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Original Article

Clinicobacteriological and Histopathological Correlation in Leprosy in a Tertiary Care Centre: A Study of 220 cases

A Chavda[1], H Shah[2], M Rathwa[3], J Chauhan[4]

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The burden of leprosy has reduced drastically with the use of multi-drug treatment. However, the goal of appropriate diagnosis for therapeutic purposes remains an important issue. A study of 220 newly diagnosed cases of leprosy was conducted using the Ridley-Jopling classification of leprosy. For histopathology routine Haematoxylin & Eosin staining as well as a Fite-Faraco staining for acid-fast bacillus was done. The data regarding age, sex, clinical findings like type, number, morphology, site of lesion and neural involvement, histopathological features like an invasion of the epidermis, involvement of subepidermal zone, character and extent of granuloma, lymphocytic infiltrate, epithelioid cells, Langhans giant cells, foamy macrophages, involvement of arrector pilorum and presence of *Mycobacterium leprae* on Fite-Faraco stain and slit skin smears were collected and analysed. Out of these 220 clinically diagnosed cases of leprosy, borderline tuberculoid leprosy (37.27%) was the most common type followed by lepromatous leprosy (29.09%), borderline lepromatous leprosy (24.54.%), mid borderline (5%) and tuberculoid leprosy (4.09%). The overall correlation of clinical diagnosis with histopathology was seen in 79.55%, and the maximum concordance 88.89% was found in Borderline lepromatous leprosy patients. By Fite-Faraco staining 120 out of 220 showed AFB positivity. Overall clinical diagnosis correlated with histopathology was seen in 79.55% (175/220). Higher proportion of multibacillary (MB) cases was diagnosed by bacteriological and histopathological examination including Fite-Faraco staining. Hence, to arrive at a conclusive diagnosis and optimum treatment, it might be preferrable to consider multiple parameters like clinical features, bacillary index, and histopathological findings and use of special stains rather than relying on a single criterion. Any improved criteria should be based on therapeutic gains to be proved in proper follow-up studies.

Keywords: Leprosy, Clinical, Histopathology, Bacteriology, Correlation, Fite Faraco, Granuloma

Introduction

Leprosy is a chronic, granulomatous disease caused by *Mycobacterium leprae* (Semwal et al 2018). It is well known that the clinical presentation of leprosy depends on the immunity of the host. The skin and peripheral nerves are most commonly affected (Semwal et al 2018). Leprosy occurs in all age groups and both genders but is known to have a propensity among adults with a male predominance (Archana & Fernandes

[1] Dr. Archana Chavda, MD (Dermatology), Assistant Professor, Department of Dermatology, Dr. M. K. Shah Medical College & Research Centre, Chandkheda, Ahmedabad, Gujarat, India
[2] Dr. Hiral Shah, MD (Dermatology), Associate Professor, Department of Dermatology, Government Medical College, Vadodara, Gujarat, India
[3] Dr. Mukesh Rathwa, MD (Dermatology), Assistant Professor, Department of Dermatology, Government Medical College, Vadodara, Gujarat, India
[4] Dr. Jatin Chauhan, MD (Dermatology), Assistant Professor, Department of Dermatology, Maharishi Markandeshwar College of Medical Sciences & Research, Sadopur, Haryana, India
Place of work: Government Medical College, Anandpura, Vadodara-390001, Gujarat, India
Corresponding author: Dr Archana Chavda, **Email:** archanachavda747@gmail.com,
ORCID ID: https://orcid.org/0000-0003-0947-6390

2020). A high risk of transmission for contacts, either through the skin or nasal mucosal droplets is present (Archana & Fernandes 2020). Leprosy manifests as a spectrum of clinical findings that correlate with the histopathological changes and the different grades of cell-mediated immune status of individuals. At one end of the spectrum is tuberculoid leprosy, which presents with few lesions and a paucity of organisms. At the other end is lepromatous leprosy in which there are numerous lesions with multiple bacilli and an absence of cellular immunity (Giridhar et al 2012). In 1966, Ridley-Jopling classified leprosy according to the clinical, bacteriological, immunological and histological categories into tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL) and lepromatous leprosy (LL) (Ridley & Jopling 1966). Following this, WHO proposed a classification of paucibacillary and multibacillary leprosy in 1982, which was based on clinical findings and the bacteriological index, this criteria is now updated (WHO 2018). Diagnosing and classifying leprosy solely based on clinical examination of the skin lesions could lead to inappropriate treatment and multi-drug resistance, an ongoing therapeutic conundrum (Archana & Fernandes 2020). Evaluation of histopathological findings in skin biopsies and special staining may help to arrive at a definitive diagnosis and classification, which would help the clinician to administer appropriate and adequate anti-microbial therapy (Lucus & Ridley 1989, Semwal et al 2018). The present study was conducted to study the spectrum of leprosy and correlate the clinical phenotype with histopathological findings. Clinical features indicate only the gross morphology of lesions which do relate to underlying pathological changes, while the histopathological features indicate the accurate response of tissue and bacteriological examination along with the bacillary load. It is expected that various histopathological changes and bacteriological assessment may help in arriving at better therapeutically relevant diagnosis and classification as sometimes clinical diagnosis may not justify the duration of treatment that a particular leprosy patient attending a good clinical setting may have the access. While lot of literature of correlations among clinical, bacteriological, and histopathological features of leprosy exists, it would be important to continue to investigate this aspect in different settings for improving the patient care. This study aims at analysing the newly diagnosed cases of leprosy attending our tertiary care centre with a specific focus on histopathological correlation and bacteriological index of granuloma.

Materials and Methods

A total of 220 patients, diagnosed clinically according to Ridley-Jopling classification as the leprosy of all age groups in the Dermatology OPD of SSG Hospital, Vadodara from June 2020 to April 2023 were included in this descriptive study. Clinical examination included type, number, site and morphology of lesion(s) and neural involvement (NLEP 2019). Slit-skin smears (SSS) were taken from skin lesion and right ear lobes from all patients, stained with a modified Ziehl- Neelsen stain and average BI was calculated on a Ridley's logarithmic scale (Prasad 2005). Skin biopsies were taken from the skin lesion with a 3 mm disposable punch. Specimen fixed in formalin, were sent for the histopathological examination and stained with Hematoxylin & Eosin and Fite-Faraco stain. After Fite-Faraco staining bacteriological index (BI) was calculated by Ridley's logarithmic scale. The histopathological examination included assessment of the invasion of the epidermis, involvement of the subepidermal zone, character and extent of granuloma, lymphocytic infiltrate, epithelioid cells, Langhans giant cells, foamy macrophages, involvement of nerve and arrector pilorum (Ridley & Jopling 1966) and presence

of AFB (*Mycobacterium leprae*) by special (Fite-Faraco) stain.

Results

Of the 220 clinically diagnosed cases of leprosy the majority 51.36% (n=113) were in the age group of 21-40 years. Males constituted a significant portion of the study population accounting for 61.36% (n=135) and the male: female ratio was 1.5:1 (135:85).

The major proportion of cases that is, 117 out of 220 cases (53.18%) showed erythematous nodules/ plaques followed by hypopigmented macules in 103 cases (46.82%). Out of the 103 cases who presented with hypopigmented patches, 75 cases (72.82%) of borderline tuberculoid leprosy, 12 (11.65%) cases of borderline lepromatous leprosy, 9 cases (8.74%) of tuberculoid leprosy and 7 cases (6.79%) of lepromatous leprosy were seen. Among patients who presented with hypopigmented patches, 81.56% (TT-8.74% and BT-72.82%) were towards the tuberculoid pole of leprosy (Fig. 1) ; those with erythematous plaques or nodules, 84.62% (BL-35.90% and LL-48.72%) were towards the lepromatous pole (Fig. 2).

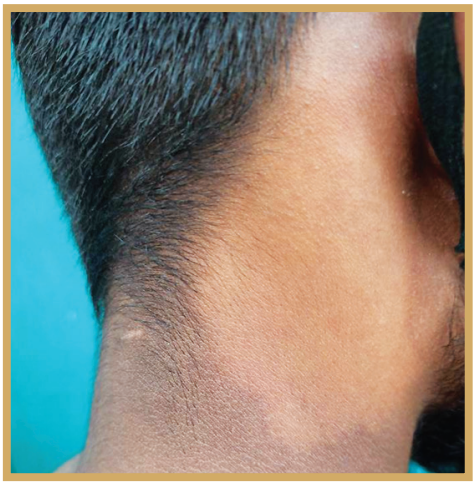

Fig. 1 : Single hypopigmented patch over right side of neck in tuberculoid leprosy.

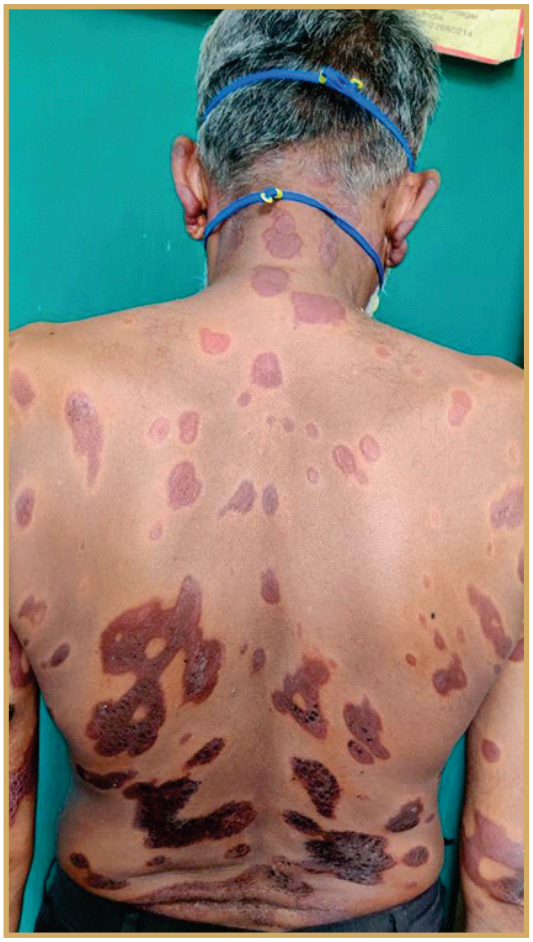

Fig. 2 : Multiple erythematous plaque (inverted saucer shaped) over back in a borderline lepromatous leprosy patient.

All cases of lepromatous and borderline lepromatous spectrum had multiple skin lesions while all cases of tuberculoid leprosy had single lesion. The duration of skin lesions was <6 months in 86 cases (39.09%), 6 month-1 year in 78 cases (35.45%), and >1 year in 56 cases (25.45%). Among 9 tuberculoid leprosy patients, 3 cases (25%) presented within 6 months to 1-year duration and 6 cases (75%) presented with > 1 year duration. In borderline tuberculoid leprosy out of 82 cases, 43 cases (52.44%)

presented within 6-month duration, 29 cases (35.36%) within 6 months to 1 year duration and 10 cases (12.19%) had >1 year duration. In mid borderline leprosy out of 11 cases, 2 patients (18.18%) presented within 6-month duration, 3 patients (27.27%) within 6 months to 1 year duration and 6 cases (54.54%) had >1 year duration. For borderline lepromatous leprosy, the time duration of the presentation was within a 6-month duration in 23 cases (42.59%), within 6 months to 1-year duration in 18 cases (33.33%) and with > 1-year duration in 13 cases (24.07%) out of 54 cases. In lepromatous leprosy, 18 cases (28.12%) presented within the 6-month duration, 23 cases (35.94%) within 6-month to 1-year duration and 23 cases (35.94%) with > 1-year duration out of 64 cases.

The most common clinical subtype was borderline tuberculoid leprosy 37.27% (82) followed by lepromatous leprosy 29.09% (64), borderline lepromatous leprosy 24.54% (54), mid borderline leprosy 5% (11) and tuberculoid leprosy 4.09% (9).

Table 1 : Histopathology findings in the leprosy cases included in the study.

Histopathology findings		TT (9)	BT (84)	BB (3)	BL (73)	LL (51)
Epithelioid cells		9 (100%)	75 (89.29%)	3 (100%)	24 (32.88%)	-
Lymphocytes	Peripheral rim	9 (100%)	70 (83.33%)	-	-	-
	Interspersed	-	23 (27.38%)	3 (100%)	68 (93.15%)	25 (49.02%)
Langhans giant cells		9 (100%)	68 (80.95%)	1 (33.33%)	10 (13.70%)	-
Clear subepidermal zone (grenz zone)		-	-	3 (100%)	61 (83.56%)	43 (84.31%)
AFB (Fite Faraco stain)		-	14 (16.67%)	1 (33.33%)	57 (78.08%)	48 (94.12%)
Foamy macrophages		-	-	3 (100%)	73 (100%)	51 (100%)
Arrector pilorum involvement		5 (55.56%)	50 (59.52%)	1 (33.33%)	19 (29.03%)	21 (41.18%)
Peri appendageal involvement		2 (22.22%)	74 (88.09%)	1 (33.33%)	17 (23.29%)	15 (29.41%)

Table 2 : Bacteriological index in tissue sections in different types of leprosy on Fite-Faraco staining (220 clinically classified).

	Bacteriological Index (Ridley Scale)						
	0	1+	2+	3+	4+	5+	6+
TT (9)	9 (100%)						
BT (84)	70 (83.33%)	11 (13.1%)		3 (3.57%)			
BB (3)	2 (66.67%)		1(33.33%)				
BL (73)	16 (21.92%)	14 (19.18%)	15(20.55%)	7 (9.59%)	13 (17.81%)	6 (8.22%)	2(2.74%)
LL (51)	3 (5.88%)	4 (7.84%)	2 (3.92%)	3 (5.88%)	13 (25.49%)	17 (33.33%)	9(17.65%)

The most common histopathological subtype was borderline tuberculoid leprosy constituted 38.18% (84) of the cases, while, borderline lepromatous leprosy was seen in 33.18% (73) cases, lepromatous leprosy was seen in 23.18% (51) cases, tuberculoid leprosy was seen in 4.09% (9) patients and mid borderline leprosy was seen in 1.36% (3) patients.

In the present study, on histopathology (Table 1), 100% (9/9) patients diagnosed as tuberculoid leprosy (TT) showed epithelioid cells, peripheral rim of lymphocytes and langhans giant cells, 89.29% (75/84) patients diagnosed as borderline tuberculoid (BT) showed epithelioid cells, 83.33% (70/84) showed peripheral rim of lymphocytes, 27.38% (23/84) showed interspersed lymphocytes and 80.95% (68/84) showed langhans giant cells in borderline tuberculoid leprosy, in borderline lepromatous leprosy 100% (46/46) cases showed foamy macrophages, 93.48% (43/46) cases showed interspersed lymphocytes and 13.70% (10/73) cases showed langhans giant cells, in lepromatous leprosy 100% (73/73) cases showed foamy macrophages and 83.56% (61/73) showed subepidermal clear zone (grenz zone). we observed involvement of arrector pilorum muscle in 43.63% (96/220) patients and peri appendageal involvement in 49.54% (109/220) patients. Fite-Faraco stain was negative in all the cases of tuberculoid leprosy (TT). 16.67% (14/84) of borderline tuberculoid leprosy were positive for Fite-Faraco stain. 78.08% (57/73) of borderline lepromatous and 94.12% (48/51) lepromatous leprosy cases showed positivity for Fite-Faraco stain.

Amongst cases of borderline tuberculoid leprosy, 11 (13.1%) cases showed bacillary index (BI) 1+ and 3 (3.57%) showed BI 3+. In borderline

Table 3 : Slit-skin smears results in different clinically diagnosed types of leprosy.

Clinical type	Positive (119)		Negative (101)	
	Number of cases	Percentage	Number of Cases	Percentage
TT (9)	-	-	9	100%
BT (84)	5	5.95%	79	94.05%
BB (3)	1	33.33%	2	66.67%
BL (73)	62	84.93%	11	15.07%
LL (51)	51	100%	0	00.00%

Table 4 : Correlation between clinical and histopathological diagnosis.

Clinical diagnosis	Histopathological diagnosis					Percentage
	TT (9)	BT (84)	BB (3)	BL (73)	LL (51)	
TT (9)	6	3				66.67%
BT (82)	3	69		10		84.15%
BB (11)		6	2	3		18.18%
BL (54)		4	1	48	1	88.89%
LL (64)		2		12	50	78.13%

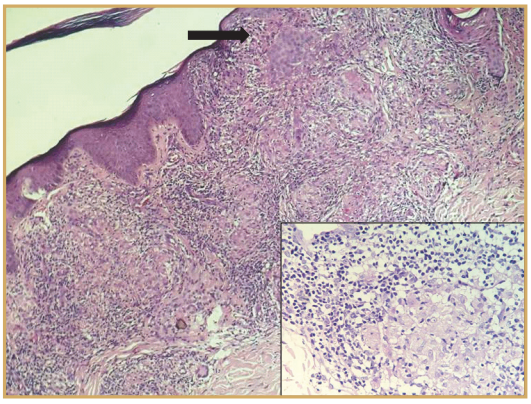

Fig. 3 : Epithelioid cell granuloma with peripheral rim of lymphocytes better visualised in inset image (Haematoxylin and Eosin, 40x) & affinity towards epidermis in tuberculoid leprosy (Haematoxylin and Eosin, 10x).

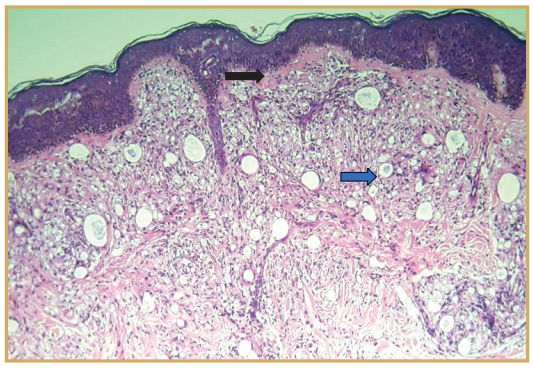

Fig. 4 : Grenz zone (black arrow) & foamy macrophages & globi with bluish pink material in vacuoles (blue arrow) in lepromatous leprosy (Haematoxylin and Eosin, 10x).

lepromatous Leprosy, 14 (19.18%) cases showed BI 1+,15 (20.55%) showed 2+, 7(9.59%) showed BI 3+, 13 (17.81%) showed BI 4+, 6 (8.22%) showed 5+ and 2 (2.74%) showed 6+.In Lepromatous leprosy, 4 (7.84%) cases showed BI 1+, 2 (3.92%) showed 2+, 3 (5.88%) showed BI 3+, 13 (25.49%)showed 4+, 17 (33.33%) showed 5+ and 9 (17.65%) showed 6+ (Table 2).

The slit-skin smears (SSS) were positive in 54.09% (119/220) cases. 84.93% (62/73) were from borderline lepromatous leprosy and 100% (51/51) from lepromatous leprosy patients (Table 3). All the slit skin smears were negative in tuberculoid leprosy patients. Nine cases of borderline tuberculoid leprosy were Fite - Faraco stain positive and slit skin smear-negative for acid fast bacilli.

In our study, an overall clinicohistopathological correlation of 79.55% (175) was observed. A maximum clinicohistopathological correlation of 88.89% (48/54) was observed in borderline lepromatous leprosy (BL) followed by 84.15% (69/82) in borderline tuberculoid leprosy, 78.13% (50/64) in lepromatous leprosy, 66.67% (6/9) in tuberculoid leprosy and 18.18% (2/11) in mid borderline leprosy (BB) (Table 4).

Discussion

Leprosy is an infectious disease with a complex pathogenesis dependent on the immune status of the host (Archana & Fernandes 2020). The clinicohistopathologic manifestations are a result of immunopathology, virulence of lepra bacilli and the host response could range from almost none to a marked immune response at the polar end of the spectrum. When the host response is minimal, progressive damage to the nerves, eyes and skin is potentially permanent and morbid. The present study classified cases according to the Ridley & Jopling classification (Ridley & Jopling 1966). Tuberculoid leprosy is a result of the good immune response of the host and clinically presents as hypopigmented, anaesthetic, well-defined lesions; lepromatous leprosy is a manifestation of the poor immune status of the host where the patient presents with numerous ill-defined skin lesions in symmetric distribution and frequent involvement of peripheral nerves.

On histopathology of the skin biopsies, tuberculoid leprosy exhibits numerous well-formed granulomas with erosion into the epidermis, numerous lymphocytes peripheral to the epithelioid cell aggregates and the absence of a subepidermal clear zone because the subepidermal zone is occupied by granuloma (Fig. 3). Lepromatous leprosy due to the high bacillary load has a characteristic histopathological appearance of sheets of foamy macrophages with the presence of a clear subepidermal zone and bluish-pink material in the foamy cells present as globi, lymphocytes are fewer and forming clusters which do not extent to the edge of granuloma (Fig. 4). Borderline tuberculoid leprosy has multiple granulomas in a curvilinear fashion which follows a neurovascular bundle with a variable number of lymphocytes and when lymphocytes are fairly numerous, clusters are present within the granuloma rather than peripheral to it and no invasion of the epidermis, mid-borderline cases have a mixture of epithelioid cells and foamy macrophages; borderline lepromatous leprosy, is rich in foamy cells with few epithelioid cells and lymphocytes are numerous and infiltrate the granuloma and cover the whole focus and extend to its periphery. This suggests that the predominant cell type of the granuloma, bacterial load & the number and distribution of lymphocytes in the lesion are the basis of histopathological classification.

Only mononuclear (& polymorphs) cells are phagocytic, mononuclear cells are difficult to distinguish from lymphocytes, after ingesting mycobacterium leprae their cytoplasm swells and evolves as macrophages, having difficulty in digesting the organisms they eventually immobilized and aggregate to form a granuloma. A granuloma, therefore, in leprosy always signifies either the site of mycobacterium leprae or the site of its destruction, by contrast, plasma cells and lymphocytes are drawn to the vicinity of the lesion, often without being closely localized at the bacterial site and are a poor guide either to the diagnosis of leprosy or exact site of the organisms.

Bacterial load is a pointer to the position within the two halves of the spectrum, and also a check on the cell type, if the bacterial content does not correlate with the cell type it may be that the cell type has been misconstrued, the patient had been treated or a faulty stain technique, particularly useful in the middle part of the spectrum, in which the immune state is most unstable and classification is sometimes difficult.

Plasma cells are not very useful for classification, they are sometimes fairly numerous in lepromatous leprosy and borderline lepromatous leprosy, usually fairly scanty in borderline tuberculoid and tuberculoid leprosy.

Demonstration of AFB in the specimens from patient may be a better marker for classifying the patient into PB and MB types. In our study 9 patients of BT leprosy negative for AFB in slit-skin smears were positive for the same by Fite-Faraco staining. It shows that acid-fast bacilli are better seen in biopsies than in slit skin smears. However, three LL patients were positive for acid-fast bacilli in slit - skin smear but were negative for AFB in the tissue section by Fite-Faraco staining. These bacilli in the histopathological section may be missed or technical error in staining method. Thus, no method is fool-proof. Depending upon the availability combined approach would be better.

The present study showed a male preponderance of 61.36% (135), similar to the results reported in other studies (Giridhar et al 2012, Banushree et al 2016, Semwal et al 2018, Archana & Fernandes 2020). This has been attributed to an increased risk of exposure due to occupation-associated mobility. The most commonly affected age groups in this study were between

21-40 years (113), followed by 41-60 years (71); this age range was even observed by Banushree et al (2016) and Archana & Fernandes (2020).

Borderline tuberculoid leprosy was the most common clinical subtype in our study accounting for 37.27% (82). The predominance of borderline tuberculoid leprosy could be due to earlier detection and increased accessibility to medical care. The clinico-histopathological correlation we obtained was 79.55% (175), which was similar to the results obtained by Banushree et al (2016) with a correlation of 79.44 % and Mathur et al (2011) with a correlation of 80.4% and Archana & Fernandes (2020) with a correlation of 83.42%. Mid-borderline lesions showed 18.18% (2/11) (minimum) correlation and borderline lepromatous leprosy showed 88.89% (48/54) (maximum) correlation. Contrasting results were seen by Semwal et al (2018), Banushree et al (2016) and Giridhar et al (2012) where they obtained maximum histopathological correlation for the polar spectrum of leprosy. This discordance at polar LL end requires in-depth investigations and meta-analysis of published data. For more than 70-80 years, clinicians and pathologists have tried to establish correlation between clinical features of leprosy and histopathology. These efforts have continued in the recent years as well (Ridley & Jopling 1966, Lucus & Ridley 1989, Mathur et al 2011, Giridhar et al 2012, Manandhar et al 2013, Banushree et al 2016, Semwal et al 2018, Archana & Fernandez 2020). However, the goal is still illusive and there is a big unacceptable range of overall concordance of 50-100% (Misra & Kumar 2023). Our study provides data from our settings. Expertise and experience could be important factors. It would be important to establish better diagnostic criteria based on characteristics at cellular level.

The discordance between clinical and histopathological findings can be because the clinical examination is subjective with a lack of uniformity, whereas histopathological demarcation is almost accurate based on the characteristics. Although polar stable ends of the spectrum exhibits specific histopathological findings, an overlap is commonly observed in borderline cases with the least stability in mid-borderline leprosy. Thus, clinical, histopathological and microbiological parameters need to be considered together for accurate diagnosis. Molecular assays will improve the sensitivity and should be part of future investigations. These correlations should be analysed in the context of therapeutic gains by carrying out long term follow-up studies.

Conclusion

In our study, correlation of clinical diagnosis with histopathology was seen in 79.55% (175/220) cases included in the study. While the maximum concordance 88.89% (48/54) was found in borderline lepromatous leprosy and highest discordance was found in mid-borderline leprosy. Fite-Faraco staining of histopathology sections enhanced the detection of AFB especially in BT cases. Hence, to arrive at a conclusive diagnosis, it might be preferrable to consider multiple approaches like clinical features, bacillary index, and histopathological findings and use of special staining like Fite-Faraco rather than relying on a single criterion.

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