European Journal of Molecular & Clinical Medicine

ISSN 2515 – 8260 Volume 09, Issue 04, 2022

Original Research Article

An Understanding of A Clinico Pathological Correlation In Leprosy In A Tertiary Care Teaching Institution.

Nishant Saxena[1], Nimisha Saxena[2] Shilpa Mittal[3,] Harsh Sharma[4]*,

[1]Assistant Professor, [1]Dept. of ENT, G.S.V.M College and Hospital Research Center, Kanpur, Uttar Pradesh, India.

[2]Associate Professor, [2]Dept. of Biochemistry, K.D Medical College and Hospital Research Center, Mathura, Uttar Pradesh, India.

[3]Professor, [3]Dept. of Biochemistry, Al-falah School of Medical Science and Research Centre, Dhauj, Faridabad, Haryana, India.

[4]Associate professor, *Dept. of Dermatology, K.D Medical College and Hospital Research Center, Mathura, Uttar Pradesh, India.

Corresponding Author – Dr. Harsh Sharma, Associate Professor, Dept. of Dermatology, K.D Medical College and Hospital Research Center, Mathura, Uttar Pradesh, India.
Email I'd - drharsh1311@rediffmail.com

A B S T R A C T

Background: Leprosy caused by mycobacterium leprae is a chronic granulomatous disease that mainly affects peripheral nerves and skin. Depending upon the immune status of the individual it manifests various clinical and pathological forms. Histopathology plays an important role in the diagnosis of clinically suspicious cases and helps in exact classification of various subtypes and types which therefore helps in deciding the treatment plan and cure. Various inflammatory disorders also mimics clinically to leprosy therefore exact diagnosis plays an important role for early treatment. So this study is undertaken to correlate the clinical diagnosis with histopathological findings which plays crucial role in patient management.

AIM: To establish the correlation of clinically diagnosed cases of leprosy with histopathological findings with the help of microscopy, special stain and immunofluorescence.

Materials and Methods: This will be a prospective, cross-sectional study for a duration of 2 years (from June 2019 to June2021). All the newly diagnosed leprosy patients attending dermatology OPD will be enrolled for the study. An informed and written consent will be taken from each of them, following which relevant history concerning the disease will be recorded. All clinically diagnosed patients with Leprosy with age >18 years who are willing to give written informed consent to participate in the study will be included.
Out of total patients attending Derma OPD 100 patients were diagnosed clinically as leprosy from June 2019 to June 2021 . Cases with age above 18 years were included. Skin biopsy were taken and sent for Hand E stain, Fite Faraco (FF) stain and immunofluorescence. P value was deduced and kappa statistics was used to further strengthen the results.

Results: About 35% cases of leprosy belonged to borderline tuberculoid (BT). Out of this correlation of clinically diagnosed cases with histological findings was established in 80% cases. Maximum correlation was established in LL patients i.e 95%. fite faraco staining showed positivity in 25 out of 100 person and auramine rhodamine showed 46 out of 100.

Conclusion: Our study showed only minor disagreement between clinical findings and histopathological results. Therefore results of our study suggest that single criteria is not sufficient to support the diagnosis rather use of other contributory factors like bacillary index and immunofluorescence should also be taken into account to give conclusive diagnosis.

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

Introduction:

Leprosy is a chronic infectious granulomatous disease caused by Mycobacterium leprae affecting about 30% population, of India(1). Leprosy principally affects skin, peripheral nerves, bones, joints, testes and eyes (2). Immune response to leprosy varies in different individuals resulting in varied clinicopathological forms of Leprosy(3). In rural areas still only method available for diagnosis of leprosy is clinical examination alone(4), but to make precise diagnosis histopathological examination of skin biopsy and demonstration of bacilli in the sections provided is a must. Bacillary index is important to assess the outcome of the patient and this is dependant on clinicopathological type of leprosy. Ziehl-Nielson's stain is used on slit skin smear to demonstrate acid fast bacilli but Fite Faraco(FF) method(5) has better sensitivity as compared to Ziehl -Nielson. FF stain is used along with Hematoxylin and Eosin (6)to detect the bacilli in histopathological sections. FF stain method required minimum 1000 per cubic millimeter bacilli in order to see a single bacilli which is a cumbersome technique and can cause false reporting(7).to overcome this many studies have suggested Auramine rhodamine immunofluorescence (AR) study on tissue sections(8). Clinical diagnosis involves only gross examination whereas histopathological examination adds to more precise and well defined classification of lesions based on immunological response.it also helps in determining progression and regression of lesions(8). But biopsy alone cannot provide definitive diagnosis like in tuberculoid and indeterminate types. It needs a close communication between a pathologist and clinician for better understanding and classification of lesions and its subtypes. To further aid to diagnosis besides clinical examination and bacilloscopic examination, skin biopsy plays crucial role in differential diagnosis separating from other granulomatous lesions. Diagnosis of leprosy and classification of its subtypes not only requires clinical examination of skin lesions, peripheral nerve and skin smear examination but also needs histopathological examination. Therefore in doubtful cases like borderline cases confirmation is made not only on clinical examination but also with the help of histopathological examination. Still if some disparity exists more advanced diagnostic methods are taken into account and diagnosis and classification is made accordingly to prevent improper treatment. Ridley and Jopling described precise criteria for histopathological typing to define the subtypes of leprosy. Several studies have been conducted to show the correlation of clinically defined diagnosis of leprosy subtypes with histopathological defined subtypes of leprosy. Some studies showed strong association and need for histopathological classification of leprosy alongwith clinical diagnosis but some others show little advantage of histological classification so studies have not been uniform and shows disparity which sometimes require more advanced methods for the confirmation. Leprosy results in neural damage and disabilities and deformities and associated with social stigma therefore correct diagnosis plays a pivotal role in diagnosis , treatment and assessing the prognosis of the patient. This study has been conducted to show clinical and histopathological correlation to make a correct and definitive diagnosis and classification of leprosy .The aim of this study is show correlation of histopathological diagnosis with clinical diagnosis of leprosy.

Materials And Methods:

This is a cross-sectional comparative study conducted in K.D.M.C.H, Akbarpur, Mathura after taking approval from ethical committee with 100 cases studied during the duration of 2 years (from June 2019 to June2021) attending dermatology OPD . Patients undergoing treatment for leprosy or those which biopsies were not adequate showing leprae reactions were excluded from the study. Informed consent has been taken from all the patients segregated on the basis of Ridley -Jopling scale and were subjected for skin biopsies. Punch biopsies taken from newly clinically diagnosed cases with skin lesions and sent for histopathological examination with haematoxylin-eosin staining and Fite-Faraco stain. History of the patient taken, clinical examination of the skin lesion, type of skin lesion and slit skin smear (SSS) stained with Ziehl-Neelson stain were all recorded and kept for further reference. Histopathological examination and classification was done according to Ridley and Jopling. Mycobacterium leprae which appears solid,rod-shaped and bright yellow green on fluorescent microscopy was taken as diagnostic(7,8,9). Statistical analysis was done using SPSS 20.0. P value was calculated by using chi-square test. As a strength of agreement for clinical and histopathological correlation Kappa statistics was used.

Observations and results: This study included 100 skin biopsies from the patient attending dermatology department and diagnosed on this basis of clinical examination as leprosy and also confirmed by histopathological examination. Demographic datas were collected which shows that mainly patients of leprosy fall in age group of 9 years to 76 years , majority of them comes in age group of 21-30 years i.e 20% and only 2% in age group less than 10 years . the most common in age group 31-40 is TT whereas BT is most common in age 21-30 , BB in 51-60, and BL and LL in 61-70 age group.

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)

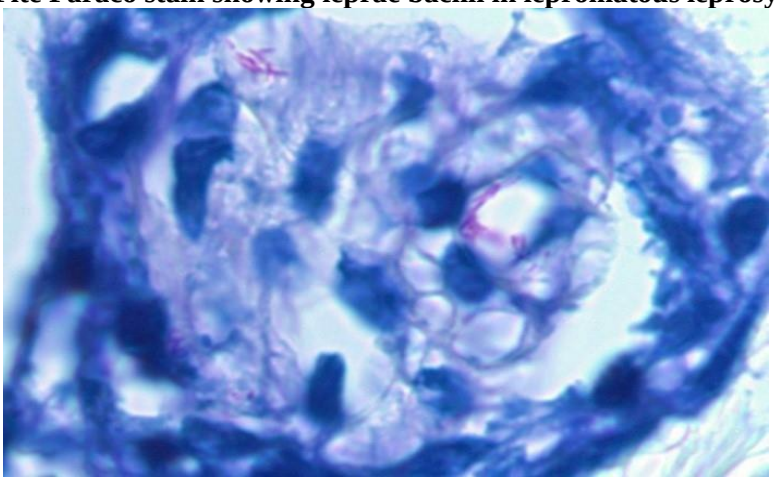
70% were males out of 100 cases taken, majority belonged to (30%) BT group. Whereas 30% belonged to TT in histological examination. [Table 2]

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%)

Skin biopsies taken from the patients showed 21% positivity for acid -fast bacilli in FF stain among these none were positive in FF stain for TT and BT.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 FF. [Figure 1].

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



We have determined bacillary index, it was found to be 3 or >3 in LL , 1or < 1 in TT and in borderline it ranges between 1-3. Those who were negative in FF 2 of 30 TT and 3 of 28 BT were found to be AR positive. Midborderline cases were 5 out of which 3 cases were AR positive of which 1 case was also FF positive and 2 were FF negative[Total 4]cases. Out of 10 cases of borderline leprosy ,7 were AR positive ,3 were FF positive and 4 were negative [Total 7] cases. Out of 28 cases of lepromatous leprosy 25 were AR positive [Figure 2] to which 17 were FF positive and 8 were FF negative[Total 11]cases.[Table 3].

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

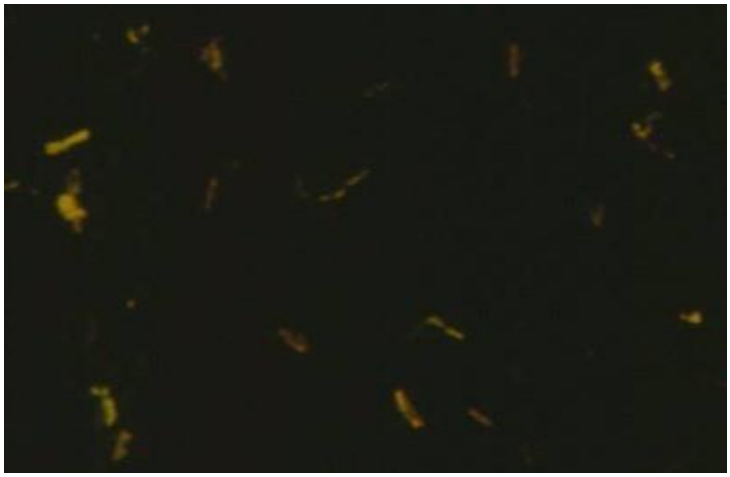


Table 3: Distribution of the cases of Hansen`s disease along with FF and AR staining results.

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) 77% Percent of agreement was found between the clinical and histopathological types .Strong correlation was seen at polar ends of leprosy , with 92.85% in LL and 79.31% in TT. The weak correlation was seen 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

Good strength of agreement was seen for LL leprosy and for TT and BL, moderate for BT and was found lowest 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 poses as major health issues in some parts of the world like Asia, Africa and Latin America(10). Leprosy was eradicated in 2006, still it continues to be major health issues in several districts in developing India. The highest prevalence rate is seen in some of the districts like Vidarbha, Amravati, Gondia and Wardha with more than one(10). The prevalence is influenced by many factors like shifting of population from rural areas to urban areas country to country other factors also play important role like overcrowding, malnutrition, poverty and inadequate treatment increases prevalence of the disease(1). Clinicopathological disparity is commonly seen in leprosy which otherwise influence the treatment of the patient. Third disparity is owing to different clinical presentation and histopathological sections showing compact granuloma with gaint cells at one end and foamy macrophages with diffuse infiltration of dermis at the other end which is due to difference in immune response varying from individual to individual to M. leprae. Lepsrosy cases are classified based on the immune response according to Ridley and Jopling classification. This is in accordance with the clinical , pathological and bacterial findings(4,11). This study not only takes into account of Ridley and Jopling classification but also aims at a higher level and incorporates FF and AR to increase the level of accuracy in classifying the subtypes of leprosy as it is more wise to use multiple criterias than relying on one. Leprosy cases are classified 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 this study we have choosen the age group from 8 to 78 years . Most commonly affected age group falls in age group of 21-30 years. It has been seen that less than 2% belongs to age less than 10 years . this demographic distribution of age has been seen in other studies as well(12,13,14,15,16,17,18,19,20). This age distribution of leprosy can be attributed to difference in exposure and immune response which leads to varied opportunity for infection in children and adult. There are some other factors which also contributes to this age distribution like long and varied incubation period(19). In this study there were 70% were males and 30% were females. This is similar to other studies where M:F ratio was found quite similar to this i.e males more than females affected with this disease(12,17,19) . Only one study done by Mathur MC et al (14)Sunita Goyal et al (18)showed equal incidence in male and females. One study done by Suri SK et al(21) showed slight female preponderance . Explanation to this male preponderance is probably more exposure of males to industrialization ,urbanization owing to more contact at job places and outdoor chores whereas females may be lesser numbers due to less exposures as most of them are confined to indoor activities and social factors leading to less reporting also. The most type of leprosy is BT(30%) followed by TT(29%) and the least common is BB(03%) .All the studies done previously also show that BT is the common type of leprosy(15,16,17,22) whereas some researchers also found that TT(13,14,19) and LL(18,23) as the most common subtype. In our study we found borderline as the most common subtype . But contrary to this in histopathology the most common type is TT(30%)[Figure3&4] followed by LL(28%)[Figure 5& 6] . whereas no case showed the histoid leprosy in our study. Out of all clinically classified cases of BT ,three were found as TT and two were of BL on histopathology. Whereas out of 25 clinically diagnosed case of TT , 13 were defines as BT and three cases on histopath showed no evidence of leprosy, they were reported as superficial perivascular dermatitis. Clinically and histopathologically both BT and TT have only slight difference . On clinical examination both of these entities have well -defined lesions with partial or complete loss of sensation and can present with or without thickening of the nerve and have scant acid fast bacilli. On histopathological examination also both of these show similar granulomatous reactions so to differentiate between the two based on clinical and pathological findings is difficult. Therefore the present criteria is inadequate in differentiating these two. Minor disagreement between clinical and histopathological findings have been seen in one group in 29 cases(38.66%) and major disagreement in two or

more groups in 9 cases (12%) ,while in three cases of tuberculoid leprosy (4%) which were clinically diagnosed histology was found to be non-specific. Ridley and Jopling criteria found minor disagreement in 21 patients(25.6%) and major difference in 5 patients(6%).

In our study disagreement between clinical and histopathological diagnosis was seen in 5 cases (29%) of borderline lepromatous leprosy. Out of these 5cases 4 were clinically diagnosed as BL but on histopath were diagnosed as TT and one case was classified as BT. Similarly 3 cases of TT and 2 cases of BT showed features of indeterminate leprosy. Some authors also found similar variations in clinical and histopathological diagnosis in tuberculoid group of leprosy . it is found that many lepromatous and tuberculoid leprosy shows indeterminate histopathological features after treatment. One case of LL and three cases of BL were classified as BT leprosy. Similarly , Bhatia AS et. al showed two cases of BL and three cases of LL as BT on histopathology.

This discordance between clinical and histopathological diagnosis is because clinical diagnosis was made on the basis of Ridley -Jopling classification before the histopathological diagnosis was even made. Factors that influence histopathological diagnosis are selection of cases , number of cases of each type of leprosy , duration of the lesion, nature and depth of the biopsy .quality of the section taken ,number of acid-fast stained sections examined ,immunological status and type of treatment the patient is on. So the chances of difference between these two diagnostic modalities i.e clinical and histopathological is more when biopsy is taken at early stage. Of course interobserver variation is always there both at clinical and histopathological level(19,25). The discordance can also be attributed to the type and site of lesion and lepra reactions because site selection plays key role in histopathological diagnosis since clinically different lesions from the same patient when taken for biopsy shows different type of histopathology(19). Selection of site and type of lesion plays key role in making histopathological diagnosis. Different types of leprosy shows overlap both clinically histopathologically and immunologically therefore correlation of clinical ,histopathological features alongwith bacteriological index is more important in accurately diagnosing the type of leprosy than considering only one parameter for making the diagnosis(12).

Histopathological and clinical disconcordance was maximum seen in midborderline (66%) compared to TT and LL due to differential immune response and histopathological criteria for differentiating polar ends of leprosy(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]

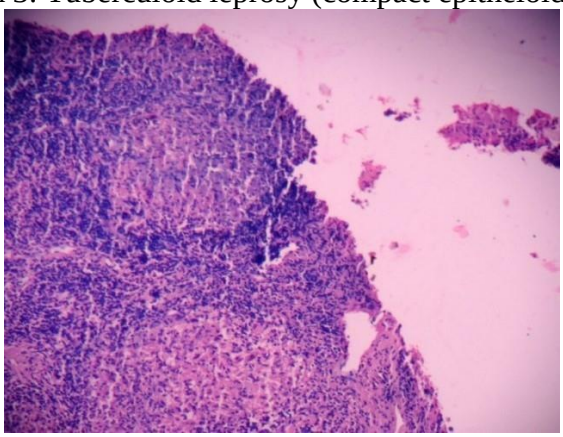
As there are always some overlaps between different types of leprosy, both clinically and histopathologically, correlation of clinicopathological features along with bacteriological index is more useful for accurate typing of leprosy rather than considering only one parameter.[12]

Various diagnostic modalities are available like slit -skin smears, nasal swabs and formalin fixed paraffin embedded tissue for determining bacteriological index by ZN nad FF staining but in paucibacillary leprae cases they detection rate is very poor so nowadays AR staining is used which is more sensitive to aid clinical diagnosis. AR is a fluorescence -based method to detect leprae bacilli(26,27,28).

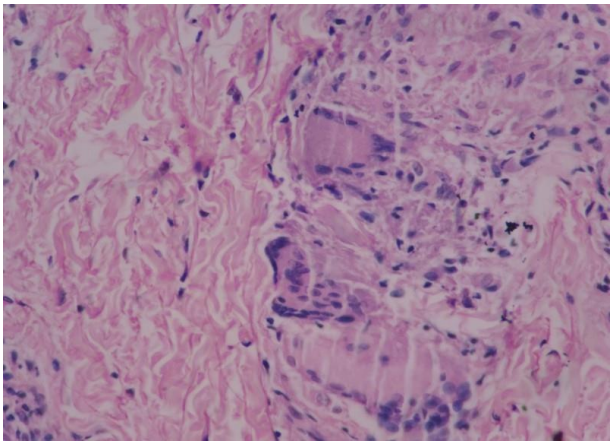
Out of 100 cases ,AR method showed 40 positive cases whereas FF stain showed only 21 cases as positive for M. leprae bacilli. Two cases of TT and three cases of BT were positive by AR which showed negative result with FF stain.[Table3].Above results shows that AR method of detection is more sensitive compared to FF staining method (26-30).As FF stain uses albumin and phenol which can lead to erroneous observation (31). Gupta et al studied oral candida prevalence and species specificity in leprosy(32). Various articles related to

leprosy were also reported (33-35)

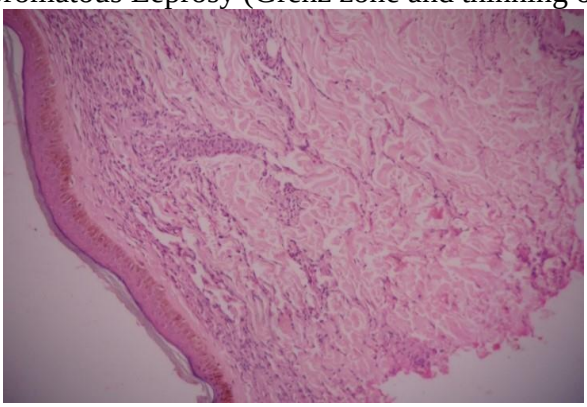
Photomicrograph 3: Tuberculoid leprosy (compact epitheloid granuloma)(H&E 10x)



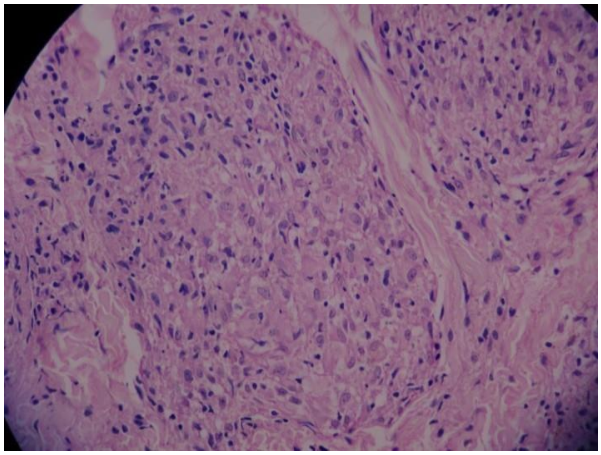
Photomicrograph 4: Tuberculoid leprosy (Langhans Giant cells) (H&E 40x)



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%

This study showed 77% of concordance between clinical and histopathological diagnosis which is better than the other studies. [15], [19],[24] This can be attributed to proper selection of the site of biopsy which results in high concordance rate of our study. Utmost correlation was noted in LL (92.85%) followed by TT & BL [Figure 7]. Other studies also showed highest percentage of concordance in LL followed by TT and least is shown in mid borderline leprosy. [Table 8]. [12], [14], [15], [16], [17], [18], [22], [24]

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

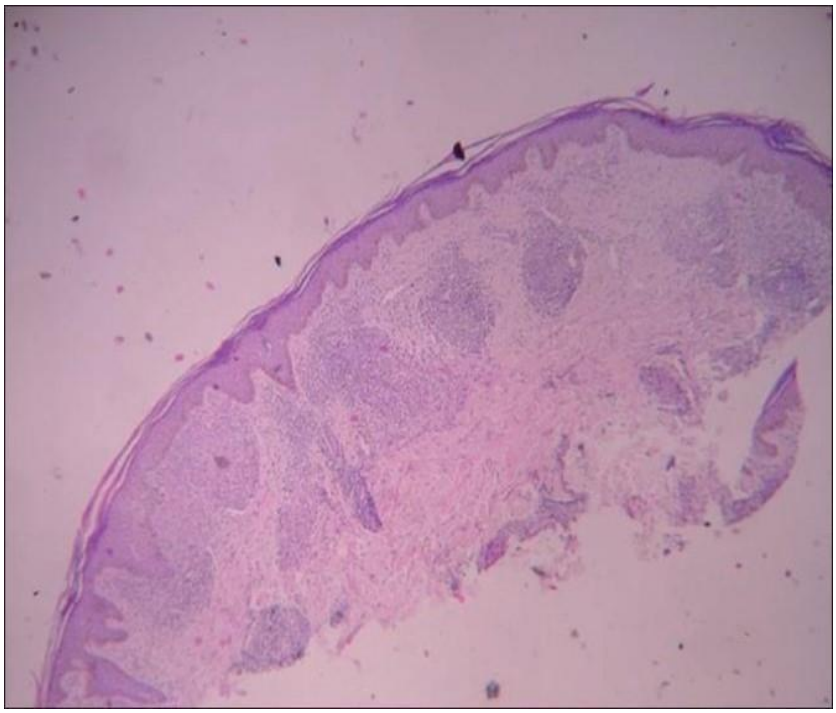


Table 8: A comparative study of correlation in different histopathological types of leprosy by various authors.

Comparativ estudy	Present study	Manandhar Uet al,[19]	Sunita Goyal et al,[18]	B. Mehta et al,[16]	S. Bijjaragiet 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%

This study used kappa statistics for determining clinicopathological correlation.

Conclusion:

This study concludes that clinical and histopathological findings shows concordance at the polar ends of leprosy when compared with the borderline cases therefore all clinically diagnosed cases of leprosy must be subjected to histopathological examination for better and accurate diagnosis and treatment. In this study we also found that fluorescent methods are better than FF staining, so that it can be used as supplement tool for the FF stain for detection of bacilli.. It is important to control leprosy , associated deformities for better quality of life . Leprosy transmission must be controlled, so to achieve this it is important that timely and accurate diagnosed must be made using various modalities available as single parameter cannot be relied upon to decide for subtyping of leprosy therefore clinical diagnosis alongwith histopathological examination and also utilizing immunofluorescence for determining bacillary load is of extreme importance.

CONFLICTS OF INTERESTS:

There are no conflicts of interest.

References

1. Walker SL, Lockwood DN. The clinical and immunological features of leprosy. Br Med Bull 2006;78:103-21.
2. Panday AN, Tailor HJ. Clinocohistopathological correlation of leprosy.IndJ DermatolVenerolLeprol 2008;74:174-6.
3. Sengupta U. (2001). *IADVL Textbook and Atlas of Dermatology* (2nd ed., p. 1573). Bhalani publishing house.
4. Ridley DS, Jopling WHO, Classification of leprosy according to immunity: a five group system. Int J Lep. 1996;34:255-7.
5. Jopling, W., &Mcdougall, A. (1999). Hand book of leprosy (5th ed., p. 60). CBS.
6. Job CK, Chacko CJ. A modification of Fite`s stain for demonstration of M. leprae in tissue sections. Indian J Lepr.1986;58(1):17-8.
7. Deepa SA, Surekha B H et al.Evaluation of Fluorescent Staining for Diagnosis of Leprosy and its Impact on Grading of the Disease: Comparison with Conventional Staining, J ClinDiagn res. 2016;10(10):23-6.
8. Lucus SB, Ridley DS. Use of histopathology in leprosy dignosis and research. Lep Rev. 1989;60:257-62.
9. Bancroft JD, Gamble M. (2001). Theory and Practice of Histological Techniques (5th ed., pp. 127- 128). Churchill Livingstone.
10. Lastória JC, Abreu MA. Leprosy: review of the epidemiological, clinical, and etiopathogenic aspects -part 1. An Bras Dermatol. 2014;89(2):205-18.
11. Kuper SW, May JR. Detection of acid-fast organism in tissue sections by fluorescence microscopy. J PatholBacteriol. 1960;79:59-68.
12. Moorthy B N, Kumar P, Chatura K R, Chandrasekhar H R, Basavaraja P K. Histopathological correlation of skin biopsies in leprosy. Indian J DermatolVenereolLeprol 2001;67:299-301.
13. Jha R, Karki S. Limitations of clinico-histopathological correlation of skin biopsies in leprosy. JNepal Health Res Counc. 2010;8(1):40-3.
14. Mathur MC, Ghimire RB, Shrestha P, Kedia SK. Clinicohistopathological correlation in leprosy. Kathmandu Univ Med J (KUMJ). 2011;9(36):248-51.
15. Bijjaragi S, Kulkarni V, Suresh KK, Chatura KR and Kumar P. Correlation of clinical and histopathological classification of leprosy in post elimination era. Indian J Lepr. 2012;84:271-5.
16. Mehta B, Desai N, Khar S. Clinical and Pathological Correlation in Leprosy. The Internet Journal of Dermatology. 2012;9(1).

17. Shivaswamy KN, Shyamprasad AL, Sumathy TK, Ranganathan C, Agarwal V. Clinicohistopathological correlation in leprosy. Dermatol Online J. 2012;18(9):2
18. GoyalSunita, Shah N, Shah F R, Shah C K, Shah J M. Clinicopathological correlation with histomorphological spectrum and bacillary index of skin biopsy in leprosy. Biennial Journal ofGAPM, ISSN 2229-4074.
19. Manandhar U, RC Adhikari, G Sayami. Clinico-histopathological correlation of skin biopsies in leprosy. Journal of Pathology of Nepal. 2013;3,452-8.
20. Kumar A, Negi SR, Vaishnav K. A study of clinico-histopathological correlation of leprosy in a tertiary care hospital in western district of Rajasthan. J ResMed Den Sci. 2014;2(3):43-8.
21. Suri SK, Iyer RR, Patel DU, Bandil S, Baxi S. Histopathology and clinicohistopathological correlation in Hansen`s disease. J Res Med Den Sci. 2014; 2(1):37-44.
22. Badhan R, Kundal RK, Raj RT, Bahl RK, Bal MS. A clinico-pathological correlation study of leprosyin a tertiary care teaching institute in Northwest Punjab, India. American J Med Sci and Medicine. 2014;2(5):99-108.
23. Chauhari B, Mehta R. Clinicohistopathological correlation in Leprosy. Inter J Sci Res.2012;1(5):104- 5.
24. Mitra K, Biswas S, Saha B, Dasgupta A. Correlation between clinical and histopathological criteriafor the classification of leprosy. Indian J Dermatol. 2001; 46(3):135-7
25. Bhatia AS, Katoch K, Rangarajan B, Narayanan, Gopal R, Mukherjee A *et al*. Clinical and histopathological correlation in the classification of Leprosy. International Journal of Leprosy. 1993;61(3):433-8.
26. A N., ANagarajappa , D Prabhu. Sensitivity of Fluorescent Microscopy in detecting Mycobacterium leprae in tissue sections. The internet journal of pathology. 2010;11(2).
27. Nayak SV, Sivarudrappa AS, Mukkamil AS. Role of fluorescent microscopy in detecting Mycobacterium.Leprae in tissue sections.AnnDiagn Pathol.2003;7(2):78-81.
28. Jariwala HJ and Kelkar SS. Fluorescence microscopy for detection of M.leprae in tissue sections. Int JLepr Other Mycobact Dis. 1979;47(1):33-6.
29. Bhatia VN, Rao S, Saraswathi G. Auramine staining in histopathological sections. Indian J Lepr. 1987;59(4):386-8.
30. Mansfield RE. An improved method for the fluorochrome staining of mycobacteria in tissues and smears. Am J ClinPathol. 1970:53(3):394-406.
31. De Faria LL. Fluorescent staining of M.leprae in tissue sections comparison with FiteFaraco. Int J Lepr. 1974:42(1):52-4.
32. Gupta, Bharti, Shekhar Gupta, Minal Chaudhary, A. Thirumal Raj, Kamran Habib Awan, and Shankargouda Patil. “Oral Candida Prevalence and Species Specificity in Leprosy.” *DM DISEASE-A- MONTH* 66, no. 7, SI (July 2020). https://doi.org/10.1016/j.disamonth.2019.100920.
33. Singh, Adarsh Lata, S. J. Vagha, Amit Agrawal, S. R. Joharapurkar, and Brij Raj Singh. “Current Scenario of Leprosy at Tertiary Care Level Hospital of Rural Central India.” *INDIAN JOURNAL OF DERMATOLOGY VENEREOLOGY & LEPROLOGY* 75, no. 5 (October 2009): 520–22. https://doi.org/10.4103/0378-6323.55409.
34. Van Veen, Natasja H. J., Ton A. R. Schreuders, Willem J. Theuvenet, Amit Agrawal, and Jan Hendrik Richardus. “Decompressive Surgery for Treating Nerve Damage in Leprosy.” COCHRANE DATABASE OF SYSTEMATIC REVIEWS, no.1(2009). https://doi.org/10.1002/14651858.CD006983.pub2.
35. Zodpey, SP, BS Bansod, SN Shrikhande, BR Maldhure, and SW Kulkarni. “Protective Effect of Bacillus Calmette Guerin (BCG) against Leprosy: A Population-Based Case-Control Study in Nagpur, India.” *LEPROSY REVIEW* 70, no. 3 (September 1999): 287–94.