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A clinico-histopathological analysis and bacillary index in a study of skin biopsy of leprosy

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Abstract--Introduction: Leprosy is one of the leading causes of physical disabilities contributing to intense social stigma resulting in human discrimination. This chronic infectious disease caused by *Mycobacterium leprae* principally affects skin and peripheral nerve. It can also include muscle, eyes, bones, testis and internal organs. Histopathology study and bacillary index is important in understanding the disease progression, diagnosis, varied manifestation and complications. Method: All cases attending the skin OPD were examined clinically and skin biopsy specimen was obtained from clinically diagnosed cases of Leprosy and stained with Hematoxylin & Eosin and modified FiteFaraco (AFB). The clinical diagnosis correlated with histopathology in all 100 cases. Result: The age of the patients was ranged from 4 to 80 years. The male to female ratio patients was 3 to 1. Borderline Tuberculoid was the most common presentation. Highest parity was observed in BT and Histoid leprosy. Clinico- histopathological agreement was seen in 76(76%) cases. Conclusion: The clinical and histopathological features along with bacteriological index are useful than any single parameter in arriving definitive diagnosis and classification of the leprosy.

Keywords---clinico-histopathological, Leprosy, disease.

Introduction

Leprosy, also known as Hansen's disease, is one of the oldest diseases of mankind. Leprosy still remains an important public health problem in many parts of Asia, mainly in India. In our country despite declaring leprosy elimination at national

level in January 2006 it is still a disease of endemic in many states. The total estimated global new cases detected in 2009 were 2,27,849 and India account 1,33,717 (58.7%) cases.

Depending on the immune status of the host, Leprosy presents in various clinical – pathological forms. Leprosy can be diagnosed by various methods including detailed clinical examination of the skin lesions and peripheral nerves, histopathological section and demonstration of bacilli by modified FiteFaraco procedure.

Ridely and Jopling have suggested immunological basis of leprosy and classified into five types as Tuberculoid Leprosy (TT), Borderline Tuberculoid Leprosy (BT), Mid borderline leprosy (BB), Borderline Lepromatous Leprosy (BL), and Lepromatous Leprosy (LL). This classification is accepted worldwide and is highly recommended. Though the clinical diagnosis is based on characteristic hypopigmented patches with sensory loss, great variations are seen in interpretation of these hypopigmented skin lesions both clinically and histopathologically.

So along with provided detailed clinical information and bacilloscopic examination, skin biopsies play an important role in the diagnosis of leprosy. Histopathological examination also helps us to ascertain the immunological status of the individual by which we can predict the response to the treatment. This research is taken to study the correlations between the clinical and histopathological diagnosis of leprosy patients, and to evaluate the importance of skin biopsy for the diagnosis of leprosy.

Aims of the Study

- To study the histopathological features of leprosy in skin biopsies.
- To categorize them into various types based on microscopy.
- Bacillary index to correlate with clinical presentation whenever possible and prognostic assessment.

Materials and Methods

A minimum of hundred patients of Leprosy belonging to all the age groups and both sexes were randomly selected and included in the study after taking their consent. In each patient detailed history, thorough general and local examination was done as per the standard protocol followed for examining a patient with leprosy. Skin biopsy was done in all cases for histopathological study with patients consent.

Inclusion Criteria

- Skin biopsies obtained from patients clinically diagnosed as new cases of leprosy or old cases not responding to therapy.
- All ages of patients which are clinically suspicious **of leprosy are included.**

Exclusion Criteria

- Inadequate biopsy material.
- All skin biopsies except clinically diagnosed leprosy will be excluded.

History

Detailed history of age , sex, occupation and socioeconomic status was taken and presenting complaints like skin lesions ,numbness, trophic ulcers and deformities were noted.

Clinical Examination

A detailed general examination was carried out in all the patients. Local examination of skin lesions was carried out with particular references to the number, shape, size, surface, margins, satellite lesions, supplying nerves, sensation, sweat loss, hair loss and trophic changes. All the peripheral nerves were palpated for enlargement and tenderness. The patients were clinically diagnosed as Tuberculoid (TT), Borderline Tuberculoid (BT), Borderline Borderline(BB), Borderline Lepromatous(BL), Lepromatous.

Routine Investigations

All patients were investigated routinely like Hb%, total count, differential count, ESR, Platelet count, bleeding time, clotting time and ELISA for HIV.

Histopathological Examination

Skin Biopsy⁷⁶

Importance of skin biopsy

- To confirm the diagnosis
- To classify leprosy
- To identify the complications like reaction
- To help in the management

Study Tools:

- Gloves
- 10%Formalin
- Biopsy Container
- Scalpales
- Forceps
- Cotton swab
- Cassetes
- Paraffin wax
- Microtone machine
- Hot water bath

- Clean glass slides
- DPX
- Haematoxylin& Eosin stain
- Zheil and Nelson stain
- Microscope

Skin biopsies for the study were obtained ny punch biopsy by the dermatologist after taking written consent from the patient and sent to department of pathology in 10% formalin. After adequate fixation for about 8-12 hours, the biopsies were submitted in to routine processing, following which the paraffin embedded section of 5 micron thickness were stained with hematoxylin and eosin for morphological analysis.

Hematoxylin and Eosin stained sections of skin biopsies will be examined for

1. Epidermal atrophy
2. Epitheloid and macrophage granulomas
3. Number and distribution of lymphocytes, histiocytes and foam cells.
4. Infiltration of nerves, blood vessels and adnexa
5. Grenz zone

Sections stained with modified Fite's stain will be examined for acid fast bacilli in all cases. Histopathological findings will be graded into Polar tuberculoid (TT), Borderline tuberculoid (BT), Mid-borderline (BB), Borderline Lepromatous(BL), Polar Lepromatous(LL) based on Ridley and Jopling scale.

Site

In indeterminate leprosy the biopsy should be taken from the middle of the lesion, where the lesion is active. If multiple lesions are present, the most active lesion will be selected and biopsy is taken from edge of the lesion.

Size

The elliptical piece of skin with size of 1.5 cm long and 0.6cm wide with the depth of dermis and subcutis will be taken.

Fixatives

- Lowy's fixative(FMA)
- Formaldehyde(40%)- 100ml
- Mercuric chloride- 20g
- Glacial acetic acid- 30 ml
- Distilled water-1000ml

The biopsy specimen should be kept in this solution for 2 hours and then transferred to 70% ethyl alcohol, in which it can be stored for long time. The following stains are done:

- Haematoxylin &Eosin stain
- Fite – Faraco stain

- Gomarimethamine silver stain
- S-100 STAIN

Skin biopsy specimen was obtained from all clinically diagnosed cases of leprosy and was subjected to the following staining techniques

- Haematoxylin & Eosin stain
- Modified Fite's stain

Haematoxylin and eosin staining procedure:

- After removing paraffin wax slides were emersed in absolute alcohol for 2 minutes
- Washed in water
- Stained with haematoxylin for 15 minutes
- Washed in water
- 1-2 dips were given to absolute alcohol
- Washed in water
- Counter stain 1% aqueous eosin for 3 min
- Dehydrated
- Mount with DPX

Modified Fite's Stain-to demonstrate morphology, site and number of AFB

Procedure:

- Section is brought to the water
- Stained in CarbolFuschin for 30 minutes
- After washing with water differentiated in 3% HCL in 70% alcohol for 5-10 minutes
- Counter stain with 0.1% methylene blue for 10 to 15 seconds
- Washed in water, dehydrated and mounted.

Statistical Analysis Plan

Data obtained were analysed using appropriate statistical package suggested by the statistician.

Observation and Results

Incidence of clinical diagnosis

In our study all the patients were thoroughly examined clinically and diagnosed. Out of 100 cases.

TABLE 1: Incidence of Clinical Diagnosis

Clinical diagnosis	Frequency	Percentage
Tuberculoid leprosy	04	4.0
Borderline tuberculoid	39	39.0
Mid borderline	4	4.0

Borderline lepromatous	20	20
Lepromatous leprosy	21	21.0
Histoid leprosy	3	3.0
Erythema Nodosum Leprosum	9	9.0
Total	100	100.0

Incidence of Histopathological distribution

In our study skin biopsy was taken from all the 100 patients and stained with H&E stain. Out of 100 patients 26(26%) patients were histologically diagnosed as Inconclusive to Leprosy and its variants.

Age distribution

In our study, the youngest patient was 4 years old and the eldest was 80 years old. The maximum number of patients (36%) showing clinical activity in this study belonged to the 21-30 years age group whereas the least number of patients(2%) belonged to the less than 10 years age group.

Table 2: age distribution

Age	frequency	Percentage
0-10	02	02
11-20	16	16
21-30	36	36
31-40	17	17
41-50	13	13
51-60	10	10
ABOVE 61	06	06
TOTAL	100	100

Sex distribution

In the present study, male patients comprised 75% and female patients comprised 25% of the total patients. Male to female ratio was 3:1

Table 3: Sex distribution

Sex distribution	Frequency	PERCENTAGE
Male	75	75.0
Female	25	25.0
TOTAL	100	100.0

Complaints

In this study of 100 patients, 51(51%) patients had the complaints of hypopigmented skin lesion. 36(36%) patients had raised lesions. Numbness and

hypopigmented lesions in 8(8%) patients and swelling & hypopigmented lesions in 5(5%) patients.

Table 4: Complaints

Complaints	Frequency	Percentage
Hypopigmented LESIONS	51	51.0
Raised lesions	36	36.0
Hypopigmented lesions & numbness	8	8
Hypopigmented lesions & swellings	5	5
Total	100	100

Site distribution of skin lesions

In our study among 100 patients, majority of patients had lesions over trunk. 38(38%) on trunk, 23(23%) cases had lesions on upper limbs, 24(24%) on the lower limb, 14(14%) on the head & neck, 1(1%) patients had lesions over multiple sites of the body.

Table 5: site distribution

Site	Frequency	Percent
TRUNK	38	38.0
LOWER LIMB	24	24.0
UPPER LIMB	23	23.0
HEAD & NECK	14	14.0
MULTIPLE SITES	1	1.0
TOTAL	100	100.0

Acid fast bacilli

In our study 30(30%) patients showed smear positivity whereas 70(70%) showed smear negativity. 2(6.66%) BT patient, 7(23.33%) BL, 16(53.33%) Histoid and 2(6.66%) ENL patient showed smear positivity. All Tuberculoid patients were smear negative.

AFB	Frequency	Percentage
Negative	70	70.0
Positive	30	30.0
TOTAL	100	100

Bacillary index

Table 6: bacillary index

Bacterial index	Frequency	Percentage
<or=3+	6	20

>3+	24	80
TOTAL	30	100

Discussion

In the present study, Ridley- Jopling classification was used to classify leprosy histopathologically in all cases. Indeterminate leprosy was not included for analysis. Histoid leprosy is considered as variant of lepromatous leprosy and it was included in LL specimen.

Age Distribution

In the present study more number of patients belong to the age group of 21-30 years (36%). In a study done by Moorthy BN et al ¹, majority of patients were between 20-29 years (20.70%). Singh et al² found the disease in 48% of patients belonging to the age group of 21-40 years. Santaram and porichha³ found majority of patients were 21-40 years.

Thus, the age incidence in the present study correlates well with the other studies. The disease is more common in this age group because of their mobility and increased opportunity for contacts.

Sex Distribution

In the present study 75% of patients were males and 25% were females .

Similarly santaram and poricha found the disease in 80% of males.

Singh et al found the disease in 69% males.

Similarly Moorthy et al, Girdhar M et al and Nitesh et al found the disease to be more common in males.

The disease is more common in males because of their outdoor works and higher chances of getting infection.

Summary

- Majority of patients (36%) belongs to the age group of 21-30 years.
- Male patients comprised 75% and females were 25%.
- 65% of patients had the disease duration less than 1 year, 33% had the duration between 1-5 years and more than 5 years in the 2% cases.
- 51% patients had the complaints of hypopigmented skin lesions. 36% patient had raised lesion, numbness and hypopigmented lesion is 8% patients and swelling and hypopigmented lesions in 5% patients.
- 1% patient had the lesion over multiple site of the body, 23% patients had lesion on upper limb, 24% on the lower limb, 38% on the trunk and 14% on head and neck.
- Borderline tuberculoid was the most common clinical presentation comprising of 39%.
- 30% patient showed acid fast stain positivity and 70% were smear negative. 2(6.66%) Bt patient, 7(23.33%) BL, 16(53.33%) LL, 3(10%) Histoid and 2(6.66%) ENL patient showed smear positivity.

- Out of 100 patients 30% patients were histologically diagnosed as BT, 16% as LL, 12% as BL, 3% as BB and 4% as TT.
- Clinico-histo-pathological agreement was seen in 74% cases and disagreement was seen in 26% cases.

Out of 4 patients clinically diagnosed as TT, 2(50%) patients had histopathological correlation. Out of 39 patients clinically diagnosed as BT, 24(61.53%) patient had correlation. 25% of BB patients had clinic- histo-pathological correlation, 50% BL and 42.85% of LL patients had clinic- histopathological correlation.

Conclusion

In our study:

- The higher incidence in the age group 21-30 years.
- The incidence was higher in males because of their more physical activity and thus more chances of getting infection.
- The duration of illness was less than one year in majority of patients.
- Most common complain was hypopigmented skin lesions.
- The higher number of patients were borderline tuberculoid. This is because these these patients come early for the treatment because of its neurological symptoms.
- AFB positivity was found in 30% of patients.
- Clinico- histopathological correlation was seen in 74% of the patients. Maximum correlation was seen in borderline tuberculoid leprosy.

In conclusion it can be said that leprosy still continues to be a domestic, national, global burden and is present in different clinico-pathological forms. Many cases can be diagnosed clinically; especially lepromatous pole of the disease, however, other types of leprosy pose a significant problem in clinical diagnosis. Histopathological examination of lesions confirms the exact subtype of the disease and facilitate the institution of accurate mode of therap. So, correlation of clinical and histopathological features along with bacteriological index is more useful for accurate typing of leprosy than considering single parameter alone.

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