"Clinicopathological Correlation Of Different Types Of Leprosy, Based On Histomorphology, Special Stain And Immunofluorescence: In A Rural Setup From Central India"

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Abstract:

Background:

Leprosy is a chronic granulomatous disease and it manifests in varied clinical forms which can mimic many nonrelated inflammatory diseases. So early and precise diagnosis is very important for adequate and early treatment of disease, thus the study is undertaken to determine the extent to which clinical types are correlated with histopathological aspects, which is extremely crucial in patient management.

Aims: To establish histopathological types of leprosy cases on the basis of microscopy, special stain and immunofluorescence and to correlate it with clinical presentations.

Materials and Methods: In total 100 patients diagnosed clinically as leprosy were studied. Cases of all age groups were included. Skin biopsy were taken from suspected cases and subjected to H and E stain, fite faraco (FF) stain and immunofluorescence. P value was calculated and kappa statistics was used as a strength of agreement.

Results: Clinically maximum 30(30%) of the cases belongs to Borderline tuberculoid (BT) leprosy. The overall correlation of clinical diagnosis with histopathology was seen in 77(77%) cases. The utmost concordance (92.85%) was found in LL patients. By fite faraco staining, 21 cases out of 100 shows positivity and by auramine rhodamine staining 40 cases out of 100 shows positive results.

Conclusion: We noticed minor disagreement between clinical and histopathological diagnosis. So rather than using single criteria, other contributory factors like determination of bacillary index using special stain and immunofluorescence must be consider to arrive ultimate diagnosis.

Keywords: Auramine Rhodamine, Fite Faraco, Hematoxylin and Eosin, Leprosy.

Introduction

Leprosy is a chronic infectious granulomatous disease. Thirty percent patients are from India. [1] It is caused by mycobacterium leprae and principally affects skin, peripheral nervous system, bones, joints ,eyes ,testes etc.[2] Leprosy presents itself in various clinicopathological forms which depends on immune response of the host to the disease.[3] Diagnosis of leprosy is still based on clinical examination alone,[4] particularly in rural setup. To arrive at precise and reliable diagnosis, good histopathological examination of skin biopsy along with demonstration of bacilli in the sections must be implemented. Bacillary index is based on clinicopathologic type of leprosy and is important factor to determine patient outcome. Conventionally Ziehl – Nielsen's stain is used on slit skin smears to demonstrate acid fast bacilli, but many authors suggested that it has lower sensitivity when compared to fite faraco (FF) staining method. [5] FF stain is routinely used along with Hematoxylin and Eosin (H&E) stain to detect leprae bacilli in histopathological section.[6]In FF method, bacillary density required to see single bacilli is about 1000 per cubic millimetre. This leads to observer fatigue and thus false negative reporting. [7] Auramine rhodamine immunofluorescence (AR) study on tissue sections can overcome this problem. There are many studies showing comparison of FF and AR stain on slit skin smears, but studies on tissue sections are scarce in literature. [7]

Clinical classification use only gross appearance of the lesions for typing, but histopathological parameters are well defined, precise and consider immunological manifestation for classifying lesions. Histology also identifies response to treatment in term of progression and regression of lesion.[8] Thus this study was carried out to establish the importance of histopathological examination along with advance staining in diagnosing difficult leprosy cases where clinical diagnosis is not sufficient.

Methodology

After receiving approval from institutional ethical committee, this prospective, analytical study was conducted at our hospital over a period of two years. Patients with clinical diagnosis of leprosy visiting dermatology inpatient and outpatient department were enrolled for study. Patients already treated with anti-leprosy medications, biopsies which are inadequate not including part of subcutaneous fat, biopsies which didn't show histology of the leprosy (nonspecific), and biopsies which suggest leprae reactions were not included in this study. Total 135 skin biopsies were received in histopathology section, after exclusion 100 patients were studied.

After obtaining informed consent, leprosy cases were segregated clinically based on Ridley - Jopling scale and were subjected for biopsies from active skin lesions. Staining was carried out by H&E stain and special staining by FF and AR for identification of leprae bacilli by standard protocol. [7], [8], [9]For immunofluorescence study fluorescent microscope (Nikon – E 200 – with blue filter) was used. Skin biopsies of a typical LL and normal individual were used as controls. *Mycobacterium leprae* which appears solid, rod shaped and bright yellow green on fluorescent microscopy was considered diagnostic. Bacillary fragments were not taken into consideration. Bacillary index is determined by number of bacilli visualised in 100X objective in a limited microscopic field and is graded from zero to six+.

Statistical analysis: Data was collected and analysis was done by SPSS 20.0. P value was calculated for clinicopathological correlation and to compare bacillary index of FF and AR stain on sections by using chi-square test. Kappa statistics was used as a strength of agreement for clinical and histopathological correlation.

Observations and results:

This study included a total of 100 skin biopsies received from dermatology department and reported as leprosy on histopathological examination. The demographic data showed that age of the patients fall in between 08 years to 75 years, majority of them (20%) were seen in age group of 21- 30 years and minimum cases (2%) were present in an age group of less than ten years. TT is most common in age group of 31 – 40 years, BT is common in 21 – 30 years of age group, BB in 51 – 60 years, BL and LL in 61 – 70 years of age group [Table 1].

Table 1: Distribution of Histopathological diagnosis according to the age.

Sr.No.	Age (yrs)	Histological diagnosis						(%)
		TT	BT	BB	BL	LL	Total	
1.	0 – 10	01			01		02	02%
2.	11 – 20	06	01		01		08	08%
3.	21 – 30	04	09	01	02	04	20	20%
4.	31 – 40	07	04	01	01	04	17	17%
5.	41 – 50	06	05		01	06	18	18%
6.	51 – 60	05	03	02	01	05	16	16%
7.	61 – 70	01	04	01	03	08	17	17%
8.	71 – 80		01			01	02	02%
	Total	30	27	05	10	28	100	100%

(TT = Tuberculoid, BT = Borderline tuberculoid, BB = Borderline Borderline, BL = Borderline lepromatous, LL = Lepromatous)

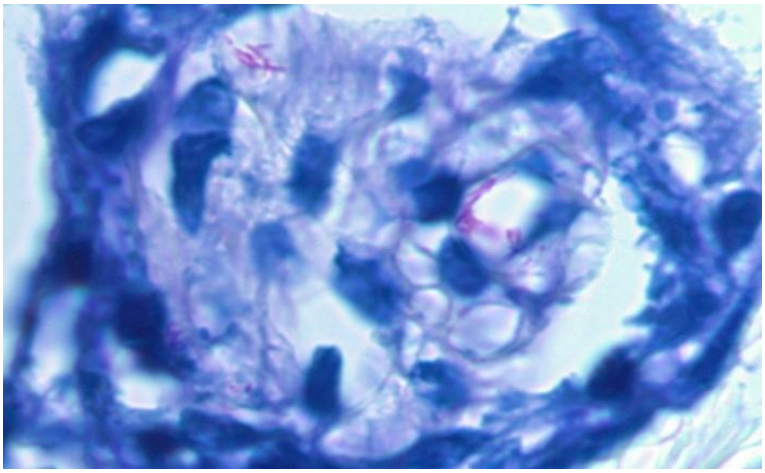
Out of 100 cases 70% were males. Clinically majority of the patients (30%) were from BT group. Whereas histologically majority of the cases (30%) belonged to TT group [Table 2].

Table 2: Distribution of the cases of Hansen`s disease

Type	TT	BT	BB	BL	LL
Clinical	29 (29%)	30 (30%)	03 (03%)	10 (10%)	28 (28%)
Histo-pathological	30 (30%)	27 (27%)	05 (05%)	10 (10%)	28 (28%)

All skin biopsies, 21 % were positive for acid fast bacilli in FF stain. None of the cases of TT and BT were positive for fite faraco. 1 out of 4 cases of BB, 3 out of 7 cases of BL and 17 out of 28 cases of LL shows positivity with fite faraco [Figure 1].

Photomicrograph 1: Fite Faraco stain showing leprae bacilli in lepromatous leprosy (H&E 100x)



Bacillary index (BI) was 3 or>3 in LL cases, 1 or <1 in TT cases and it ranges from 1-3 in borderline cases. From FF negative cases, 2 of 30 TT and 3 of 28 BT were AR positive. Out of 5 cases of midborderline leprosy, 3 cases were AR positive which included 1 case which was FF positive and 2 cases which were FF negative [Total 4] cases. Out of 10 cases of Borderline lepromatous leprosy, 7 cases were AR positive which included 3 cases which were FF positive and 4 cases which were FF negative [Total 7] cases. Out of 28 cases of Lepromatous leprosy, 25 cases were AR positive [Figure 2] which included 17 cases which were FF positive and 8 cases which were FF negative [Total 11] cases [Table 3].

Photomicrograph 2: Auramine Rhodamine staining – leprae bacilli (100x)

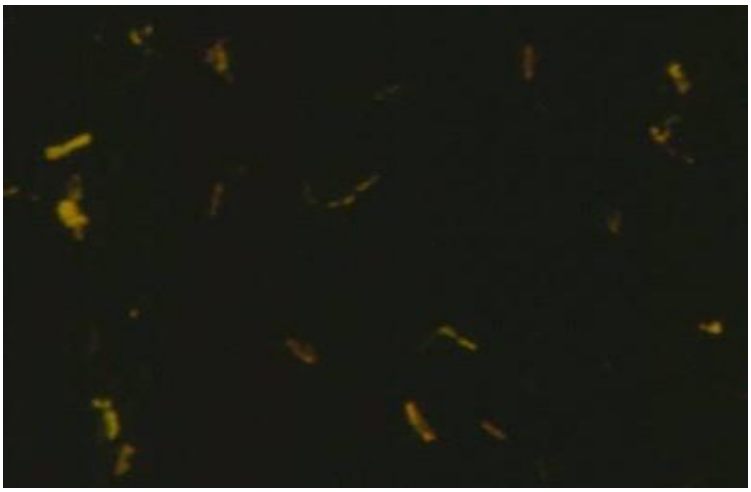


Table 3: Distribution of the cases of Hansen`s disease along with FFand AR staining results.

Sr.No.	Type of Leprosy	Histopathological diagnosis	FF Positive/Negative	AR Positive/Negative
1.	TT	30	00/30	02/28
2.	BT	27	00/27	03/24
3.	BB	05	01/04	03/02

4.	BL	10	03/07	07/03
5.	LL	28	17/11	25/03
	Total	100	21/79	40 /60

Leprosy cases diagnosed by AR were significantly more than FF (p value < 0.0003) [Table 4].

Table 4: Comparison of Fite Faraco staining results with Auramine Rhodamine results

Leprosy cases	FF positive	AR positive	P value *
100	21	40	0.0003

*P value (using z test for proportion)

Percent of complete agreement between the clinical and histopathological types was 77%. Strong correlation was noted at spectral ends, with 92.85% in LL and 79.31% in TT. The correlation was weak in the borderline leprosy with 66.66% in BT, 70% in BL and least, 33.33% in BB [Table 5].

Table 5: Clinical and Histopathological correlation of leprosy

Type	Clinical cases	Histopathological diagnosed cases					% of concordance	% of discordance	P value
		TT	BT	BB	BL	LL			
TT	29	23	06				79.31% (23/29)	20.68% (6/29)	<0.001
BT	30	07	20	03			66.66% (20/30)	33.33% (10/30)	0.01
BB	03		01	01	01		33.33% (01/03)	66.66% (02/03)	0.41
BL	10			01	07	02	70% (7/10)	30% (03/10)	0.07
LL	28				02	26	92.85% (26/28)	7.14% (2/28)	<0.001
Total	100	30	27	05	10	28	77%	23%	<0.001

Strength of agreement was almost perfect for LL leprosy and was substantial for TT and BL, Moderate for BT and was found lower in BB group[Table 6].

Table 6: Different subtypes of leprosy with Kappa statistics

Type of leprosy	**Kappa index value**	**Agreement**
TT	0.688	Substantial
BT	0.583	Moderate
BB	0.221	Fair
BL	0.667	Substantial
LL	0.901	Almost perfect

Discussion:

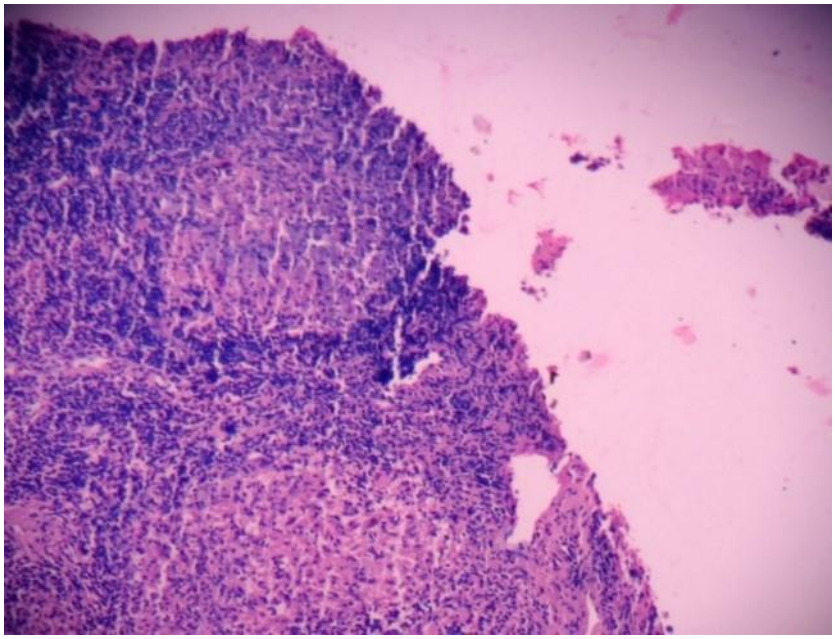
'**Leprosy work is not merely medical relief; it is transforming frustration in life into joy of dedication, personal ambition into selfless service.'**

Mahatma Gandhi.

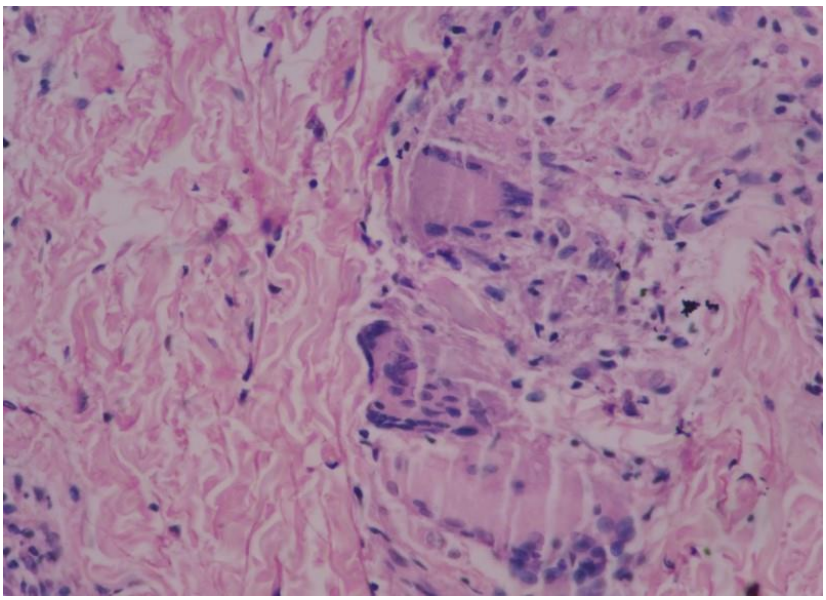
In few parts of world like Asia, Africa, and Latin America, leprosy still continues to be a major health problem. [10] It is discovered hundred years ago and achieved elimination at national level in 2006, still continues one of the major health problem in around 19% districts of developing India. Within Vidarbha region, Amravati, Gondia, and Wardha are the districts with high prevalence rate of more than one.[10] Many factors, such as movements of population from rural areas to urban areas and country to country, overcrowding, poverty, malnutrition and ultimately inadequate treatment accentuate the prevalence of the disease.[1] Clinicopathological discordance is commonly seen in leprosy patients and has an influence over their treatment. Disparity occurs as, clinically it has varied presentation and histopathological sections shows compact granuloma with giant cells at one end of spectrum and foamy macrophages with diffuse infiltration of dermis at other end, depending on immune response of host to the infectious agent M .leprae. Ridley and Jopling classification was commonly used by authors to type leprosy cases, as it is based on immunity of patient and is well correlated with clinical, pathological and bacterial findings. [4],[11] There are many studies concerned with the aim of correlating clinical and histopathological types of leprosy using Ridley and Jopling classification, but in our study we aimed at next level by using FF and AR to increase the accuracy in subtyping leprosy cases, as it is more reliable to use multiple parameters than using single one.

In our study, age of the patients varied from 08 years to 75 years. Most common age group affected by leprosy is 21to30 years and only 2% of cases belong to pediatric age group that is less than 10 years of age. This demographic distribution was similar to other studies. [12], [13], [14], [15], [16], [17], [18], [19], [20] This age distribution may be due to differences in exposure, opportunities for infection, immunological differences in children and adults. Other factors associated with leprosy like long and varied incubation period can be responsible for this age distribution. [19]. In this study 70% of the patients were males and 30% were females with the sex ratio of 2.3. This finding is similar to the studies done by other authors showing M:F of more than one,[12], [17], [19]whereas study did by Mathur MC et al,[14]Sunita Goyal et al,[18] showed equal incidence in both males and females. There is a slight female predominance in the study of Suri SK et al. [21] Males are commonly affected than females probably because of industrialization, urbanization and more opportunities for contact, whereas numbers may be lesser in females due to social factors leading to underreporting of the cases. [22] In our study, BT(30%) is most common clinical type of leprosy followed by TT (29%) and least common type is BB (03%). According to different studies BT was a most common subtype of leprosy. [15], [16], [17], [22] In contrast to our findings Kumar A et al, [22]found BB (25.10%) as a most common subtype of leprosy, whereas other researcher observed TT, [13], [14], [19]and LL, [18], [23] as a most common subtype. Similar to previous studies, [15], [16], [17], [20], [22],[23]we found that maximum cases belonged to borderline group. On histopathology the most common subtype is TT (30%) [Figure 3 & 4] followed by LL (28%)[Figure 5 & 6]. In our study we have not found any case of indeterminate leprosy. During histopathological examination, no case showed features of histoid leprosy.

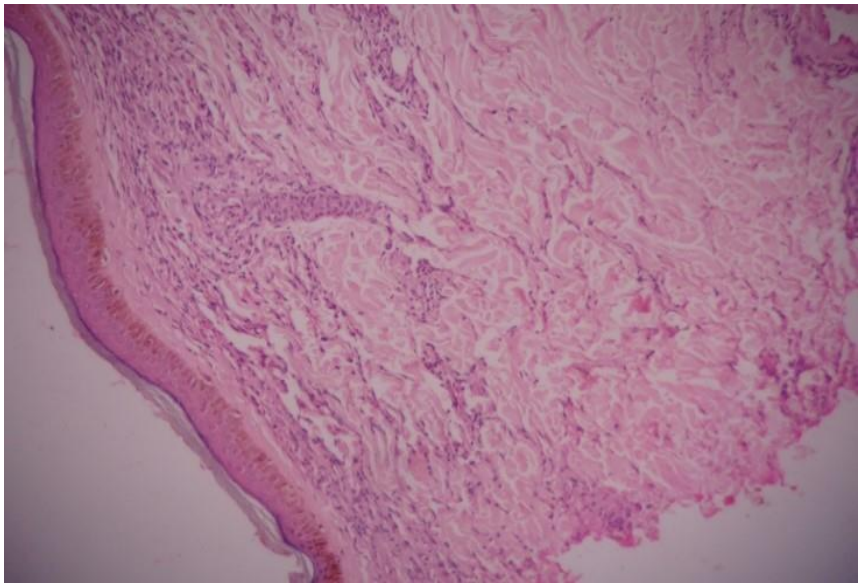
Photomicrograph 3:Tuberculoid leprosy (compact epitheloid granuloma)(H&E 10x)



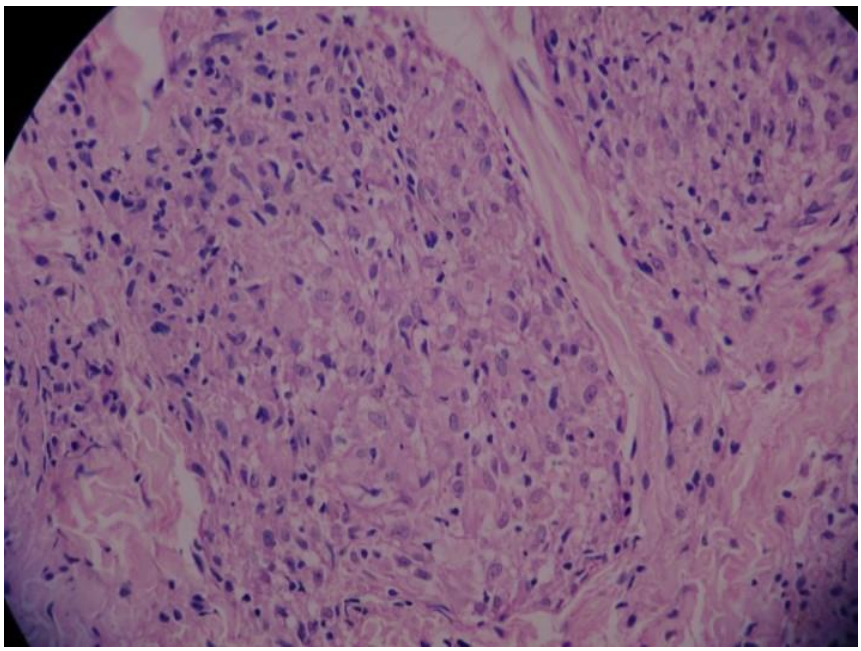
Photomicrograph 4:Tuberculoid leprosy (Langhans Giant cells) (H&E 40x)



Photomicrograph 5: Lepromatous Leprosy (Grenz zone and thinning of epidermis) (H&E 10x)



Photomicrograph 6: Lepromatous Leprosy (Foamy macrophages infiltrating the dermis) (H&E 40x)



According to many authors, clinical and histopathological concordance for different types of leprosy ranges from 33% - 82% [Table 7].

Table 7: Comparative study in clinicopathological correlation by different authors.

Various studies	Number of cases	Clinicohistopathological correlation
Moorthy BN et al,[12]	372	62.63%
Mathur MC et al,[14]	115	80.4%
S. Bijjaragi et al, [15]	171	57.3%
B. Mehta et al,[16]	100	70%
K N Shivaswamy et al,[17]	182	74.7%

Sunita Goyal et al,[18]	51	68.62%
Manandhar U et al,[19]	75	45.33%
Kumar A et al,[20]	423	62.90%
Suri SK et al,[21]	45	75.5%
RavneetBadhan et al,[22]	60	75%
B. Chauhari et al,[23]	120	70.83%
KalyaniMitra et al,[24]	92	57.6%
Bhatia AS et al,[25]	1272	69%
Present study	100	77%

We observed 77% of concordance between clinical and histopathological diagnosis which is better than the other studies. [15], [19],[24]A proper selection of optimum lesion for biopsy might have been responsible for the high concordance rate in our study. Utmost correlation was noted in LL (92.85%) followed by TT & BL [Figure 7].Similar to our study highest percentage of clinicopathological correlation was observed in LL followed by TT and least in midborderline leprosy [Table 8]. [12], [14], [15], [16], [17], [18], [22], [24]

Photomicrograph 7: Borderline Tuberculoid leprosy (Granulomas infiltrating dermis is) (H&E Scanner view)

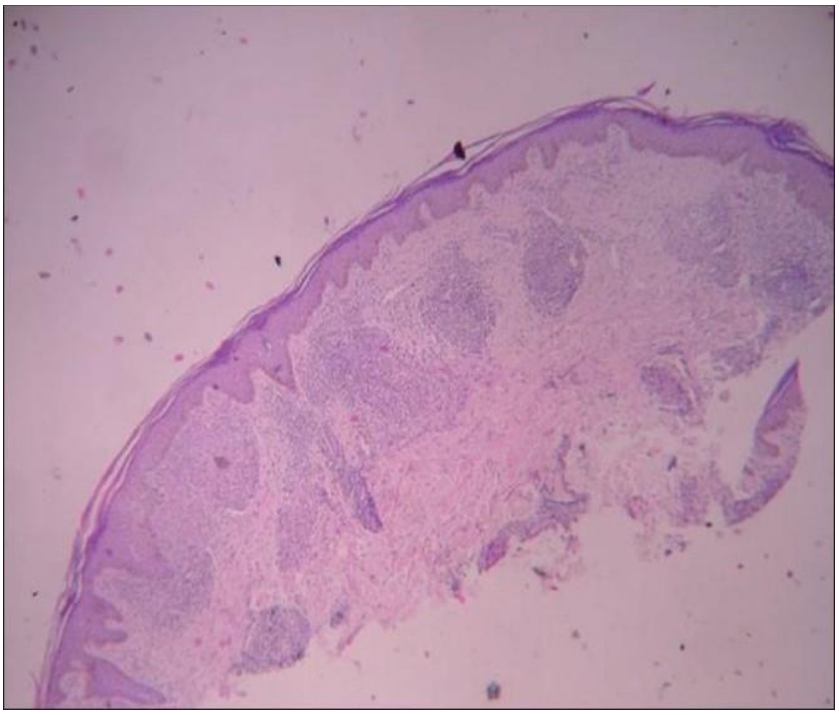


Table 8: A comparative study of correlation in different histopathological types of leprosy by various authors.

Comparative study	Present study	Manandhar U et al,[19]	Sunita Goyal et al,[18]	B. Mehta et al,[16]	S. Bijjaragi et al,[15]	B. Chauhari et al,[23]	Mathur MC et al,[14]	Moorthy BN et al,[12]
Year	2015	2013	2012	2012	2012	2012	2011	2001
No. of cases	100	75	51	100	171	120	156	372
TT	79.31%	24%	75%	75%	75%	86.2%	73.2%	46.15%

BT	66.66%	63.15%	33.33%	58.6%	57.3%	50%	89.7%	66.34%
BB	33.33%	0.00	20%	33.3%	16.7%	28.6%	64.7%	50%
BL	70%	57.14%	37.5%	71.4%	40%	63.3%	72.4%	70%
LL	92.85%	57.14%	85.2%	90%	76.9%	83.3%	95.2%	80%
Overall concordance (%)	77%	45.33%	68.62%	70%	57.3%	70.8%	80.4%	62.63%

In contrast Manandhar U et al,[19] showed better (63.15%) correlation in BT leprosy followed by LL (57.14%).

Instead of using simple agreement between yes and no, we used Kappa statistics to determine clinicopathological correlation. Kappa statistics was selected because it corrects for chance and is used to determine the strength of agreement between two diagnostic methods. In our study agreement was almost perfect for LL. Study did by Kumar et al had similar finding.[20]

Histopathologically change of classification from clinical diagnosis was seen maximum in midborderline (66%) than TT and LL, due to continuously changing immune system and precise clinical and histopathological criteria of diagnosing polar ends of spectrum. [20] There are various factors which leads to clinicopathological disparity like different clinical criteria's for case diagnosis, fewer number of cases in borderline group, early stage of the lesion, improper selection of site for biopsy, inadequate biopsy not involving full depth of dermis and subcutaneous tissue, poor quality of the section and stain, less number of acid fast stained sections examined, patient already on treatment and immunological status at the time of diagnosis. Clinical and histopathological interobserver variation also could be a reason for overlap between different types of leprosy. [19], [25] Proper selection of the site for biopsy is important in histopathological diagnosis since clinically dissimilar lesions biopsied from same patient can show different types of histopathology.[19]

As there are always some overlaps between different types of leprosy, both clinically and histopathologically, correlation of clinicopathological features along with bacteriological index is more useful for accurate typing of leprosy rather than considering only one parameter.[12] Slit-skin smears, nasal swabs and formalin fixed paraffin embedded tissue samples are used for determining bacteriological index by ZN and FF stain. ZN and FF staining method are easily available in resource limited centres but their performance in detecting leprae bacilli is poor, especially in paucibacillary patients. Hence more sensitive method is required to aid clinical diagnosis. AR staining is a fluorescence-based method commonly used to detect mycobacterial species such as M. tuberculosis and M. leprae. [26], [27], [28] By the fite faraco staining out of 100 cases, 21 cases showed positivity, whereas by auramine rhodamine staining, 40 cases showed positive results for presence of acid fast M.leprae bacilli. Two cases of TT and three cases of BT were positive by auramine rhodamine, which were negative with FF [Table 3]. In the above mentioned cases auramine rhodamine staining method detected more leprae bacilli than by the fite faraco method. Fluorescent stains were found to be more sensitive when compared to FF staining method in detecting M.leprae in tissue sections. [26-30] Fluorescent microscopy had the advantages of speed and ease of screening and thus reduces observer fatigue. Fluorescent method has high bacillary positivity rates as compare to FF method in each subtype of leprosy.[27] Presence of artefacts from albumin and phenol might have led to erroneous observation about superiority of FF method than fluorescent staining method.[31] Gupta et al studied the Oral Candida Prevalence and Species Specificity in Leprosy [32]. Few more

related articles on leprosy were reported[33-35]. Patil et al compared efficacy of slit skin smear and fite faraco stain on histopathology specimens in cases of leprosy[36].

Conclusion:

We conclude that clinicopathologic concordance is much better at polar ends of leprosy as compared to borderline type. Therefore all patients suspected of having leprosy clinically should be subjected for histopathological examination, so that there is better allotment of them to the treatment categories. Our study proved that fluorescent method is more sensitive for calculating bacillary index as compared to FF stain. It can be used as a supplementary tool when FF stain fail to detect bacilli. To control incidence of leprosy, deformities associated with it and further transmission in the society, it is necessary to execute before time diagnosis, accurate subtyping of cases histopathologically and utilising immunofluorescence for determining bacillary load is of extreme importance.

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