement. Out of 2 cases treated for TT one showed treatment response with Type1 reaction and other had no response for treatment. Hence out of 10 completely treated cases 50% showed the evidence of response to the treatment. 50% showed no response.Out of the 2 incompletely treated cases, 1 case of BL have got upgraded to BT Leprosy and other case which had treatment for LL didn't show any evidence of improvement. In the present study 2 cases already positive for HIV who are undergoing ART treatment where diagnosed as BT and TT respectively. As per the epidemiological study conducted by Orege P A[20] & Lucas SB et al[22] and Ponnighaus JM et al[21] HIV infection has no effect on the incidence of Leprosy nor has a change in proportions of Tuberculoid vs Lepromatous patient being noted.

In this study, concurrent skin and nerve biopsies were taken from 22 patients, clinically suspected to be having leprosy. The distribution of lesions of skin and nerve were as shown in the Table- 10.Based on the histopathological spectrum of leprosy in skin and nerve, these cases could be divided into two groups. Group 1 in which spectrum of both skin and nerve were in same and Group II where there was disparity between the lesions of the two sites.

Group 1 Correlation between skin and nerve spectrum was present. There were 19 cases in this group, accounting for 86% of the cases. The distributions of these cases are given in the Table 10.

Group 2 Disparity between the skin and nerve spectrum was present. In our study, 3 cases (14% of the total cases) belonged to this group in our study. In the first case the skin was suggestive of Mid Borderline Leprosy (BB) but the nerve showed features of BT and in the rest of the two cases the skin showed features of BL with BI 3+ and the nerve showed features of LL with BI 4+

Srinivasan et al[40] observed that there was disparity in 21 cases out of 36 cases. Mukherjee et al[43] in their study of 22 cases, found disparity in 8 cases. Ashok Kumar et al[50] in their study, of 27 cases found a disparity in 15 cases (Table-11).

Statistical analysis was done, which showed an association between the histological features of skin and nerve tissue sections (P<0.001). This confirms that there is no significant difference between histological features of skin and nerve lesions in Hansen's disease.

REFERENCES:

1. John Farrow, Damien the Leper (New York: Sheed & Ward, 1951),p-89
2. Browne SG. The History of Leprosy. In : Hastings RC, editor. Leprosy. 1st ed. Edinburgh : Churchill Livingstone; 1989. p. 1-8.
3. Oliver Hasselblad, Hansen's Disease: & Present Day Understanding, (n.p, n.d.), pp. 2-3.
4. Th. M. Vogelsang, "Gerhard Henrik Armauer Hansen, 1841-1912. The discoverer of the leprosy bacillus. His life and his work." International Journal of Leprosy And Other Mycobacterial Diseases 46, no. 3 (July:SeptemberT978): 278.-
5. Tan SY, Graham C.Armauer Hansen (1841-1912): discoverer of the cause of leprosy. Singapore Med J 2008;49(7):520-21
6. skinsnes OK; Notes from history of leprosy int.j.leprosy 41 1973, pp220-237
7. Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five group system. Int J Lep 1996; 34: 255-77
8. Jopling WH, Mcdougall AC. Diagnostic Tests, In: Hand book of leprosy, 5th edn. CBS. 1999. pp.60.
9. Nayak SV, Shivarudrappa AS, Nagarajapa AH, Ahmed SM.Role of modified rapid AFB method in Histopathological sections of Hansen's disease. Ind J Dermatol Venereol Leprol 2003;69: 173-74
10. Job CK, Chacko CJG. A modification of Fite's stain for demonstration of M. leprae in tissue sections. Ind J Lepr 1986; 58: 17-19
11. Ridley DS, Jopling WH. A classification for research purposes. Lepr Rev 1962; 33: 119.
12. Shenoi SD, Siddappa K. Correlation of clinical and histopathologic features in untreated macular lesions of leprosy- a study of 100 cases. Ind J Lepr 1988; 60: 202-06.
13. Nadkarni NS, Rege VL. Significance of histopathological classification in leprosy. Ind J Lepr 1999; 7: 325-32
14. Moorthy BN, Kumar P, Chatura KR, Chandrasekhar HR, Basavaraja PK. Histopathological correlation of skin biopsies in leprosy. Ind J Dermatol Ven Leprol 2001; 67: 299-301. WHO, A guide to leprosy control, WHO,Geneva 1980,pp 12-15 Hansen's GA; Bacillus leprae, virchow's arch.pathol,79 1880 pp32-42
15. Noordeen SK. Eliminating as a public health problem. Int J Lepr 1995;63:559.
16. Britton WJ, Lockwood DNJ. Lancet 2004;363:1209- 1219 Lowy L. Processing of biopsies for leprosy bacilli. J Med Lab Technol 1956;13:558.
17. Lowy L. Processing of biopsies for leprosy bacilli. J Med Lab Technol 1956;13:558.
18. Van Brakel WH, de Soldenhoff R, McDougall AC. The allocation ofleprosy patients into paucibacillary and multibacillary groups for multidrug therapy, taking into account the number of body areas affected by skin, or skin and nerve lesions. Lepr Rev 1992;63:231.
19. Ridley DS, Radia KB. The histological course of reactions in borderline and their outcome. Int J Lepr
20. Orege PA, Fine PEM, Lucas SB, et al. A case control study of human immunodeficiency virus-1 (HIV-1) infection as a risk factor for tuberculosis and in western Kenya. Tubercle Lung Dis 1993; 74:377.
21. Ponnighaus JM, Mwanjasi LJ, Fine PEM, et al. Is HIV infection a risk factor for Int J Lepr 1991;59:221.
22. Lucas SB. HIV and [Editorial]. Lepr Rev 1993; 64:97.
23. Blum L, Flageul B, Sow S, et al. reversal reaction in HIV-positive patients. Int J Lepr 1993;61:214.
24. Chacko CJG. Role of histopathology in the early diagnosis of leprosy. Indian J Lepr. 1993;65:23-27.
25. Elder DE, Elenitsas R, Johnson Jr. BL, Murphy GF, editors. Lever's histopathology of skin. 9th ed. Delhi: Lippincott Williams & Wilkins; 2005. p.569-76.
26. Lucus SB, Ridley DS. The use of histopathology in leprosy diagnosis and research. Lepr Rev.1989;60:257-62.
27. Who ,Weekly epidemiological record 15 AUGUST 2008, 83rd YEAR / 15 AOÛT 2008, 83e ANNÉE No. 33, 2008, 83, 293–300
28. Azulay R D. Histopathology of skin lesions in leprosy. Int J Lepr 1971; 39: 244-50.
29. Fite GL, Cambre PJ, Turner MH. Procedure for demonstrating lepra bacilli in paraffin sections. Arch Pathol 1947; 43: 624-5.
30. lyer CGS. Predilection of M. leprae for nerves. Neurohistopathologic observations. lnt J Lepr 1965; 33: 634-45
31. Nayar A, Narayanan JS, Job CK. Histopathological study of early skin lesions in leprosy. Arch Pathol 1972; 94: 199-204.
32. Khanolkar VR. Pathology of leprosy. In: Cochrane RG, Davey TF, eds. Leprosy in theory and practice. Bristol: John Wright&Sons Ltd., Sayal, s. K., das, a. L., and gupta, c. M. Concurrent leprosy and HIV infection: a report of three cases. Ind. J. Lepr. Other mycobact. Dis. 69(3) (1997) 261–265.
33. Cellona, r. V., balegon, M. V. F., dela cruz, e. C., burgos, j. A., abalos, r. M., walsh, g. P., topouki, r., gelber, R. H., and WALSH , D. S. Long term efficacy of 2 year W.H.O. multi-drug therapy

(M.D.T.) in multibacillary (MB) leprosy patients. Int. J. Lepr. Other Mycobact. Dis. **71** (2003) 308–319.

34. KATOCH, K., NATRAJAN, M., BAGGA, A., and KATOCH, V. M. Clinical and bacteriological progress of highly bacillated BL-LL patients discontinuing treatment after different periods of M.D.T. Int. J. Lepr. Other Mycobact. Dis. 59 (1991) 248–254.

35. RIDLEY, D. S. Bacterial indices. In: *Leprosy in Theory and Practice*. Bristol: John Wright and Sons, Ltd.,1964. pp. 620–622

36. Cockerell CJ. Leprosy. In: Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE, editors. Pathology of Infectious Diseases. 1st ed. Stanford : Appleton and Lange; 1997. p. 605.

37. Park K. Park's Textbook of Preventive and Social Medicine. 17th ed. Jabalpur : Banarasidas Bhanot ; 2002.

38. Dharmendra. Classifications of Leprosy. In : Hastings RC, editor. Leprosy. 1ˢᵗ ed. Edinburgh : Churchill Livingstone; 1989. p. 88 – 98.

39. Antia NH, Pandya NJ. Qualitative Histology and Quantitative Bacteriology in various tissues of 50 leprosy patients. Lepr Rev 1976 ; 47 : 175 – 183.

40. Srinivasan H. Rao KS, Iyer CGS. Discrepancy in the histopathological features of leprosy lesions in the skin and peripheral nerve. Lepr in India 1982 ; 54 (2) : 275 – 82. 55

41. Haimanot RT, Mshana RN, McDougall C, Andersen JG. Sural nerve biopsy in leprosy patients after varying periods of treatment : Histopathological and Bacteriological findings on light microscopy. Int J Lepr 1983 ; 52 (2) : 163-9.

42. Ridley DS, Ridley MJ. Classification of nerves is modified by the delayed recognition of Mycobacterium leprae. Int J Lepr 1986 ; 54 (4) : 596 – 605.

43. Mukherjee A, Misra RS. Comparative histology of skin and nerve granulomas in leprosy patients. Lepr Rev 1988 ; 59 : 177 – 180.

44. Nilsen R, Mengistu G, Reddy BB. The role of nerve biopsies in the diagnosis and management of leprosy. Lepr Rev 1989 ; 60 : 28-32.

45. Pereira JH, Palande DD, Gschmeissner SE. Mycobacteria in nerve trunks of long-term treated leprosy patients. Lepr Rev 1991 ; 62 : 134 – 142.

46. Shetty VP, Suchitra K, Uplekar MW, Antia NH. Persistence of Mycobacterium leprae in the peripheral nerve as compared to the skin of multidrug treated leprosy patients. Lepr Rev 1992 ;63 :329 – 336.

47. Negesse Y, Beimnet K, Miko T, Wondimus A, Berhan TY. In leprosy the presence of mycobacteria in the nerve is an essential factor in the cycle and spectrum of Mycobacterium leprae infection. Lepr Rev 1993 ; 64 : 104 – 109. 56

48. Lakshmi TSS, Rao PN, Krishna AV, Kumar ECSV. Histopathology of skin and nerve and clinical classification in leprosy patients. Indian J Lepr 1996 ; 68 (3): 258 – 260.

49. Kaur S, Sharma VK, Basak P, Kaur I, Radotra BD. Concurrent skin and nerve histology in leprosy and its role in the classification of leprosy. Lepr Rev 1996 ; 64 : 110 – 116.

50. Ashok Kumar SK, Reddy BSN, Ratnakar C. Correlation of skin and nerve histopathology in leprosy. Lepr Rev 1996 ; 67 : 119 – 125.

51. Khan A, Koranne RV, Bajaj P, Ravi B. Clinico-histopathological correlation of skin and nerve in leprosy. J Dermatol 2004 ; 31 : 632 – 636.

52. Culling CFA, Allison RT, Barr WT. Cellular Pathology Techniques. 4th ed. London : Butterworth & Co. ; 1985.

53. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 5th edn. Edinburgh: Churchill Livingstone. 2002.pp. 127-128.

54. Sherren.J: Injuries of nerves and their treatment,William Wood and Co, NewYork, a quoted by Job CK in nerve damage in Leprosy.Int.J.Leprosy 57, 1989, page 532.

55. Metha LN, Shetty VP, Anitha AH & Irain PF; Quantitative Histologic and Ultrastructural studies of the index branch of Radial cutaneous nerve in Leprosy and its correlation with electrophysiological study.Int.J.Lepr 43, 1975, pp256-264.

56. WHO Expert Committee on Leprosy, Classification (of leprosy) for control programme; WHO Tech Rep.Ser.1988,pp14.

57. Pearson JMH and Weddell AGM: perineurial changes in untreated leprosy.Lepr Rev.46,1975 pp 51-67

58. Chandy SM, Chacko CJG & Job CK; segmental necrotizing granulomatous neuritis leprosy.Int.J.Lepr 48, 1986, pp 596-606

59. Job CK; Nerve damage in Leprosy.Inj.J.Lep 57,1989, pp532-539.

60. Ridley DS. The Bacteriological Interpretation of Skin Smears and Biopsies in Leprosy. TransR Soc Trop Med Hyg 1955; 49(5) : 449-452. 57

61. Ridley DS, Job CK. The Pathology of Leprosy. In : Hastings RC, editor. Leprosy 1st ed. Edinburgh: Churchill Livingstone; 1989. p. 100 – 114.

62. Ridley DS; skin biopsy in leprosy, Documenta Geigy 3ʳᵈ Ed, 1990 pp 13-16

63. Devasundaram B, Chacko CJG; A technical manual for smear personnel, karigiri Leprosy Education Programme 2ⁿᵈ Ed, 1992, SLR & TC Karigiri page 22.

64. Praveen Singh et al; concurrent skin and nerve histopathology; its role in the classification of leprosy 2005, pp52-53