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A study of clinico-pathological correlation of leprosy in a tertiary care center at Karamsad

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Abstract
Introduction: Leprosy is a chronic, infectious disease caused by Mycobacterium leprae. It is classified into five groups based on clinical, histological, microbiological and immunological criteria (Ridley & Jopling Classification).
Aims: To find the incidence of histopathological forms of leprosy, to categorize leprosy into various types based on microscopy and bacterial index in skin biopsies as well as with microbiological slit skin smear and to know the correlation between clinical and histopathological diagnosis of leprosy.
Materials and Methods: A cross-sectional study was conducted on 100 diagnosed cases of leprosy in department of Pathology, Shree Krishna Hospital, Karamsad, a tertiary centre in Anand, from December 2015 to July 2021.
Results: Total 100 cases of leprosy studied, majority of patients belonged in the age group 41-50 years with male predominance with most common presenting feature being hypopigmented anaesthetic patches and most common histological type of leprosy being borderline tuberculoid leprosy. The overall concordance between clinical and histopathological type was 73%. Maximum concordance was seen in tuberculoid leprosy (95.23%).
Conclusion: Most common histological type of leprosy was borderline tuberculoid leprosy and overall concordance between clinical and histopathological type was 73% with maximum concordance was seen in tuberculoid leprosy.

Keywords: leprosy, bacillary index, microbiological slit skin smear, Fite Faraco stain

1. Introduction
Leprosy is a chronic infectious disease caused by Mycobacterium Leprae. It is a slowly progressive, chronic granulomatous, infectious disease and affecting the skin, peripheral nervous system. It can affect any age and both sexes. It is also known as Hansen's disease and is indeed one of the oldest documented infectious diseases known to mankind [1].
The nine-banded armadillo (Dasypus novemcinctus) remains the only well-documented zoonotic reservoir of leprosy disease [2, 3]. The mode of transmission of leprosy is unknown, but the transmissibility is thought to be low. The mechanism is probably inhalation of bacilli, which may be excreted from the nasal passages of a multibacillary patient or possibly implanted from organisms in the soil. High numbers of bacilli of M. leprae have been documented in the nasal mucosa of infected patients (100 million bacilli) and bacterial load in the nose has been proposed to correlate with immune response in leprosy patients.[4] After inhalation, it is likely that bacilli pass through the blood to peripheral and cutaneous nerves, where infection and host reaction commence.
Cardinal signs of leprosy are hypopigmented or erythematous skin lesion with definite loss or impairment of sensation, thickening of peripheral nerves with sensory impairment and skin smear positive for acid-fast bacilli [5].
Leprosy can be diagnosed by various methods including detailed clinical examination of the skin lesions and peripheral nerves and demonstration of the Acid-Fast Bacilli (AFB) in microbiological slit skin smears by Ziehl-Nielsen staining and Modified Fite-Faraco Stain procedure, skin biopsy and histopathological examination is considered gold standard method [6].
Slit skin smears (SSS) is a safe and easy side laboratory-based technique. It is one of the three WHO cardinal signs of leprosy. It has a very high specificity and low sensitivity. Ziehl Nelson stain is used to detect acid-fast bacilli in the SSS smear.

It is used for to determine the disease activity in a patient, to assess progress of the disease and to help in follow-up patients on treatment.
Fite-Faraco stain detects M. leprae due to the acid-fastness of the bacilli due to the presence of mycolic acid in their cell wall.

2. Materials and Methods
The present study is hospital based cross-sectional study to know the correlation between clinical and histopathological diagnosis of leprosy. It is carried out at the department of Pathology, Shree Krishna Hospital, Karamsad, a tertiary centre in Anand. A total of 100 cases of leprosy reported at Pathology department during a period from December 2015 to July 2021 are enrolled in the study.
The Ridley Jopling criteria are used to diagnose and classify leprosy clinically and histopathologically. All the biopsies are fixed in 10% formalin. Serial sections of 5µ thickness are cut and stained with Haematoxylin and Eosin (H&E) along with Fite-Faraco to demonstrate Acid Fast Bacilli.

2.1 Inclusion criteria
Cases where histopathological diagnosis of leprosy is made or considered in the differential diagnosis irrespective of age and sex of the patient or nature of the lesion, are selected for the study.

2.2 Exclusion criteria
Those cases where leprosy is suspected clinically but not confirmed on biopsy are not included. Lepra reactions, already diagnosed and confirmed cases of leprosy are excluded.

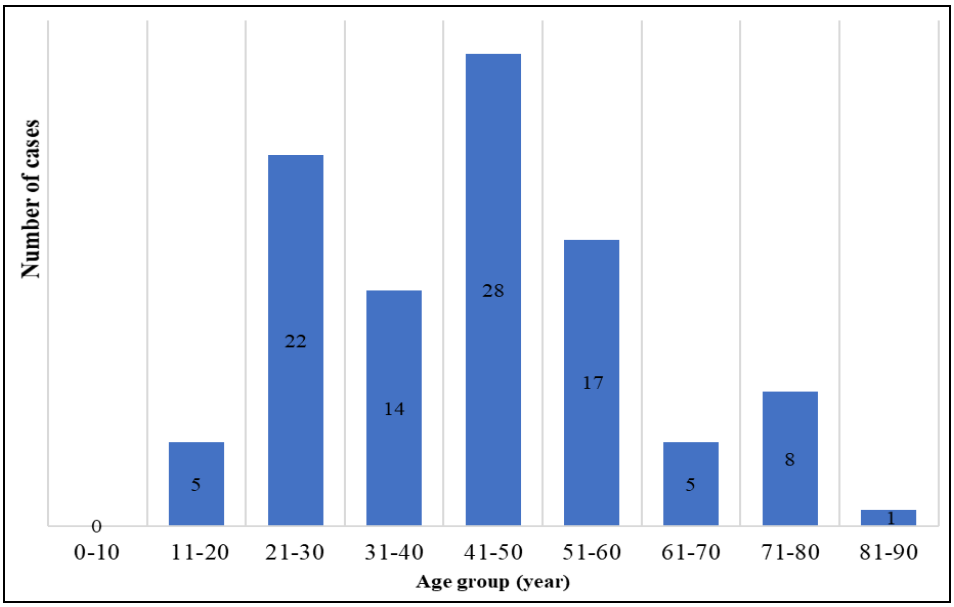
2.3 Operational definition for clinicopathological correlation
Clinicopathological correlation was defined for statistical analysis as: The requisition form should have leprosy as one of the two/three differential clinical diagnosis to be considered as clinicopathologically correlating.

2.4 Statistical analysis
The data is entered and analysed in Microsoft Excel. Descriptive statistics [Mean (SD), Frequency (%)] were used for present profile of study participant, types of leprosy and clinical findings of skin lesion. The Kappa statistics was used to assess agreement between clinical and histological diagnosis.

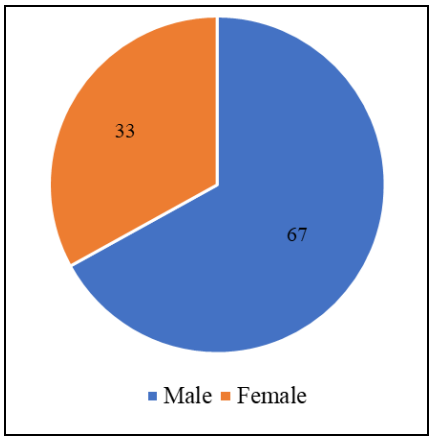
3. Results
A total 100 cases of leprosy studied, most common age group of patients is 41-50 years and the least common age group is 81-90 years as shown in Graph 1. Males are affected more as compared to female with M:F ratio of 2:1 as shown in Graph 2.
Out of total 100 cases of leprosy, 49 cases (49%) presented with hypopigmented anaesthetic patches, 34 cases (34%) presented with erythematous plaques, 8 cases (8%) presented with erythematous nodules, 8 cases (8%) presented with erythematous papules and a single case (1%) presented with erythematous macules as shown in Graph 3.
Out of 100 cases of leprosy, 37 cases (37%) were diagnosed as borderline tuberculoid leprosy, 32 cases (32%) were diagnosed as tuberculoid leprosy, 15 cases (15%) were diagnosed as borderline lepromatous leprosy, 12 cases (12%) were diagnosed as lepromatous leprosy, 3 cases (3%) were diagnosed as indeterminate leprosy and a single case (1%) was diagnosed as histoid leprosy using routine Haematoxylin and Eosin (H&E) stained slides as shown in Table 1.
Histological changes observed in epidermis is as shown in Table 2. Out of 32 cases of tuberculoid leprosy, atrophy/thinning was seen in 24 cases while 8 cases show unremarkable histology. Out of 37 cases of borderline tuberculoid leprosy, atrophy/thinning was seen in 31 cases while 6 cases show unremarkable histology. Out of 15 cases of borderline lepromatous leprosy, atrophy/thinning was seen in 14 cases while 1 case shows unremarkable histology. Out of 12 cases of lepromatous leprosy, atrophy/thinning was seen in 10 cases while 2 cases show unremarkable histology. Out of 3 cases of indeterminate leprosy, atrophy/thinning was seen in 2 cases while 1 case shows unremarkable histology. In the only case of histoid leprosy, atrophy/thinning was seen. Not a single case presented with ulceration/erosion.
Histological changes observed in dermis is as shown in Table 3. In present study of leprosy, total cases of tuberculoid leprosy were 32 (32%). Most significant dermal change seen in tuberculoid leprosy was epithelioid granuloma (93%) followed by giant cell reaction (87%) while least common change was grenz zone (3%). Total cases of borderline tuberculoid leprosy were 37 (37%). Most significant dermal change seen in borderline tuberculoid leprosy was epithelioid granuloma (86%) followed by perivascular lymphocyte (72%) while least common change was grenz zone (10%). Total cases of borderline lepromatous leprosy were 15 (15%). Most significant dermal change seen in borderline lepromatous leprosy was macrophage (80%) followed by perivascular lymphocyte and epithelioid granuloma (73%) while not a single case presented with giant cell reaction. Total cases of lepromatous leprosy were 12 (12%). Most significant dermal change seen in lepromatous leprosy was grenz zone (100%) and macrophage (100%) followed by while least common change was peri appendageal lymphocyte (41%) and perineural lymphocyte (41%) and not a single case presented with epithelioid granuloma and giant cell reaction. In a single case of histoid leprosy, dermal changes showed sheets of histiocytes, peri appendageal lymphocyte, perineural lymphocyte. In three cases of indeterminate leprosy dermal changes showed peri appendageal lymphocyte, perineural lymphocyte, perivascular lymphocyte and macrophage. Hence, most common dermal change in all types of leprosy was epithelioid granuloma and least common dermal change was grenz zone.
Out of total 100 cases, slit skin smears were done in 62 cases of which 16 cases (25.80%) showed positivity as shown in Table 4.
Out of total 100 cases, Fite-Faraco staining was performed on 62 cases of which 24 cases (38.70%) were positive for acid fast bacilli with BI of 1-6, while 38 cases (61.30%) were negative for acid fast bacilli with BI of 0 as shown in Table 5.
There were 5 cases in whom acid fast bacilli were detected by slit skin smear but not by Fite-Faraco stain because of the bacillary load in the biopsy sample was less as it was taken from a single site whereas slit skin smears were taken from multiple sites, while in 11 cases acid fast bacilli were detected by Fite-Faraco stain but not by slit skin smear. In 9 cases, acid fast bacilli were detected by both the methods slit skin smear as well as Fite-Faraco stain, while in 29 cases no

acid-fast bacilli were detected by either Fite-Faraco stain or slit skin smear method as shown in Table 6.

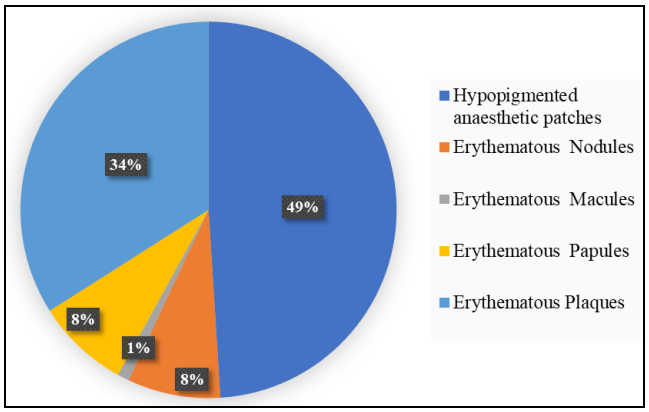
Percent of complete agreement between the clinical and histopathological types was 73%. Strong correlation was noted in tuberculoid leprosy 95.23%, followed by borderline tuberculoid leprosy and borderline lepromatous leprosy (83.33% for each). The correlation was weak in the lepromatous leprosy, histoid leprosy and indeterminate leprosy as shown in Table 7



Graph 1: Distribution of Leprosy cases according to age group



Graph 2: Distribution of Leprosy cases according to gender.



Graph 3: Percentage of various types of skin lesions on clinical examination**.**

Table 1: Incidence of histological types of leprosy

Types of leprosy	Percentage (%)
Borderline tuberculoid	37%
Tuberculoid	32%
Borderline lepromatous	15%
Lepromatous	12%
Indeterminate	3%
Histoid	1%
Total	100%

Table 2: Histological changes observe in epidermis

Epidermal change	Tuberculoid	Borderline Tuberculoid	Borderline Lepromatous	Lepromatous	Indeterminate	Histoid
Atrophy/ Thinning	24	31	14	10	2	1
Unremarkable	8	6	1	2	1	0
Ulceration/erosion	0	0	0	0	0	0
Total	32	37	15	12	3	1

Table 3: Histological changes observe in dermis

Dermal change	Tuberculoid	Borderline Tuberculoid	Borderline Lepromatous	Lepromatous	Histoid	Indeterminate
Epithelioid granuloma	30	32	11	0	0	0
Giant cell	28	16	0	0	0	0
Periappendageal lymphocyte	13	25	7	5	1	3
Perivascular lymphocyte	12	27	11	6	0	3
Perineural lymphocyte	11	25	6	5	1	3
Macrophages	9	18	12	12	1	3
Grenze zone	1	4	7	12	0	0

Table 4: Microbiological slit skin smear findings positivity in different histological types of leprosy

Histological diagnosis of leprosy	Number of positive Microbiological slit skin smears	Percentage
Tuberculoid	4 out of 20	20%
Borderline Tuberculoid	4 out of 22	18.18%
Borderline Lepromatous	3 out of 11	27.27%
Lepromatous	4 out of 8	50%
Histoid	1 out of 1	100%
Total	16 out of 62	25.80%

Table 5: Fite-Faraco positivity and bacillary index in different histological types of leprosy

Histological diagnosis of leprosy	Bacillary Index							Total
	0	1+	2+	3+	4+	5+	6+	
Tuberculoid	17 (94.45%)	1 (5.55%)	-	-	-	-	-	1/18
Borderline Tuberculoid	17 (80.95%)	3 (14.28%)	-	-	1 (4.76%)	-	-	4/21
Borderline Lepromatous	2 (16.66%)	-	2 (16.66%)	2 (16.66%)	3 (25%)	3 (25%)	-	10/12
Lepromatous	1 (10%)	-	-	-	2 (20%)	3 (30%)	4 (40%)	9/10
Indeterminate	1 (100%)	-	-	-	-	-	-	0/1
Total	38	4	2	2	6	6	4	24/62

Table 6: Comparison between detection of Acid-Fast Bacilli stained by Fite-Faraco stain and slit skin smear method

		Fite Faraco stain		Total
		Positive	Negative	
Slit Skin smear	Positive	9	5	14
	Negative	11	24	35
Total		20	29	49

Table 7: Clinico-histopathological correlation of leprosy

Type of leprosy	Provisional Clinical Diagnosis	Histological Final Diagnosis							
		Tuberculoid	Borderline Tuberculoid	Borderline Lepromatous	Lepromatous	Histoid	Indeterminate	% of concordance	% of discordance
Tuberculoid	21	20	0	0	0	0	1	95.23% (20/21)	4.76% (1/21)
Borderline Tuberculoid	36	2	30	1	2	0	1	83.33% (30/36)	16.66% (6/36)
Borderline Lepromatous	18	1	2	15	0	0	0	83.33% (15/18)	16.66% (3/18)
Lepromatous	18	8	2	0	7	0	1	38.88% (7/18)	61.11% (11/18)
Histoid	6	0	2	0	3	1	0	16.66%(1/6)	83.33%(5/6)
Indeterminate	1	1	0	0	0	0	0	0% (0/1)	1% (1/1)
Total	100	32	36	16	12	1	3	73%	27%

6. Discussion

Leprosy or Hansen's disease is a slowly progressive, chronic infectious disease caused by the Mycobacterium leprae. It is exclusively a disease of humans. The only sources of infection is a leprosy patient. It is a serious, mutilating and stigmatizing disease in many countries. Early diagnosis and treatment are the most important strategies for its control. It is an important public health problem in our country, with highest prevalence in Uttar Pradesh, Chhattisgarh, West Bengal, Bihar, Rajasthan and Maharashtra. The gold standard test for diagnosis is histopathological examination of skin lesions. It is an important tool in accurate diagnosis and classification of leprosy.

The present study assesses the correlation between clinical and histopathological diagnosis of leprosy and to categorize leprosy into various types based on microscopy, bacterial index in skin biopsies and microbiological slit skin smear examination.

The leprosy can affect all age groups. In the present study, majority of the patients were in the age group of 41-50 years. The similar studies done by Kumar Pokhrel *et al.* [7], Tiwari M *et al.* [8], Kaur *et al.* [9] and Nadia *et al.* [10] reported mean age of 31-40 years, 20-40 years, 30-39 years and 31-40 years respectively. Leprosy has a variable and long

incubation period, which is responsible for the wide age distribution.

The leprosy is common in males, with a male to female ratio of 2:1 in the present study, which is in concordance with study done by Nadia *et al*. [10] (2:1), Sikha Ghanghoria *et al*. [11] (2:1) and Neha Yadav *et al*. [12] (1.5:1), while Vasikar *et al*[13] reported slight female predominance (1:1.2). Male preponderance might be attributed to increase chances of exposure as males are more engaged in outdoor work than female. Social customs and taboos may mask actual number of cases in females.

Hypopigmented anaesthetic patches were the most common clinical presentation in present study (49%), the reason being skin and nerves are the most common sites of M. leprae infection, signs and symptoms related to the skin and nerves were common. These findings are in concordance with the studies done by Vasikar *et al*. [13] (65%), Nadia *et al*. [10] (56.7%), Jain Niharika *et al*. [14] (76%) and Manisha Atram *et al*. [15] (80.95%). Other clinical presentation in present study are erythematous plaques in 34% cases, erythematous nodules in 8% cases, erythematous papules in 8% cases and erythematous macules in 1% cases.

In present study, the most common histological type of leprosy is borderline tuberculoid (37%) which is comparable to studies done by Nadia *et al*. [10], Debeeka Hazarika *et al*. [16] and Vasikar *et al*. [13] whose cases are 34.7%, 41.66% and 35% respectively. The least common type of leprosy is histoid (1%) in present study. Neha Yadav *et al*. [12] reported lepromatous leprosy cases 38.70% as the most common type of leprosy, which could probably be due to the differences in the case selection criteria of the study.

In the present study most common histological change in epidermis is atrophy/thinning 82 cases (82%) while the rest of 18 cases (18%) show unremarkable histology. Jain Niharika *et al*. [14] reported unremarkable histology in majority of cases (51%), followed by thinned out epidermis in 49% cases. Manisha Atram *et al*. [15] also reported unremarkable histology in majority of cases (56.6%), followed by thinning and atrophy in 32.27% cases.

In present study, the most significant dermal change in all types of leprosy is epithelioid granuloma (73%) and least common dermal change is grenz zone (24%). Shikha Ghanghoria *et al*. [11] and Jain Niharika *et al*. [14] have reported similar findings with epithelioid granuloma (40% and 65% respectively), while Manisha Atram *et al*. [15] reported periappendgeal lymphocytes in majority of cases (66.66%).

In the present study of leprosy, microbiological slit skin smear examination has been done in 62 cases (62%), out of which, 16 cases (25.80%) are positive and 46 cases (74.19%) are negative for microbiological slit skin smear examination. The present study is in concordance with Shrestha A *et al*. [17] who had taken 50 cases of leprosy, out of which 25% were positive and 75% were negative for microbiological slit skin smear examination, while study done by Shruti Semwal *et al*. [18] who had taken 48 cases of leprosy**,** out of which 53.4% were positive and 46.6% were negative for microbiological slit skin smear examination. Slit-skin smear test helps in establishing an early diagnosis of Hansen's disease. Though this test has high specificity but low sensitivity [18]. The inherent problems of skin smears are in the techniques of sample collection, slides preparation and the subjective nature of the reporting.

In present study, we have observed 38.70% positivity for Fite-Faraco staining which is comparable with Shrestha A *et al*. [17] (33.33%) and Bahadure Sweta *et al*. [19] (21%), while Jain Niharika *et al*. [14] and Manisha Atram *et al*. [15] had 49% and 51.85% positivity for Fite-Faraco staining respectively.

In present study, in 49 cases both Fite-Faraco staining and Slit skin smear were examined, out of them 20 (40.81%) cases were positive for Fite-Faraco stain and 14 (28.57%) cases were positive for Slit skin smear. These findings are comparable to the studies done by Aishwarya Bhalchandra Patil *et al*. [20], who showed 16 (18.18%) cases were positive for Fite-Faraco stain and 19 (21.59%) cases were positive for Slit skin smear and Appannavar S *et al*. [21], who showed 26 (56.52%) cases positive for Fite-Faraco stain and 24 (52.17%) cases were positive for Slit skin smear. Low detection of Fite Faraco and slit skin smears could be explained by histopathological types of leprosy because in our study most common histopathological types of leprosy was borderline tuberculoid leprosy followed by tuberculoid leprosy. Such types of leprosy lack of AFB in tissues either due to killing mediated by immune responses.

We have observed 73% of concordance between clinical and histopathological diagnosis. A proper selection of representative lesion for biopsy might have been responsible for the high concordance rate in our study. Results of present study are comparable with Mathur MC *et al*. [22] (80.4%), B. Mehta *et al*. [23] (70%), K N Shivaswamy *et al*. [24] (74.7%) and B Chauhari *et al*. [25] (70.83%) while Kalyani Mitra *et al*. [26] had 57.6% clinic histopathological correlation. The discordance between clinical and histopathological diagnosis is in 27 cases (27%) noticed because the clinical examination only reflects the gross morphology of lesion, caused by the underlying pathology; whereas the specific histopathologic features in leprosy which are well defined, precise and indicate the accurate response of the tissue, while taking into account the immunologic manifestations.

In our study, highest percentage of Clinico-histopathological correlation is observed in TT (95.23%), followed by BT and BL (83.33% for each). Similar to our study B. Chauhari *et al*. [25] and Kansagra *et al*. [27] showed highest correlation in TT (86.2% and 100% respectively). But in contrary of our results, studies done by S. Bijjaraji *et al*. [28] and B. Mehta *et al*[23] reported highest clinico-histopathological correlation in LL (76.9% and 90% respectively), followed by TT (75% and 75% respectively).

7. Conclusion

Clinico-histopathological concordance was observed in 73% of cases and discordance was observed in 27% of cases. Highest Clinico-histopathological concordance found in tuberculoid leprosy was 95.23%.

Leprosy can be classified clinically by observing number and types of skin lesions and nerve involvement. However, for accurate typing of disease, biopsy and histopathological examination is considered gold standard.

Since no single criterion can be used to diagnose leprosy conclusively, so other contributory factors such as epithelioid granuloma, giant cell reaction, periappendgeal lymphocyte, perivascular lymphocyte, epidermal atrophy and bacillary index has a vital role to arrive at a definitive diagnosis of leprosy.

A combination of clinical diagnosis, histopathological examination along with microbiological slit skin smear examination and Fite-Faraco stain are imperative for proper treatment, prevention of complications and achieving

leprosy free world.

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