e-ISSN: 0975-9506, p-ISSN: 2961-6093

Available online on www.ijpqa.com doi: 10.25258/ijpqa.16.6.15

International Journal of Pharmaceutical Quality Assurance 2025; 16(6); 82-87

Original Research Article

Histopathological and Immunopathological Aspects in Cutaneous Lesions of Leprosy

Jaspreet Kaur Chatrath[1], Umang Baghel[2], Monika Gupta[3], Manju Purohit[4]

[1]Assistant Professor, Department of Pathology, RKDF Medical College Bhopal
[2]Assistant Professor, Department of Pathology, Ram Krishna Medical College Hospital and Research Centre Bhopal
[3]Junior Consultant, Vivekanand Polyclinic and Institute of Medical Sciences, Lucknow (UP)
[4]Professor & Head of Department, Department of Pathology, RD Gardi Medical College Ujjain

Received: 20-04-2025 / Revised: 19-05-2025 / Accepted: 20-06-2025
Corresponding Author: Dr. Jaspreet Kaur Chatrath
Conflict of interest: Nil

Abstract:

Methods: The present study was carried out in Department of Pathology, in Ruxmaniben Deepchand Gardi Medical College, Ujjain. In which biopsies of 51 cases of clinically and histologically diagnosed leprosy cases were included. All the cases were subjected to special stains like Fite Faracco and Auramine 'O' to know the bacterial Index.

Results: In most of the cases male's predilection is seen especially in third decade with clinical symptoms of anaesthetic hypopigmented lesion. On Histopathology most of the cases were of Borderline group (BT, BL). On Fite Faracco and Flourescent staining Histoid leprosy and Lepromatous leprosy showed highest bacterial load. In study of 51 cases, clinically and histologically diagnosed as hansen's showed a male preponderance with Male: Female ratio approximately 2:1. It was observed that Auramine 'O' stain is better than Fite Faracco.

Conclusion: Histopathological examination of skin lesions remains the mainstay in diagnosing and classifying leprosy. Majority of cases were Borderline Tuberculoid Leprosy on histology of the biopsies. Bacterial load can be easily judged by Fite Faracco and Auramine 'O' staining. Fluorescent stain, Auramine 'O' is better than Fite Faracco stain.

Keywords: Bacterial load, Fite Faracco, Auramine 'O' staining, Borderline group (BT, BL), Histoid leprosy, Lepromatous leprosy.

Introduction

Leprosy also named as Hansen's disease, is the oldest disease known to mankind. In India, leprosy was first described in susruth samhita written in 600 BC. Since ancient times in India, leprosy is known as as "Kushtaroga", whose clinical manifestations are largely confined to the skin, peripheral nervous system, upper respiratory tract, eyes, and testes. The three-cardinal sign of the disease are skin lesions, skin anesthesia and enlarged peripheral nerves. Leprosy is one of the leading causes of physical disabilities which contribute to intense social stigma resulting in discrimination of patients and their families. It is a chronic granulomatous disease caused by Mycobacterium leprae, affecting the peripheral nervous system, skin, and reticuloendothelial system.

Leprosy is important mainly because of its potential to cause permanent and progressive physical deformities with serious social stigma and economic consequences. Several factors influence the occurrence and the progress of deformity in leprosy patients, which are related to age, sex, occupation, duration, type of disease and environmental factors. It is very difficult to accurately find the actual number of cases of leprosy in the world. Case diagnosis and definition are not always and everywhere consistent and the enumeration of cases in some parts of the world is incomplete. Despite the difficulties, estimates were made by World Health Organization (WHO) from time to time.

Although in January 2006, leprosy was declared to be eliminated in India, but it is still a public health problem in the country. In India a total of 0.9 lac cases were on record as on April 2013, giving a Prevalence rate (PR) 0.73 per 10,000 population. [4] According to official reports received from 138 countries from all WHO regions, the global registered prevalence leprosy at the end of 2015 was 0.18 cases per 10,000 people. The number new cases reported globally in 2015-0.21 new cases per 10,000 people. T number of new cases indicates the degree of continued transmission infection.

The clinical manifestations of leprosy are varied and diverse and can mi a variety of unrelated diseases. Clinical presentation may vary from insignificant skin lesion to extensive disease-causing profound disability/deformities.

Leprosy has been classified in a few ways. The most common classification is of Ridley and Jopling 17 Histopathological study of leprosy is very important in understanding the disease, its varied manifestation, and complications. For the correct and adequate treatment, the diagnosis must be made early. The clinico-pathological correlation is also extremely important in-patient care and management. Fite-Faracco method is used for demonstration of lepra bacilli and it demonstrates the infective status and is helpful in deciding the treatment. Sometimes, the clinical typing of leprosy is not possible, and results obtained by slit skin smear are not satisfactory. Therefore, histopathological examination is the mainstay in all suspected cases.

Present study is to find clinical, histopathological, and special stain characteristics in cutaneous lesions of leprosy and to categorize various types based on microscopy and fluorescent tests

Materials and Methods

The present study was conducted in the Department of Pathology during the period of January 2014 to June 2017.Ethical clearance for the study was obtained from Institutional Ethics Committee, R.D. Gardi Medical College, Ujjain.

Skin biopsies were obtained from clinically diagnosed leprosy patients from Department of Dermatology, R.D. Gardi Medical College, Ujjain. Paraffin embedded tissue blocks of patients diagnosed with leprosy (between January 2014 to April 2015) were retrieved from the Department of Pathology. The clinical details of the patients were obtained from the request form and case files retrieved from the Medical Records Department. While patients diagnosed with leprosy on skin biopsies were prospectively enrolled between May 2015 to June 2017 from Department of Pathology.

Study Design: Observational study

Study Setting: Department of Pathology, R.D. Gardi Medical college, Ujjain.

Inclusion Criteria

1. All clinically diagnosed cases of leprosy.
2. All cases of leprosy under treatment.

Exclusion Criteria

1. Inadequate biopsy.

Consent: Informed written consent was taken from all the cases for skin biopsy.

Method

1. The patient was subjected to detailed history and clinical findings were noted from work-sheet.
2. Formalin fixed 4mm punch biopsy specimen was processed for paraffin embedding
3. The microscopic sections were stained with Hematoxylin and Eosin(H&E), Fite Faracco and Auramine 'O'. The light microscopy changes were examined and noted.
4. The clinical, Histopathological diagnosis were compared and correlated with Bacterial Index on Fite Faracco and Auramine "O" stain.

The leprosy bacillus is much less acid and alcohol fast than the tubercle bacillus; therefore alcohol is removed from the hydrating and dehydrating steps and 10% sulphuric acid is used as a decoloriser in place of acid /alcohol solution. The sections are also deparaffinised using peanut oil/xylene mixture, this helps to protect the more delicate waxy coat of the organisms. The Bacterial index (BI) was calculated using an oil immersion field (OIF).

Auramine dye is used as a stain and potassium permanganate is used as a counterstain after the material is decolorized with acid alcohol. The acid-Fast organisms do not decolorize after staining. A light source producing a suitable wavelength is used to excite fluorescence of the stain retained by the acid-fast organisms and this fluorescence is detected by use of either dark field or bright field microscopy. A filter system which absorbs background light but transmits the light caused by fluorescence results in easy visibility of organisms.

Tissue sections were screened immediately with Carl Zeiss microscope, with HBO 50 high pressure mercury shot- arc discharge. Excitation was with blue violet rays obtained with BG 12 primary filters; an abbe condenser was also used. Skin biopsy samples from a normal individual were used as controls. All sections were screened with 10x, 40x, 100x objectives. Only solid fluorescing organisms were considered for a definite diagnosis, excluding bacterial fragments.

The typical morphology of bacilli showing bright yellow fluorescence emitted by bacilli when interspersed with the artifact was considered the diagnostic criteria for labeling the biopsy positive for mycobacterium leprae. The clinical and histopathological diagnosis were compared and correlated with Bacterial Index on Fite Faracco and Auramine "O" stain.

Observation Chart

Table 1: Age and Sex Wise Distribution According to Classification of Leprosy

	LL		BL		BB		BT		TT		HL	
AGE	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
10-19	-	-	-	-	1	-	-	-	-	-	-	-
20-29	**2**	-	**1**	-	-	-	-	**1**	-	-	-	-
30-39	-	**2**	**1**	**2**	**4**	-	**3**	**2**	-	-	**1**	-
40-49	**2**	**2**	**1**	-	**2**	**1**	**1**	-	**1**	-	-	-
50-59	**1**	-	-	-	-	-	**2**	**2**	-	-	**1**	**1**
60-69	**3**	**1**	-	-	**1**	**1**	**2**	**1**	**1**	**1**	-	-
70 & above	-	-	**1**	-	-	-	**1**	-	-	-	-	-

Table 2: Distribution According to Site of Lesion of Leprosy

Site of Lesion	Number of Cases
Face and neck	5
Trunk and back	2
Limbs (B/L)	12
Multiple sites	34

Table 3: Distribution According to Classification of Leprosy

Type of Leprosy	No. of Patients	Percentage
Lepromatous leprosy (LL)	13	25.49%
Borderline lepromatous (BL)	6	11.76%
Mid-borderline lepromatous (BB)	10	19.60%
Borderline tuberculoid (BT)	16	31.38%
Tuberculoid leprosy (TT)	3	5.89%
Histoid leprosy (HL)	3	5.89%
Total	51	100%

Table 4: Histological Changes in Epidermis in Various Types of Leprosy

Epidermis	LL	BL	BB	BT	TT	HL
Atropy	13	5	7	10	1	2
Loss of Rete ridges	13	5	7	7	1	2
Unremarkable	-	1	3	6	1	-
Ulceration	-	-	-	-	1	1

Table 5: Histological Changes in Dermis in Various Types of Leprosy

Dermis	LL	BL	BB	BT	TT	HL
Epitheloid Granuloma	-	-	1	10	3	-
Grenz Zone	13	3	-	-	-	-
Macrophages	13	5	8	-	-	3
Lymphocytic Infiltration	8	5	2	12	3	3
Giant cells	-	-	-	5	3	-
Epitheloid cells	-	-	6	12	3	1

Table 6: Bacillary Index According to Fite-Faracco Staining

Types	0	1+	2+	3+	4+	5+	6+	Total
LL	-	**5**	**3**	**2**	**1**	**2**	-	**13**
BL	**1**	**3**	**2**	-	-	-	-	**6**
BB	**1**	**7**	**2**	-	-	-	-	**10**
BT	**10**	**5**	-	**1**	-	-	-	**16**
TT	**3**	-	-	-	-	-	-	**3**
HL	-	-	-	**1**	**2**	-	-	**3**

Table 7: Bacillary Index According to Auramine 'O' Staining

Types	0	1+	2+	3+	4+	Total
LL	-	**4**	**3**	**3**	**3**	**13**
BL	-	**2**	**3**	**1**	-	**6**
BB	**2**	**6**	**2**	-	-	**10**

BT	**12**	**3**	**1**	**-**	**-**	**16**
TT	**2**	**-**	**1**	**-**	**-**	**3**
HL	**-**	**-**	**1**	**-**	**2**	**3**

Results

1. In study of 51 cases, clinically and histologically diagnosed as hansen's showed a male preponderance with Male: Female ratio approximately 2:1.
2. Histopathological examination of skin lesions remains the mainstay in diagnosing and classifying leprosy.
3. Majority of cases were Borderline Tuberculoid Leprosy on histology of the biopsies.
4. Bacterial load can be easily judged by Fite Faracco and Auramine 'O' staining.
5. Fluorescent stain, Auramine 'O' is better than Fite Faracco stain.

Statistical Analysis: The collected data was summarized by using frequency, percentage, mean & S.D. To compare the qualitative outcome measures Chi-square test or Fisher's exact test was used. To compare the quantitative outcome measures independent t test was used. If data was not following normal distribution, Mann Whitney U test was used. SPSS version 22 software was used to analyse the collected data. p value of <0.05 was statistically significant.

Discussion

Leprosy or Hansen's disease is a slowly progressive infection caused by Mycobacterium leprae. It predominantly affects the skin and peripheral nerves. Even though its incidence is on the decline worldwide, we still get new cases every now and then which continue to be an important public health problem. We analyzed 51 skin biopsy samples of leprosy patients.

Majority of the patients in our study were males (64.70%), which is similar 10 findings made in other studies. The male predominance may be because of many factors like more opportunities for contact in males as compared to females in rural areas who usually remain indoors. Also, social customs and taboos associated with leprosy may account for smaller number of females reporting to the hospital.

Leprosy is known to occur in all ages ranging from early childhood to very old age. We found that most of the cases from our study were in the 30-39 year age group (third decade) and lowest were in first decade. Although the reason for the age distribution cannot be ascertained as it is variable, the incidence is usually higher in middle and old age and less in childhood may be because of immune status.

Loss of sensation was the commonest clinical feature (96.07%). Most of the lesions were hypopigmented (62.74%). Similar observations were made in other studies. We also found erythematous lesions (37.25%) which was like study done by Murthy et al, Kaur I et al Ulceration was seen only in two cases suggesting it is a rare feature.

Grenz zone was the commonest feature observed in all cases of LL (100%). It was seen in 50% cases of BL. Grenz zone was not seen in the other types (BB, BT, TT, HL). Diagnosis of TT was considered when there was collection of epitheloid cells, lymphocytes and large Langhans type giant cells. Usually, no AFB are seen Out of 51 cases, 3 were TT all of which showed epitheloid granulomas with giant cells and lymphocytic infiltration (100%). Out of the 51 cases, 16 were BT, among which majority showed epitheloid cells (75%) with lymphocytic infiltration around appendages. Epitheloid granulomas were observed in 62.5% cases while giant cells of Langhans type were seen in 31.25% cases. These findings are quite similar to the study conducted by Murthy NB et al in 1999 in which they found lymphocytic infiltration in 68.39% and giant cells in 45.24%. BB is an unstable form of leprosy which has mixed features of both tuberculoid and lepromatous type. In our study BB constituted 19.60% cases. Presence of macrophages around adenexa was the most common finding seen in 80% biopsies. Epitheloid cells were seen in 60% cases and lymphocytic infiltration around appendages was observed in 20% cases. Diagnosis of BL was considered when there was diffuse infiltrate of macrophages and lymphocytes involving appendages, Histology of BL is like LL. The findings are correlated with clinical picture to make accurate diagnosis.

We had 6 cases of BL type in our study, out of which 5 showed macrophage and lymphocytic aggregation around adenexa. Similar findings were observed in various other studies /621 Diagnosis of LL was considered when there was diffuse infiltrate of macrophages with few lymphocytes. Presence of Grenz zone was a characteristic feature in all cases. Diagnosis of HL was considered when there were aggregates of foamy macrophages arranged in spindle shape storiform pattern and a high load of lepra bacilli. Out of the 51 skin biopsies studied, epidermis was atrophic in most cases (74.51%). Changes were unremarkable in 21.57% cases. Atrophy of epidermis was associated with loss of Rete ridges in most cases.

The most encountered type of leprosy in our case series was BT (31.38%) while TT was least (5.89%). Borderline group (BL, BB, BT) constituted 62.74% of total cases, which is like the findings of other authors. A sizeable portion of leprosy patients will be

in a continuously changing immunological spectrum, leading to a higher prevalence of borderline group. With treatment they move towards tuberculoid spectrum and without treatment they move towards lepromatous spectrum. Increased awareness among the people because of many national programs makes them present at an earlier stage to leprosy clinics which may have contribute to increased number of borderline cases. We did not get any cases of Intermediate leprosy in our study sample. We had three cases of HL; this is a new inclusion in the recent classification system by Wade and the other studies did not use this system of classification.

In the present study, majority of patients were of MB type (58.82%). All the LL and BL cases were of MB (100%). Similar observation was made by the other authors. All the 3 biopsies of TT were of PB type (100%), similar observation was made by the other authors. Majority of the biopsies of BT were PB (87.5%). The other authors also had similar findings. In our study, majority of the BB cases were of MB type (60%), the other authors reported an even higher percentage of MB type in this group. We did not have any cases of IL in our study. All 3 cases of HL in our study were of MB (100%).BI was highest in LL types and lower in BL, BB and BT variety. This was like the observation made by Jopling. This shows that the bacilli are scanty in BT, almost always present in BB and numerous in BL and LL.

On Fite Faracco stain, all 13 cases of LL in our study had a BI of 1+ or more. Most cases in BL and BB group also showed a high BI. Even in the BT group 6 out of 16 cases showed a positive staining for Mycobacterium leprae while all 3 cases of TT had a BI of 0. On Auramine 'O' stain, out of the 13 cases of LL, 4 cases had a Bl of 1+, 3 cases had a BI of 2+, 3 cases had a BI of 3+ and 3 cases had a Bl of 4+. Similarly, all 6 cases of BL and all 10 cases of BB had a BI of 1+ or more. So Auramine 'O' staining had 100% sensitivity in identifying lepra bacilli in this group. In the BT group, out of the 16 cases, 5 had a Bl of 0 while remaining 11 cases showed presence of lepra bacilli on fluorescent microscopy. TT group had a BI of 0 in 2 out of 3 cases. HL group showed a high BI in all 3 cases.

Silva LM et al evaluated the expression of inflammasome markers (nucleotide-binding oligomerization domain-like receptor containing pyrin domain 1 [NLRP1], nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 [NLRP3], caspase 1, IL-1β, and IL-18) by immunohistochemistry in 43 samples of skin lesions of leprosy patients The evaluated markers were most upregulated in LL lesions, followed by lesions of TT leprosy and I leprosy. Differences were statistically significant between the I leprosy and LL leprosy forms and between the I leprosy and TT leprosy forms. Positive and significant correlations were found between IL-18 and caspase 1 in LL (r=0.7516, P=0.0012) and TT leprosy. The expression of inflammasome markers in LL lesions indicates the ineffectiveness of this protein complex in controlling the infection. Caspase 1 may be involved in the pyroptotic cell death in the lepromatous form of the disease. Inflammasomes may act together in the initial phase of I leprosy; this phenomenon may influence the clinical outcome of the disease.

Leprosy, a disease caused by Mycobacterium leprae, has polymorphic neurocutaneous manifestations strongly correlated with the host immune response. Peripheral neural damage can lead to sensory and motor losses, as well as deformities of the hands and feet. Froes LA et al studied clinical and immunopathological characteristics of leprosy. The aim of review was to discuss this dichotomy in light of the current knowledge of the cytokines, T helper subpopulations, and regulatory T cells involved in each presentation of leprosy.

On comparing Fite Faracco stain with Auramine 'O' stain we observed that Auramine 'O' stain had higher sensitivity in detection of Mycobacterium leprae in tissue sections. Ashalatha N, A. Nagarajappaand D. Prabhu in their study also found similar results. Jariwala and Kelkar in their study observed that fluorescent microscopy was superior to Fite Farraco in detection of Mycobacterium leprae particularly in PB cases.

Conclusion

In study of 51 cases, clinically and histologically diagnosed as hansen's showed a male preponderance with male: female ratio approximately 2:1. Histopathological examination of skin lesions remains the mainstay in diagnosing and classifying leprosy. Majority of cases were borderline tuberculoid leprosy on histology of the biopsies. Bacterial load can be easily judged by fite faracco and auramine 'o' staining. Fluorescent stain, auramine 'o' is better than fite faracco stain

Declarations:

Funding: None

Availability of data and material: Department of Pathology, R.D. Gardi Medical college, Ujjain.

Code availability: Not applicable

Consent to participate: Consent taken

Ethical Consideration: There are no ethical conflicts related to this study.

Consent for publication: Consent taken

References

1. Desiken KV, Ramu G, Job CK. Symposium of relapse in leprosy. Indian J Lepr 1995; 67 (4): 1-90.

2. Fleury RN, Bacchi CE. S-100 protein and immunoperoxidase technique as an aid in the histopathologic diagnosis of leprosy. Int J Lepr 1987; 55: 338.
3. Gainsborough N. Hall SM, Hughes RAC, et al. Sarcoid neuropathy. J Neurol 1991; 238: 177.
4. De Wit MYL, Faber WR, Krieg SR, et al. Application of a polymerase chain reaction for the detection of Mycobacterium leprae in skin tissues. J Clin Microbiol 1991; 29: 906.
5. Fine PEM, Job CK, Lucas SB, et al. The extent, origin and implications of observer variation in the histopathological diagnosis of leprosy. Int J Lepr 1993; 61: 270.
6. Chemotherapy of Leprosy, Report of a WHO Study Group, Geneva, World Health Organization, 1994 (WHO Technical Report Series No. 847)
7. Single Lesion Multicenter Trial Group, Efficacy of Single Dose Multidrug Therapy for Treatment of Single Lesion Paucibacillary Leprosy, 1997, Indian Journal of Leprosy, 69, 121-129.
8. Giridhar BK. Leprosy: Drug Treatment. In: Valia RG, Valia AR, editors, Textbook and Atlas of Dermatology, 2nd ed. Mumbai: Bhalani Publishing House; 2001: 1613-1624.
9. Job CK. Pathology of Leprosy. In: Hastings RC, Opromolla DVA, editors. Leprosy 2nd ed. New York: Churchill Livingstone; 1994: 224.
10. Verma OP. Some epidemiological features of leprosy in a rural area in Hooghly District. Lepr India 1976; 48 (4): 371-381.
11. Murthy N. Histopathological study of leprosy (unpublished doctoral dissertation Rajiv Gandhi University of Health Sciences, 2000).
12. Kaur I, Indira D, Dogra S, Sharma VK, Das A, Kumar B. Relatively spared zones in leprosy: A clinicopathologiel study of 500 patients. Int J Lepr 2003; 71 (3); 227-229.
13. Noordeen SK. The epidemiology of leprosy. In: Hastings RC, editor, Leprosy, New York: Churchill Livingstone; 1985; 15-29.
14. Shenoi SD, Siddappa K. Correlation of clinical and Histopathological features in untreated macular lesions of leprosy - a study of 100 cases. Indian J Lepr 1988; 60(2): 202-205.
15. Ashok Kumar SK, Reddy BSN, Ratnakar C. Correlation of skin and nerve histopathology in Leprosy. Lepr Rev 1996; 67: 119-125.
16. A N, A. Nagarjappa, D. Prabhu. Sensitivity of Flourescent Microscopy in detecting Mycobacterium leprae in tissue sections. The Internet Journal of Pathology. 2010 Volume 11 Number 2.
17. Jariwala HJ and Kelkar SS. Fluorescence microscopy for detection for Mycobacterium leprae in tissue sections. Internal Journal of leprosy.
18. Silva LM, Sousa JR, Hirai KE, Jr LB, Furlaneto IP, Carneiro FR, Leila de Souza Aarão T, Sotto MN, Antonio Simões Quaresma J. The inflammasome in leprosy skin lesions: an immunohistochemical evaluation. Infection and drug resistance. 2018 Nov 11:2231-40.
19. Saikia UN, Sood R. Immunopathobiology of Leprosy: From the Perspective of a Dermatopathologist. IAL Textbook of Leprosy. 2023 Jan 25:162.
20. Froes LA, Sotto MN, Trindade MA. Leprosy: clinical and immunopathological characteristics. Anais Brasileiros de Dermatologia. 2022 Jun 13;97(3):338-47.