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Original Research Article

A study of clinico-histopathological correlation in leprosy

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ABSTRACT

Introduction: Leprosy is a chronic infectious disease caused by Mycobacterium leprae. It principally affects the cooler parts of the body, mainly skin and peripheral nerves; it also involves muscles, eyes, bones, testis and internal organs. Histopathological study of leprosy is very important in understanding the disease, its varied manifestations and complications. Clinical diagnosis of early leprosy lesions offer difficulties even to experienced dermatologists and leprologists. A definitive diagnosis may be possible by histopathological examination. Histological diagnosis when available is deemed the gold standard for diagnosis of leprosy.

Aims: To diagnose the case of leprosy using skin biopsy specimen. To study the clinical and histopathological correlation among leprosy patients attending a tertiary referral center. To classify the lesion according to Ridley and Jopling (RJ) classification.

Materials and Methods: The present study was conducted over a period of 18 months from Jan 2019 to July 2020. Total 51 new suspected cases were selected on clinical ground attending dermatological OPD. These patients were subjected to skin biopsy. Histopathological classification done on the basis of Riedly-Jopling criteria.

Results: Out of total 51 cases 40 cases showing leprosy changes on histopathological examination. Majority of the patients were males (53.06%), while females (46.94%) constituted a minority with male to female ratio of 1.1:1.

Both clinically and histopathologically BT constituted the predominant group (62.7%) and (54.7%) respectively, followed by LL (15.6%).

Conclusion: Clinical diagnosis of early leprosy lesions offer difficulties even to experienced dermatologists and leprologists. A definitive diagnosis may be possible by histopathological & bacteriological examination.

1. Introduction

Leprosy is a chronic infectious disease caused by Mycobacterium leprae. It principally affects the cooler parts of the body, mainly skin and peripheral nerves; it also involves muscles, eyes, bones, testis and internal organs.[1] The discovery of lepra bacillus by Gerhard Henrik Armaeur Hansen in 1873 opened a new vista in the understanding of the disease. Although it was the first bacterium to be etiologically associated with human disease, M. leprae remains one of the few bacterial species that still has not been cultivated on artificial medium or tissue culture.[2]

The maximum incubation period reported is as long as 30 years. However, average incubation period is 5 – 7 years.[3]

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There are several ways of classification of leprosy but most widely accepted is Ridley and Jopling classification. It has proved to be widely comprehensible and is known to give a good clinical-histological correlation, as well as to have the advantage of objectivity.[4]

According to this system based on immunological, histological and microbiological parameters, leprosy patients have been grouped as:

1. Tuberculoid (TT).
2. Borderline tuberculoid (BT).
3. Borderline borderline (BB).
4. Borderline lepromatous (BL).
5. Lepromatous

The World Health Organization (WHO), recommends categorization into paucibacilly (PB) and multibacillary(MB) based on skin lesions and /or nerve trunk involvement.

There is wide variation in the clinical presentation of leprosy. These clinical presentations are depends on the immune status of an individual.[5]

1.1. Cardinal signs:[6]

1. Hypopigmented or erythematous skin lesion with definite loss / impairment of sensation.
2. Thickening of peripheral nerves with sensory impairment.
3. Skin smear positive for acid-fast bacilli.

Clinical diagnosis in some cases can be difficult which can lead to occurrence of resistant cases if treated inadequately. Skin biopsies play an important role in diagnosing and classifying different types of leprosy.[7]

2. Materials and Methods

The present study is carried out over a period of 18 months in the department of pathology Dr. SCGMC Nanded, from Jan 2019 to July 2020.

2.1. Source of data

Skin biopsies taken from subjects attending OPD and admitted in IPD under dermatology department and clinically suspected cases of leprosy.

2.2. Method of collection of data

Histopathological study of skin biopsy specimens from 51 clinically suspected leprosy patients was done. A detailed clinical history, examination findings indicating signs and symptoms of the skin lesions and provisional clinical diagnosis were collected.

Cases were selected regardless of their age, sex, religion, occupation and socio-economic status.

3. Objectives

1. To diagnose the case of leprosy using skin biopsy specimen.
2. To study the clinical and histopathological correlation among leprosy patients attending a tertiary referral center.
3. To classify the lesion according to Ridley and Jopling (RJ classification.

3.1. Inclusion criteria

patients who clinically presented with hypopigmented/erythematous macules, plaques, nodules, papules or a combination of these, along with impaired sensation for touch, pain, temperature and nerve involvement.

3.2. Exclusion criteria

Patients who were previously treated for leprosy and who presented with features of lepra reaction.

3.3. Procedure of Punch Biopsy

The skin punch biopsies measuring 0.5cmx0.5cm from the representative lesion were taken by us, and dispatched in glass or plastic vials containing 10% formalin solution. Following fixation for 12-24 hours the tissues were processed embedded in paraffin and serial sections of 4-5 microns were obtained, which were stained with Hematoxylin and Eosin for histopathological assessment and with AFB stain for identification of the bacilli.

3.4. STEPS of H & E staining

1. Deparaffinize tissue section in 3 changes of xylene for 5 minutes each.
2. Subsequently 2 changes of absolute ethyl alcohol 2 minutes each.
3. Sections were washed in running tap water for 5mins.
4. Stain tissue section with Harris haematoxylin for 5 minutes.
5. Washed briefly in running water and differentiate with 1 % acid alcohol.
6. Wash in running water and blue for 10-30 seconds in bluing agent.
7. Check microscopically for sharp blue colored nuclei If not repeat the bluing step.
8. Stain with 1 % Aqueous Eosin Y solution for 1 min.
9. Dehydrate, Clear and mount in DPX (Dibutyl Phtalatepolysterene Xylene.

3.5. Steps in AFB staining

1. Stain slides with carbol fuchsin for five minutes.
2. Rinse gently with water until water flows off clear.

3. Add 5% H2SO4 (sulfuric acid) for 3 to 5 seconds.
4. Rinse gently with water until water flows off clear.
5. Stain slides with methylene blue for three minutes.
6. Rinse gently with water until water flows off clear.
7. Allow slides for air dry before viewing.

The sections were examined for epidermal atrophy, granuloma, and infiltrates of lymphocytes, histiocytes, foam cells, infiltration of nerves, blood vessels, and adnexa, and the presence of Grenz zone. They were grouped histopathologically as per the criteria formulated by Ridley and Jopling. Subsequently, a correlation was made between the histopathologic and clinical classification.

4. Results

The present study included 51 skin biopsies from the patients who were clinically diagnosed to have leprosy. Out of total 51 cases 40 cases showing leprosy changes on histopathological examination.

11 cases of the 51 cases studied had varied diagnosis on histopathology in terms of chronic dermatitis, CNSIL (Chronic nonspecific inflammatory lesion), Inflammatory lesion, Morphea, Xanthoma etc.

In the present study 26 (50.98%) of the patients were in the 2^{nd} to 4^{th} decades 12 (20.53%) of the patients in 4^{th} to 6^{th} decades, 5(8.19%) in the 6^{th} to 8^{th} decades and the least number of patients 6(11.76%) were seen in the age group of <20yrs.

Male predominance was seen in all types of leprosy. Except in BT type where 22 cases were females in comparison with 21 males.

The most common site involved in leprosy in our study is lower limb (32.5%) followed by upper limb (27.5%) multiple sites (20%). The least common site involved was the back (1.96%).

Out of the 40 study subjects 7.5% of the study subjects had 1+, 5% of the subjects had 2+ and 2.5% had 3+ and 5+. Majority of the study subjects 82.5% did not show any acid fast bacillus.

Among the 51 cases studied, BT (54.90%) constituted the major group, followed by LL (15.69%), BL (3.92%) TT (1.96%), and BB (1.96%).Table 2

21.57% of the subjects had different HPE diagnosis in terms of chronic inflammation, chronic dermatitis, Xanthoma, Morphea, pemphigus and pitiriyasis.

In the present study, out of 40 cases, 32 (80.00%) suffered from anesthesia (loss of sensation), 40 (100%) had hypopigmented patches at presentation, 13 (32.50%) had thickened nerves, 10(19.60%) presented with erythematous patches, 7(17.50%) had a combination of lesions (macules and papules), 03(7.5%) of them had nodules none had limb deformities and 1(2.50%) had trophic ulcers.Table 3

In the present study out of 40 cases, 18 (45.00%) showed an unremarkable epidermis, 13(32.50%) showed a atrophic epidermis and 2 (5.00%) cases had an ulcerated epidermis. Table 4

In this study one case which was clinically diagnosed as BT, was confirmed to be of BB type on histopathological study. Of the 33 cases clinically diagnosed as BT, histopathological study confirmed 26 (78.79%) as

BT type, 1 (3.03%) as TT type, 1 (3.03%) as BB type, 2 (6.06%) as BL type, 3 (9.09%) as LL type.

One case which was clinically diagnosed as BL type was confirmed on biopsy as LL.

Of the 6 cases clinically diagnosed as LL, 4 (66.67%) was confirmed on biopsy as LL type, 2 (33.33%) as BT type.

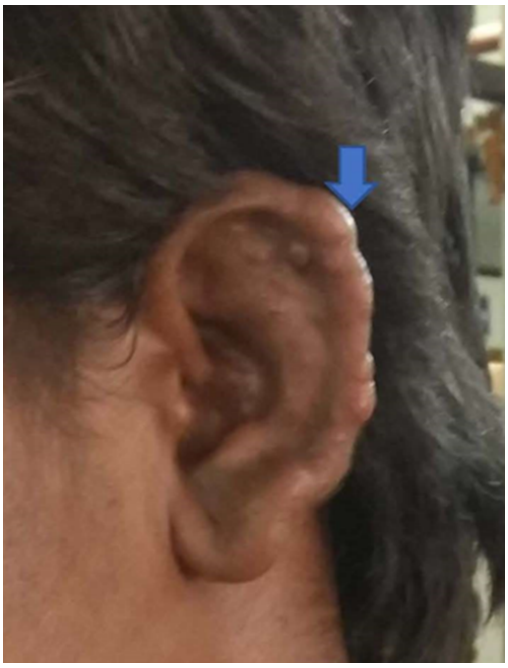

Fig. 1: Histoid leprosy with multiple nodules over ear

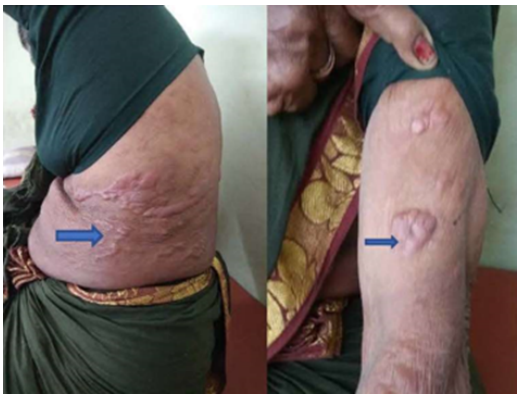

Fig. 2: Multiple nodules in histoid leprosy case over back and upper arm

Table 1: Age and sex distribution of clinically diagnosed cases of leprosy (n=51)

	TT		BT		BB		BL		LL		IL		Total			
Age	00		43		00		01		06		01		51		M+F	%
(Yrs.)	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
<20	0	0	3	2	0	0	0	0	0	0	1	0	4	2	6	11.76%
21-40	0	0	9	15	0	0	0	0	2	0	0	0	11	15	26	50.98%
41-60	0	0	5	4	0	0	0	0	1	2	0	0	6	6	12	20.53%
61-80	0	0	4	1	0	0	1	0	1	0	0	0	6	1	7	13.73%
Total	0	0	21	22	0	0	1	0	4	2	1	0	27	24	51	100.00%

Table 2: Showing histological types of leprosy (n=51)

Histological types	Frequency	Percentage
TT	01	1.96
BT	28	54.90
BB	01	1.96
BL	02	3.92
LL	08	15.69
IL	00	00
Others*	11	21.57
Total	51	100.0

Others*: Chronic inflammation, Chronic dermatitis, Xanthoma, Morphea, Pemphigus and Pitiriyasis.

Table 3: Showing Clinical features in various types of leprosy: ((n=40)

Clinical features	Types No.	TT 01	BT 28	BB 01	BL 02	LL 08	Total 40
Hypopigmented patches		1(100)	28(100%)	01(100%)	02(100%)	08(100%)	40 (100%)
Erythematous patches		00	05 (17.86%)	00	01(50%)	02(25%)	10(25.00%)
Combined (macules and papules)		00	03(10.71%)	00	00	04(50%)	07(17.50%)
Anaesthesia		1(100)	20(71.43%)	00	02(100%)	07(87.5%)	32(80.00%)
Nerve thickening		01(100)	03(10.71%)	01(100%)	01(50%)	06(75%)	13(32.5%)
Nodules		00	00	00	00	03(37.5%)	03(7.5%)
Trophic ulcer			01(2.5%)	00	00	00	12.50%
Limb deformities		0	00	00	00	00	00

Table 4: Showing Epidermal changes in different types of leprosy: (n=40)

Epidermal changes	Types No	TT 01	BT 28	BB 01	BL 02	LL 08	Total 40
Unremarkable		0	14(50%)	1(100%)	1(50.00%)	2(25.00%)	18(45.00%)
Atrophic		1(100%)	04(14.29%)	0	0	7(87.5%)	13(32.50%)
Ulcerated		0	0	0	0	0	2(5.00%)

Table 5: Showing dermal changes in different types of leprosy: (n=40)

Clinical features	Type No	TT 01	BT 28	BB 01	BL 02	LL 08	Total 40
Grenz zone		0	0	0	1(50.00%)	7(87.50%)	8(20.00%)
Lymphocytes Arrector pilorum	**around**	1(100%)	1(3.57%)	00	1(50.00%)	1(12.50)	4(10.00%)
Adnexa		1(100%)	20(71.42%)	1(100%)	2(100%)	7(87.50%)	31(77.5%)
NV bundles		00	17(60.71%)	1(100%)	2(100%)	6(75.00%)	26(65.00%)
Macrophages Arrector pilorum	**around**	1(100%)	4(14.29%)	00	00	1(12.50%)	6 (15.00%)
Adnexa		1(100%)	12(42.85%)	00	2(100%)	5(62.5%)	20 (50.00%)
NV bundles		1(100%)	12(42.85%)	00	00	2(25.00%)	15 (37.50%)
Giant cells		00	14(50.00%)	00	2(100%)	2(25.00%)	18 (45.00%)
Granulomas		00	18(64.29%)	00	1(50.00%)	5(62.50%)	24(60.00%)

Table 6: Correlation of clinical and histopathological diagnosis of leprosy (n=40)

Histopathlogical diagnosis						
Clinical diagnosis	**BB**	**BL**	**BT**	**LL**	**TT**	**Total**
BL	0	0	0	1(100%)	0	1(100%)
BT	1(3.03%)	2(6.06%)	26(78.79%)	3(9.09%)	1(3.03%)	33(100%)
IL	0	0	0	0	0	0
LL	0	0	2(33.33%)	4(66.67%)	0	6(100%)
Total	1(2.50%)	2(5.00%)	28(70.00%)	8(20.00%)	1(2.50%)	40(100%)

Table 7: Comparison of histopathological types of leprosy

Types	**Nadakarni et al[8] (1999)**	**Moorthy NB et al[9] (2001)**	**Pandya AN et al[10] (2008)**	**Sharma A et al[11] (2008)**	**Mathur MC et al[12](2011)**	**Shivaswamy KN et al[13] (2012)**	**Present Study**
TT	460(17.4%)	26(6.98 %)	2(4.25%)	20(8.09%)	43(30.7 %)	25(18.4%)	1(2.5%)
BT	969(36.7%)	269(72.31%)	11(23.4%)	87(35.22%)	39(27.86%)	53(39.9%)	28(70%)
BB	326(12.3%)	2(0.53%)	3(6.38%)	45(18.21%)	7(5%)	2(3.6%)	1(2.5%)
BL	300(11.4%)	40(10.70%)	6(12.76%)	16(6.48%)	22(15.71%)	15(11%)	2(5%)
LL	165(6.3%)	10(2.69%)	10(21.27%)	25(10.12%)	21(15%)	19(13.9%)	8(20%)
IL	420(15.9%)	25(6.72%)	15(31.91%)	54(21.86%)	8(5.71%)	22(16.1%)	00(00%)
Total	2640	372	47	247	140	136	40

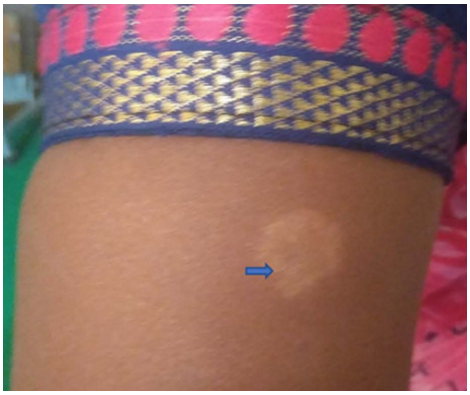

Fig. 3: Hypopigmented patch intuberculoid leprosy case

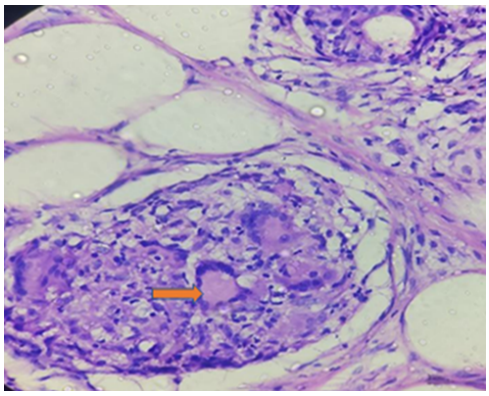

Fig. 4: Borderline TuberculoidCase: Section shows dense inflammatory infiltrate of lymphocytes Langhans type of giant cells, H & E stain High power (40x)

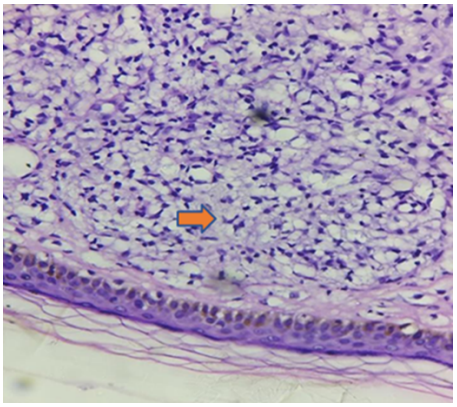

Fig. 5: Histoid Leprosy Case: Section showing atrophic epidermis and foamy macrophages in subepidermal region. H& E Stain High power (40x)

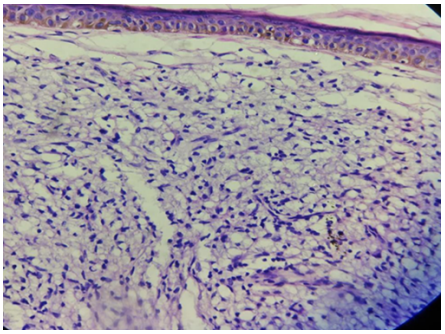

Fig. 6: Histoid Leprosy Case: Section showing clearing zone (Grenz zone) at Dermo-epidermal junction. H& E stain high power (40x)

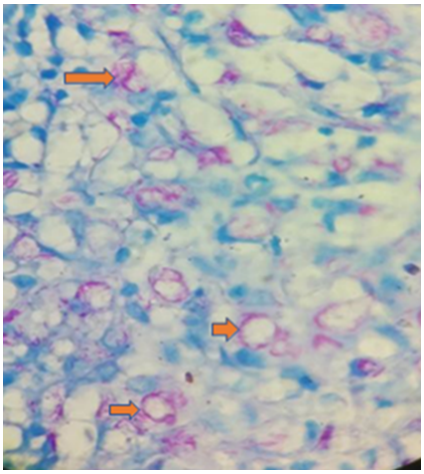

Fig. 7: Histoid leprosy Case: Section showing cigar shaped bundles of lepra bacilli in globi and clusters with positive AFB staining (100x)

5. Discussion

Histopathological examination of skin lesion is an important tool in accurate diagnosis and classification of leprosy and still remains the gold standard.

The present study was undertaken in the Department of Pathology, at DR SCGMC NANDED, over a period of 20 months from January 2019 to March 2020. The aim was to study the histopathological features of leprosy in skin biopsies and to categorize them into various types based on microscopy and to correlate them with clinical presentations whenever possible.

During the study period a total of 51 skin biopsies were received, among which 40 skin biopsies were of leprosy, which constituted (78.43%) of the total skin biopsies.

Out of total 51 clinically suspected leprosy cases, 11 were showing changes other than leprosy. So histologically 40 cases were diagnosed as leprosy.

The most commonly encountered type of leprosy was BT 28 biopsies (70%), second common type was LL 8 biopsies (20%), IL – 0 biopsies (0%) was the least encountered type.

Borderline group constituted the major spectrum 31 biopsies (77.5%), which included BT, BB, and BL similar to findings of other authors.

Increased awareness of the people to leprosy because of many national programmes makes them to present at an earlier stage to leprosy clinics, which may contribute to increased number of borderline group of leprosy.

6. Conclusion

1. A disease like leprosy needs an appropriate classification because of its varied clinical manifestations ranging from the presence of single hypopigmented patch to deformities.
2. The variation in different studies may be due to different criteria used to select the cases and difference in number of cases of each type. Various factors also influence the histopathological diagnosis such as differences in sample size, choosing the biopsy site, age of the lesion, immunological and treatment status of the patient at the time of biopsy.
3. There is no independent gold standard for diagnosis of leprosy. Considering any of the clinical signs, clinical types, histopathological parameters or histopathological types as a gold standard is not ideal
4. Clinical diagnosis of early leprosy lesions offer difficulties even to experienced dermatologists and leprologists. A definitive diagnosis may be possible by histopathological & bacteriological examination.

7. Conflict of Interest

The authors declare no relevant conflicts of interest.

8. Source of Funding

None.

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