Microscopic Vision on Leprosy in Northern Karnataka

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Received 07 February 2014; Accepted 16 February 2014; Published 18 February 2014

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Abstract

Leprosy still continues to be a public health problem in India. The cases were selected regardless of their age, sex, religion, occupation and socio economic status. Pathological examination helps in confirming the clinical diagnosis. Clinically exact typing of leprosy is difficult and even slit-skin smear yields poor results. Majority of the cases were seen in second and third decade. Patients of both sexes were affected and it was more in males than in females. Patients from different religions were affected among these most of them belonged to Hindu religion.

Key words: Leprosy; Histopathology; Diagnosis

1. Introduction

Leprosy continues to be a major public health problem in Asia and Africa. Control of leprosy mainly based on identifying and destroying the causative organism. For effective treatment and control, the diagnosis of leprosy should be done at the earliest and should be accurate. Pathological examination helps in confirming the clinical diagnosis. Clinically exact typing of leprosy is difficult and even slit-skin smear yields poor results. Thus histopathological examination is necessary for both accurate and exact typing.
2. Materials and Methods

The present study was undertaken from 2002–2012 in the Department of Pathology, Navodaya Medical College, Raichur. Histopathological studies of 270 skin biopsy specimens of leprosy patients were done.

All the biopsy specimens were received along with requisition for histopathological study containing clinical history, signs and symptoms of skin lesions, results of slit skin smears for AFB with BI in some cases and probable clinical diagnosis.

Cases were selected regardless of their age, sex, religion, occupation and socio economic status. Details of patient history and clinical examination were noted of the patients who clinically presented with hypopigmented /erythematous maculas, plaques, nodules, papules or a combination of these, along with impaired sensation for touch, pain, and temperature and nerve involvement.

Biopsy tissues were immediately fixed in 10% formalin for 12-24 hours. The tissue were processed, embedded in paraffin wax and cut into thin sections of 4-5 microns.

Sections were stained with routine hematoxylin and eosin along with special staining for AFB by Fite Faraco methods, and Auramine – Rhodamine fluorescent stain, wherever necessary.

**H and E staining procedure:**
1. Wax was removed by placing sections in xylene 3-5 minutes.
2. Two changes of absolute alcohol 1-2 minutes.
3. Washed in running tap water 10 minutes.
4. Slides stained with Harris hematoxylin 10 minutes.
5. Dipped in acid alcohol for differentiation
6. Washed in tap water for 10 minutes (bluing)
7. Counterstained with eosin 2 mins and washed in running tap water for 2 to 3 mins.
8. Sections were dehydrated in alcohol, cleared in Xylene and mounted with DPX.

**Special stain for M. Leprae in paraffin section (Fite-faraco stain):**
1. Wax was removed over two changes of xylene peanut oil (3:1) mixture 7 mins for each change.
2. Blotted with fine filter paper.
3. Sections washed in running water for 5 mins.
4. Stained with strong carbol fuchsin for 30 mins.
5. Water wash 2 mins.
6. Decolorized in 1% acid alcohol to reach a pale pink colour.
7. Water wash 2 mins.
8. Counter stained in methylene blue 5 to 6 dips.
9. Water wash until section becomes pale blue.
10. Section dehydrated in absolute alcohol 3 changes.
11. Cleared in Xylene 2 changes and mounted in DPX.

**BACTERIAL INDEX (BI)**

BI was for study of AFB stain BI was assessed in the same way as in a smear. Using an oil immersion objective the following scale was used.

1+ 1 to 10 bacilli in 100 fields
2+ 1 to 10 bacilli in 10 fields
3+ 1 to 10 bacilli in 1 field
4+ 1 to 100 bacilli in 1 field
5+ 100 to 1000 bacilli in 1 average field
6+ > 1000 bacilli in 1 field.
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Auramine - Rhodamine stain:
1. Deparaffinisation was done with 1:3 peanut oil : Xylene mixture
2. Auramine – Rhodamine stain was used to flood the slides and kept in the incubator at 65 for 15 mins.
3. Slides were washed in running tap water for 2 mins.
4. De-colorization was done in 0.5% HCL in 70% ethanol for 2 mins.
5. Washed in running tap water for 2 mins.
6. Counterstained with 0.5% aqueous potassium permanganate.
7. Washed in running tap water for 2 mins.
8. Dehydrate in absolute alcohol.
Controls – Typical lepromatous leprosy biopsy.

Figure 1: Showing age and sex distribution in leprosy

3. Observations
The present study was carried out in the Raichur for a period of ten years; i.e from 2002 to 2012. During this period 40471 specimens were received in the Histopathology section, Department of Pathology, out of which 782 were skin biopsies. Out of these histopathological study of 270 skin biopsy specimens from skin lesions of leprosy patients was done.

In the present study, patients in the age group of 20-29 years were affected most with 60 cases, followed by 10-19 years with 58, 40-49 years 50, 30-39 yrs 44, 50-59 years 22, 0-9 years 18 and 60 years and above with 18 cases. Males were affected most with 180 cases and females with 90, with Male : Female ratio of 2:1 (Figure 1).

Out of 270 leprosy patients Hindus were 216, Muslims were 50, and Christians were only 4 cases (Figure 2).
The commonest presenting feature was hypopigmented patches seen in 186, followed by erythematous patches with 64, and combinations of both were seen in 20 cases. The most common cutaneous lesions observed were macules in 146, followed by plaques in 34, papules in 16 and nodules in only 4 cases. Various combinations of macula, papule, plaque and nodule were seen in 70 cases. The margins of cutaneous lesions were well...
defined in 156, and ill defined in 114 cases. Cases showing less than 5 lesions all over the body were 244 cases, and more than 10 lesion in 26. Loss of sensation was seen in 206 cases and thickened peripheral nerves were present in 130 (Figure 3).

![Figure 4: showing clinical diagnosis in the present study.](image)

![Figure 5: showing histopathological types of leprosy.](image)

Various clinical diagnosis were given, among these borderline tuberculoid leprosy was the most common with 132, followed by tuberculoid leprosy in 52, indeterminate leprosy 24, 10 cases each; in borderline leprosy 24, 10 cases each; in borderline leprosy; and borderline lepromatous; leprosy, and lepromatous leprosy in 12 cases. In rest of the cases, the clinical
In the present study the commonest histopathological type of leprosy was borderline tuberculoid leprosy in 88, followed by tuberculoid leprosy in 78, indeterminate leprosy; in 74, lepromatous leprosy in 12 cases only. The borderline leprosy cases were not diagnostic morphologically (Figure 5).

4. Discussion

In the present study the most affected age group was 20-29 years constituting 60 and the second peak in the age group of 10-19 years with 68 which are similar to the observations made by Guha et al [1] 108 and 80 respectively.

Patients in the age group of 40-49 years constituted 50 cases, 30-39 years 44, similar results were observed by Seghal et al [2] 307 and 196 respectively. Patients less than 9 years 18 and more than 50 years were affected least; similar results were seen in the studies of Seghal et al [2] 43 and 123 respectively, and Guha et al [1] 25 and 52 respectively.

In the present study there was male predominance with 2:1 male to female ratio which is similar to observations madr by Guha et al [1] (1.72:1) and Sehgal V N et al [2] (4.39:1) while the study done by Chaturvedi et al [3] showed female preponderance with a ratio of 1:1.33.

In the present study almost all patients had skin lesions. Among which hypopigmented patches were seen in 182 and erythematous patches were seen in 64, which were similar to the study done by Kar.P.K et al[4] with 88 in 14. Nodules were observed in 12(8%) cases in the present study, similar results were observed by Verma et al [5] 1 (7%). Loss of sensation was seen in 103 (76%) of cases in the present study while Kar.P.K et al[4] and Verma et al[5] observed 31 and 14 (25.8% and 97% respectively). Nerve thickening was seen in 65(48%) and trophic ulcer in 3 (2%) cases in the present study while Verma et al[5] observed 2 and 3 (13% and 20% respectively).

In the present study the commonest histopathological type of leprosy was borderline tuberculoid constituting 88 cases, similar results were observed by P.K.Kar et al [4] and Nadkarni N.S et al [6] 38 and 969 respectively. This was followed by tuberculoid leprosy with 78 similar results were seen in the studies done by Shenoi S D et al [7] and Nadkarni N S et al [6] 22 and 460 respectively. Intermediate leproy in 74, similar results were seen in P.K. Kar et al [4] and Nadkarni N.S et al[6] respectively. Borderline lepromatous leprosy and lepromatous leprosy constitute very few cases in the present study.

5. Conclusion

Histopathological study of 270 biopsy specimens, which were taken from skin lesions
of the patients suspected to be suffering from leprosy, which constituted 0.34% of all biopsies evaluated. All the age groups were affected.

Majority of the cases were seen in second and third decade. Patients of both sexes were affected and it was more in males than in females. Patients from different religions were affected among these most of them belonged to Hindu religion. The most common type of leprosy histopathologically diagnosed was borderline tuberculoid leprosy followed by tuberculoid and intermediate leprosy.

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