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A study of histopathological analysis and clinico-pathological correlation of leprosy in urban industrial area

Tushar Kambale[1], Kanika Jain[1], Komal D Sawaimul[1,*], C R Gore[1]

[1] *Dept. of Pathology, Dr. D.Y Patil Medical College, Hospital and Research centre, Dr. D.Y Patil Vidyapeeth, Pimpri, Pune, Maharashtra, India*

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ABSTRACT

Background: Leprosy is a chronic infectious granulomatous disease caused by Mycobacterium leprae, with a prevalence rate corresponded to 0.2/10000. Skin and peripheral nerves are mainly affected exhibiting spectrum of clinical and histopathological features based on the immunological status of the individual. The diagnosis is made from adequate clinical information combined with histopathology.
Aims: The aim of study to asses the clinical and histopathological features and there correlation in diagnosing the cases of leprosy.
Materials and Methods: A cross-sectional study of 2 years was conducted on 99 cases of skin biopsies histopathologically diagnosed with leprosy. Adequate clinical history was obtained and biopsies were stained with hematoxylin and eosin and modified Fite Faraco stain. A clinicopathological correlation was then attempted along with statistical analysis.
Results: Out of the total 99 leprosy cases, maximum cases (34.34%) belonged to third decade of life with Male to Female ratio of 1.75:1. About 58.59% of the total cases of leprosy showed decreased sensations and nerve thickening was seen in 79.80% cases. Clinically, 45.46% lesions were erythematous plaques. Borderline tuberculoid leprosy (31.68%) was the most frequently diagnosed subtype of leprosy. Evaluation of agreement and correlation between clinical and histopathological classification of leprosy showed an overall agreement of 57.57%. Correlation was more in stable poles i.e. TT tuberculoid pole (TT) and lepromatous pole (LL).
Conclusion: Leprosy is still a prevalent disease which raises the concern about therapeutic approaches and various health programmes. It may not have a classical clinical picture always. Many factors contribute in clinicopathological discordance. Hence, diagnostic efficacy can be improved by clinic- histopathological corroboration which includes morphological examination of the skin lesion along with proper clinical history and correlation of these findings with histopathological examination findings.

1. Introduction

Leprosy or Hansen's disease is a chronic infectious granulomatous skin disease which is caused by Mycobacterium leprae affecting primarily the peripheral nerves, skin, mucous membranes and all other cooler parts of the body and having a high potential for disability which has significantly impact in developing countries.[1,2] Clinical, pathological and immuno-prognostic parameters seen in leprosy depend on the host immune response which also determines its classification.[1–3] In 2018, there were 2.08 lakh new cases of leprosy registered by WHO globally and the prevalence rate corresponded to 0.2/10000.[1] In 2017, India, Brazil and Indonesia accounted for 80.2% of the leprosy cases reported worldwide.[2]

* *Corresponding author*.
E-mail address: komal.sawaimul@dpu.edu.in (K. D. Sawaimul).

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A detailed clinical examination of skin lesions along with peripheral nerves and slit-skin smear examination is needed for the early diagnosis and adequate treatment in leprosy patients. However, in some early and borderline leprosy cases, clinical basis is not sufficient to label the case. Hence histopathological examination and demonstration of acid-fast bacilli is essential for confirmation of diagnosis in leprosy. Also labeling of cases as paucibacillary and multibacillary is a prerequisite as there is difference in the treatment regimens. Hence an unerring and early diagnosis is essential which can be achieved by correlating the clinical and histopathological features so that adequate treatment based on the type of leprosy can be given. [3,4]

The present study was undertaken to assess the clinical and histopathological features and there correlation in diagnosing the cases of leprosy.

2. Materials and Methods

A cross-sectional study of total 99 cases of skin biopsies which were clinically suspected and histopathologically confirmed as leprosy was carried out in the Department of Pathology, Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune, over a period of 2 years from September 2018 to August 2020. All the cases are taken from the urban industrial area of Pimpri region. In all the diagnosed cases of leprosy, case records were reviewed for the detailed clinical history including, age, sex, duration of disease, location, type of lesion, examination findings and clinical diagnosis.

2.1. Inclusion criteria

All the cases which are clinically suspected and confirmed histopathologically as leprosy.

2.2. Exclusion criteria

All the skin biopsy specimens with diagnosis other than leprosy were excluded.

All skin biopsy specimens were received in 10% formal saline and were fixed and processed. Multiple sections of approximately 5μm thick were prepared from the paraffin blocks with the help of microtome and stained with routine Hematoxylin and Eosin stain and Fite-Faraco stain for Acid Fast Bacilli. In addition, wherever available the corresponding slit-skin smear was also reviewed. Institute ethical committee clearance was obtained before the start of the study. Individual patient consent was also obtained.

Detailed histomorphological features were studied and diagnosis was made using the Ridley–Jopling scale, wherever applicable for the leprosy as tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL) and lepromatous leprosy (LL). A clinicopathological correlation was then attempted.

Statistical analysis was performed using suitable statistical methods.

3. Results

Out of total 538 skin biopsies received over 2 years period, 99 (18.40%) cases were clinically diagnosed and histopathologically proven as leprosy [Tables 1 and 2]. Maximum number of patients belonged to age group of 21-30 years (N=34; 34.34%) followed by 31-40 years (N=30; 30.30%). The age range of the study participants varied from 8 to 75 years with a mean age of 36.39 years. Out of total 99 cases, 63 (63.64%) cases were male while 36 (36.36%) were female with overall male to female ratio of 1.75:1 [Figure 1].

Clinically, the most common type of lesion seen were erythematous plaques (N=45; 45.46%) followed by patch (N=33; 33.33%) and nodule (N=11; 11.11%). Combinations of these lesions were seen in the remaining 10.10% cases. Multiple sites were mostly involved (34.34% cases) while upper extremity (22.22% cases) was the most common specific site involved followed by trunk (18.18% cases) and face (11.11%). Neck was the least common site of involvement (1.01%) [Figure 2]. About 58.59% of the total cases of leprosy had shown decreased sensations while remaining 41.41% had an intact sensation. Thickening of the nerve was seen in 79 (79.80%) cases while no nerve thickening was seen in the remaining 20 (20.20%) cases. [Table 3]

Based on these findings 32 cases (31.68%) were clinically diagnosed as Borderline Tuberculoid (BT) while 29 cases (28.71%) were diagnosed specifically as Intermediate (I) type of Hansens. This was followed by Lepromatous (LL) type of leprosy with 6 cases (6.46%) and the remaining 32 (31.68%) cases formed the other subtypes of leprosy.

The histopathological diagnosis of all 99 cases was made using the Ridley–Jopling scale wherever possible. These cases were histopathologically diagnosed as TT [Figure 3], BT [Figure 4], BB [Figure 5], BL [Figure 6], LL [Figure VII], HL, IL, lepra type 1 reaction and lepra type 2 (ENL) reaction subtypes of leprosy. Out of these, BT was the most common diagnosis (N=27; 27.30%) followed by indeterminate (IL) leprosy (N=23; 23.23%), LL (N=13; 13.13%), tuberculoid (TT) leprosy (N=1; 11.11%) and histioid (HL) leprosy and borderline lepromatous (BL) leprosy (N=8; 8.08% each). Reactions constituted 7.07% of the cases out of which type I reactions were 3.03% and ENL were diagnosed in 4.04% cases. There was only 1 case of pure neuritic (PN) leprosy and mid borderline (BB) leprosy. [Table 2]

Fite-Faraco staining [Figure VIII] for lepra bacilli (multibacillary) showed positive lepra bacilli in 47.48% cases and no bacilli (paucibacillary) in 52.52% cases. Bacillary index of 6 was seen in 10 (10.10%) cases of which

8 (80.00%) cases were of HL and 2 (20.00%) cases were of LL. Bacillary index of 0 was seen in 52 (52.52%) cases of which BT and IL had 18 (34.61%) cases each followed by TT with 11 (21.15%) cases. [Table 4]

Clinically, maximum cases i.e. 32 cases were diagnosed broadly as BT Hansens of which histologically BT was seen in 11 cases, 13 were IL cases, 1 was HL, 4 TT, 1 BL and 2 Type 2 lepra reaction. In case of 29 clinically diagnosed cases of IL, 6 cases showed concordance of 20.68% histopathologically. Out of 6 clinically diagnosed LL, 4 were histologically LL whereas 1 each turned out to be BT and IL. Thus clinico-pathological correlation in LL was 66.66%. Concordance in cases of TT was 85.71%, BL and BB was 66.66%, BT was 59.37%, PN was 50%, and IL was 55.17%.

On histopathological examination, changes in epidermis and dermis are seen on the skin biopsies. Changes in epidermis are either presence or absence of atrophy, hyperplasia, acanthosis, orthokeratosis, hyperkeratosis, ulceration and spongiosis. Changes seen in dermis are either presence or absence of perivascular and perineural inflammatory infiltrate, Grenz zone, ill-formed to well formed epithelioid cell granulomas, foamy histiocytes and Langhans giant cells. Epidermal atrophy was seen in 51 (51.51%) cases while 14 cases (14.14%) showed other epidermal changes like hyperkeratosis, spongiosis or orthokeratosis. Epithelioid granulomas are seen in 8(72.72%) of the 11 cases of TT and 7 (25.92%) of the 27cases of BT leprosy. All the 6 cases of LL and 5(62.5%) of the 8 cases of BL showed Grenz zone.

Table 1: Distribution of clinically diagnosed cases of leprosy

S.No.	Clinical Diagnosis of leprosy	No. of cases	Percentage
1	TT	7	6.93
2	BT	32	31.68
3	BL	3	2.97
4	LL	6	5.94
5	Histioid	3	2.97
6	Indeterminate	29	28.71
7	Pure Neuritic	2	1.98
8	Lepra reaction Type 1	7	6.93
9	Lepra reaction Type 2(ENL)	10	9.9
	Total	99	100

4. Discussion

In our study we found 99 cases clinically diagnosed and histopathologically confirmed as leprosy. Majority of the cases were in third (34.34%) and fourth decade (30.30%) of life [Figure 1]. This could be attributed to young population coming in the region for jobs in the industrial areas. These results were comparable to the studies done by Sharma S

Table 2: Distribution of histopathologically diagnosed cases of leprosy

S.No.	Histopathological Diagnosis of leprosy	No. of cases	Percentage
1	TT	11	11.11
2	BT	27	27.30
3	BB	1	1.00
4	BL	8	8.08
5	LL	13	13.13
6	Histioid	8	8.08
7	Indeterminate	23	23.23
8	Pure Neuritic	1	1.00
9	Lepra reaction Type 1	3	3.03
10	Lepra reaction Type 2(ENL)	4	4.04
	Total	99	100

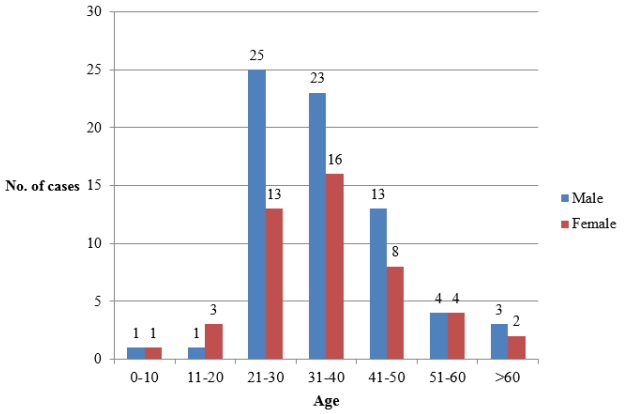

Fig. 1: Distribution of histopathologically diagnosed of all leprosy cases according to age and sex

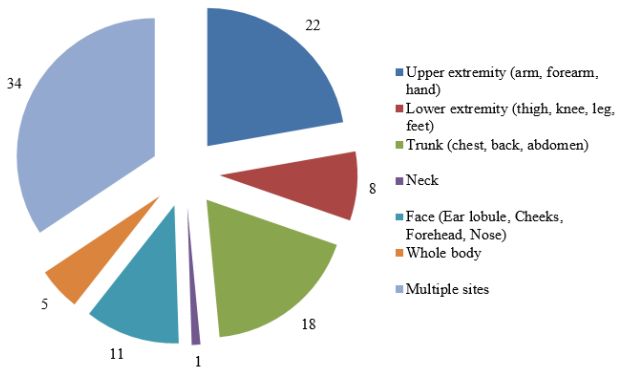

Fig. 2: Distribution of lesions of leprosy according to site

Table 3: Distribution of lesions of leprosy according to number of nerves involved

Histopathological Diagnosis	No. of Nerves					
	0	**1**	**2**	**3**	**4**	**>5**
TT	3	4	3	1	0	0
BT	5	9	7	3	3	0
BB	0	1	0	0	0	0
BL	1	5	1	1	0	0
LL	3	3	5	0	2	0
Histoid	5	0	1	0	2	0
Indeterminate	9	9	4	0	0	1
Pure Neuretic	0	1	0	0	0	0
Lepra Type 1	0	1	0	0	0	2
ENL Lepra Type 2	0	1	2	0	1	0

Table 4: Distribution of lesions of leprosy according to bacillary index

Histopathological Diagnosis	Bacillary Index (BI)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
TT	11	0	0	0	0	0	0
BT	18	6	2	0	1	0	0
BB	1	0	0	0	0	0	0
BL	0	0	0	1	5	2	0
LL	0	0	0	1	3	7	2
Histoid	0	0	0	0	0	0	8
Indeterminate	19	4	0	0	0	0	0
Pure Neuretic	0	1	0	0	0	0	0
Lepra Type 1	1	2	0	0	0	0	0
ENL Lepra Type 2	2	0	0	0	1	1	0
Total	52	13	2	2	10	10	10

Table 5: Correlation between clinical and histopathological diagnosis of cases of leprosy

S.No.	Clinical Diagnosis	Histopathological Diagnosis											
		TT	**BT**	**BB**	**BL**	**LL**	**HH**	**IL**	**PN**	**Type 1**	**Type 2 ENL**	**Total**	**% of agreement**
1	TT	6						1				7	85.71
2	BT	4	19		2	2	2	3				32	59.37
3	BL				2			1				3	66.66
4	LL			1		4		1				6	66.66
5	HH					1	2					3	66.66
6	IL		8		4	6	3	16				29	55.17
7	PN						1		1			2	50
8	Type 1							1		3		7	42.85
9	Type 2(ENL)	1									4	10	40
	Total	11	27	1	8	13	8	23	1	3	4	99	57.57

Table 6: Comparative study of the clinicopathological correlation (in percentage %) with different studies.

	Kar PK et al[5]	**Moorthy et al.[6]**	**Kalla G et al[7]**	**Bhushan et al[8]**	**Bhatia AS et al[9]**	**Nadkarni NS et al[10]**	**Present study**
TT	87.5	46.2	76.7	100	50	97	85.71
BT	60.9	66.5	44.2	83.13	77	95	59.37
BB	54.5	50	37	50	25	89	66.66
BL	53.8	70	43.7	65.22	43	87	66.66
LL	71.4	80	75.6	83.33	91	98	66.66
IL	81.2	20	-	-	35	-	55.17

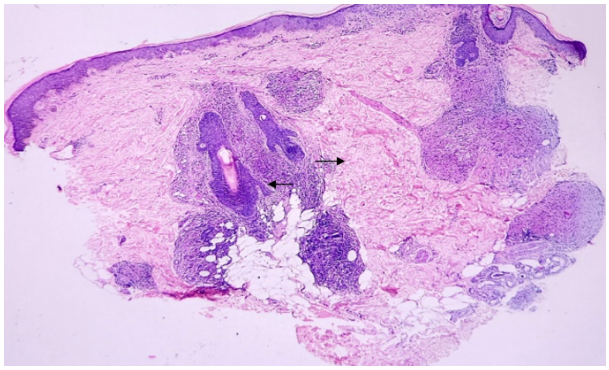

Fig. 3: Tuberculoid Leprosy (TT). Photomicrograph shows thinning of epidermis and erosion with granuloma surrounding periadnexal structures (arrow) (H & E, x100)

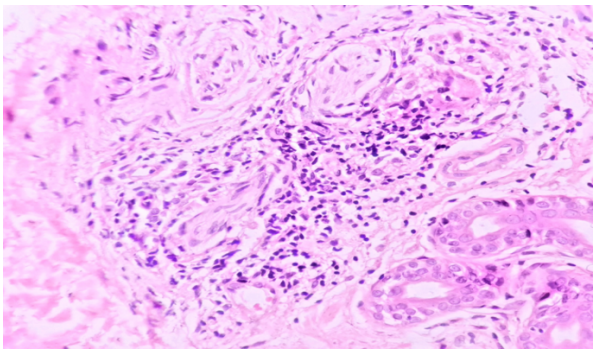

Fig. 4: Borderline Tuberculoid Leprosy (BT). Photomicrograph shows granuloma with peripheral lymphocytes around the neurovascular bundle (H & E, x400)

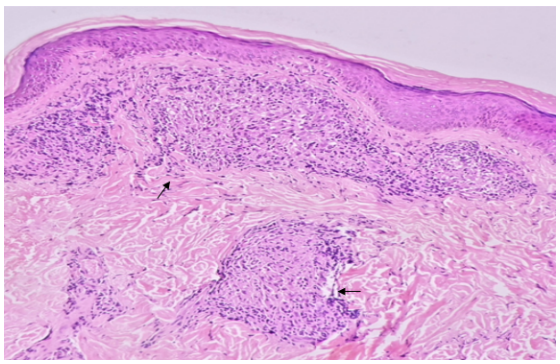

Fig. 5: Mid Borderline Leprosy (BB). Photomicrograph shows uniform activation of macrophages to epitheloid cells (arrow). Dermal edema is prominent between the inflammatory cells. (H & E, x400)

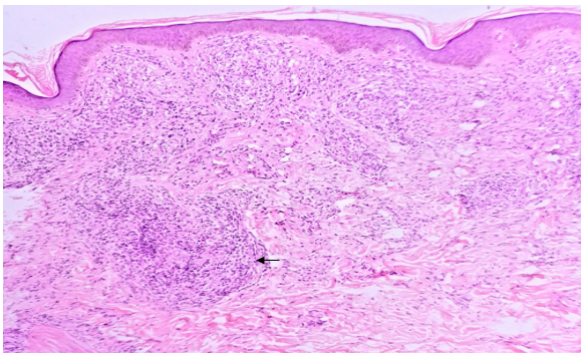

Fig. 6: Borderline Lepromatous Leprosy (BL). Photomicrograph shows predominantly lymphocytes and poorly to moderately defined granulomas (arrow). (H & E, x100)

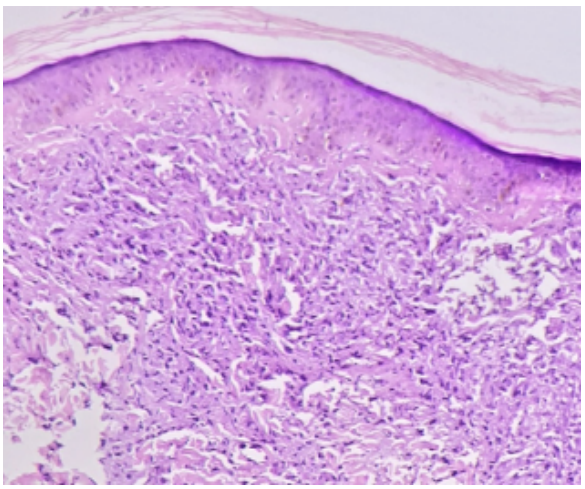

Fig. 7: Lepromatous Leprosy (LL). Photomicrograph show macrophages in the dermis with no granuloma formation. A clear grenz zone is seen under the epidermis (H&E, x100)

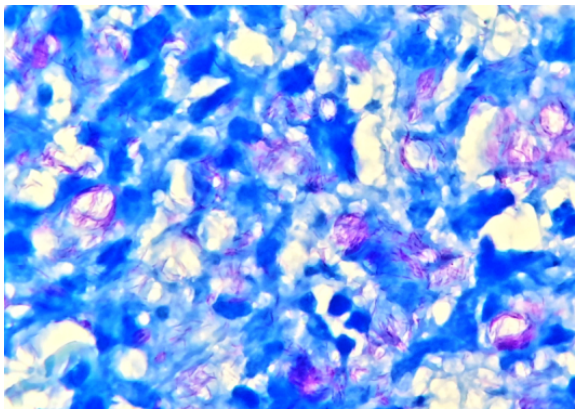

Fig. 8: Lepromatous Leprosy (LL). Photomicrograph shows macrophages containing a mixed population of solid and fragmented bacilli (Fite-Faraco Stain, x1000)

et al.[3] and Kalla G et al.[4] where the most common age range was third and fourth decade constituting 46.20% and 56.00% cases, respectively. Major proportion of leprosy cases were males, with male to female ratio of 1.75:1 [Figure 1] which is in accordance with Gautam K et al.[11] study (1.8:1) and Pailoor J,[12] Moorthy BN et al[6] and Semwal S et al.[13] The male preponderance in many studies is attributed to occupational factors and their lifestyle, which increases their risk for acquiring infection as compared to many females with inhibition from reporting either due to the social taboos or customs.

Leprosy lesions have varied sites of involvement and in this study they were commonly seen in upper extremity (22.22%), followed by involvement of trunk (18.18%) [Figure 2]. Extremities were most commonly affected (46.34%) in different studies like Khamankar ST et al.[14] Overall, nerve involvement 73.73% was the most common clinical feature, decreased sensation (58.58%) was 2^{nd} common feature seen. Plaque lesion (45.45%) was the 3^{rd} common feature observed. As in our study most of the lesions were present over the exposed areas like extremity and trunk, early noticing and reporting of lesions was found. Decreased sensation and plaque are the most common presentations seen in our study which is comparable to other studies like Moorthy BN et al,[6] Khamankar ST et al.[14] and Giridhar M et al.[15] We encountered nerve involvement in most of the cases. This may be due to more number of cases belonging to left side of Ridley and Jopling classification.

On histopathological examination, changes in epidermis and dermis are seen on the skin biopsies. Epidermal atrophy was seen in 51 (51.51%) cases while 14 cases (14.14%) showed other epidermal changes like hyperkeratosis, spongiosis or orthokeratosis. Epithelioid granulomas are seen in 8(72.72%) of the 11 cases of TT and 7 (25.92%) of the 27 cases of BT leprosy. All the 6 cases of LL and 5(62.5%) of the 8 cases of BL showed Grenz zone. This is in accordance with studies done by Roy P et al.,[16] Bhatia AS et al.[17] and Nadkarni NS et al.[18]

All the 99 cases were histopathologically diagnosed using the Ridley–Jopling classification, wherever applicable. BT (27.30%) was the most common subtype seen in our study and similar findings were seen in study by Bal et al.[19] (55.20%), Dhar et al.[20] (66.66%), Gautam K et al.[11] (47.60%), Chakrabarti S et al.[21] (57.94%) and Kumbar R et al.[22] (52.74%). TT was the most common subtype encountered in Agarwal D et al.[5] (20.00%) and BL was most common subtype in Suri J et al.[7] (27.86%).

Overall 47.48% cases showed positive Fite-faraco staining for lepra bacilli (multibacillary) which is in accordance with Nayak SV et al.[8] (44.64%). Permi HS et al.[9] showed positive lerpa bacilli in 25.74% cases. Paucibacillary cases were 52.52% in our study [Table VI]. Santos VS et al.[10] found that 78.34% of leprosy cases were paucibacillary and 21.66% of cases were multibacillary. This study is in accordance with our study, in showing that paucibacillary cases being more common than multibacillary cases. Classifying leprosy on the basis of multibacillary and paucibacillary forms is necessary since the dosage of medications and duration of treatment differs.

Evaluation of agreement and correlation between clinical and histopathological sub-types of leprosy showed overall agreement of 57.57% [Table 5]. In the study done by Santos VS et al.[10] overall agreement in case of leprosy was 58.10%. The present study also showed that the correlation was more in stable poles i.e. TT tuberculoid pole (TT) which was 85.71% and lepromatous pole (LL), BL and BB with 66.66% agreement followed by BT (59.37%). Similar results were seen in the study conducted by Kar and Arora;[23] they observed the highest correlation in stable poles i.e. TT (87.50%) and LL (71.40%) followed by IL (81.20%), BT (60.90%), BB (54.50%), and BL (53.80%) [Table 6]. In the study conducted by Moorthy B N et al.[6] the correlation was more in lepromatous pole (LL and BL) with 75% agreement than that of tuberculoid pole (TT and BT) which was 56%. Kalla et al[24] in a study on 736 patients observed the highest parity in LL and TT group (76.70% and 75.60%), respectively. Bhushan et al.[25] Nadkarni et al.[26] in their study found out maximum concordance in LL and TT cases with 100% agreement in both LL and TT cases and 98% in LL and 97% in TT cases respectively. Similarly Bhatia AS et al.[17] study found out maximum concordance in LL (91%) followed by BT (77%) cases.[Table 6]

Such discordance between clinical and histopathological diagnosis between different studies and between the same study can be attributed to the different inclusion criteria for study cases, the sample size, site of biopsy, immunological status and the treatment regimen of the patient at the time of biopsy and also the classification used by the clinicians to make the clinical diagnosis without taking histopathological examination into consideration.[17,18]

5. Limitations

The details about the patient treatment and follow up after their diagnosis were not recorded in the study.

6. Conclusion

Among all the skin biopsies received in the study period, leprosy accounted for 18.40% indicating that leprosy is still a prevalent disease which raises the concern about therapeutic approaches and various health programmes. Leprosy may not have a classical clinical picture always due to overlapping of various leprosy subtypes. Number of factors influence biopsy outcome and contribute in clinicopathological discordance like representative biopsy site, individual immune-status and morphological features of the lesions. Hence, current study focuses on correlation of clinical and histopathological features along with

bacteriological index which will guide the clinicians and pathologists for accurate diagnosis and better management of the patients. Thereby breaking the chain of infection and preventing transmission of leprosy.

7. Conflict of Interest

The authors declare that they have no conflict of interest.

8. Source of Funding

None.

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Author biography

Tushar Kambale, Professor

Kanika Jain, Resident

Komal D Sawaimul, Associate Professor

C R Gore, Professor and HOD

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