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RESEARCH ARTICLE
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EVALUATION OF MAST CELL DENSITY IN TISSUES OF LEPROSY & ITS COMPARISON WITH CLINIC-PATHOLOGICAL PARAMETERS

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ABSTRACT

Background: Leprosy, sometimes referred to as Hansen's disease (HD), is a persistent infection caused by Mycobacterium leprae. Mast cells in leprosy have been recently studied & are under consideration for future research. the present study was done to assess Mast Cell Density in tissues of leprosy & compare with clinic-pathological parameters.

Methods: The retrospective study was conducted at Hi Tech Medical College, among 100 cases of skin leprosy patients during the study period of one year. The mean quantity of mast cells per high power field was determined.

Results: The mean age of patients was 43.5±3.8 years. Out of 100 cases; 76% were male & 24% were female. The most common type of leprosy seen were paucibacillary (60) & mutibacillary (40). No reaction was found in 64 cases whereas type I reaction was seen 26 cases & type II reaction was seen in 10 cases. The number of mean mast cell found in no reaction was 22.3±10.1, in Type I reaction was 10.4±3.9 & in Type II reaction was 12.7±4.1. The histopathological slides shows most common type was borderline lepromatous in 22% cases & borderline in 20% cases. Significant association ($p<0.05$) between number of mast cell in each area by leprosy reaction type was seen.

Conclusion: Infiltrating mast cells contribute to lesion progression, as seen by the increased mast cell density in cutaneous leprosy lesions. Border line leprosy with interstitial region had considerably greater MCD. Increased mast cell counts indicate a better disease prognosis in cutaneous leprosy lesions.

Keywords- inflammation, leprosy, mast cell, mycobacterium leprae, reaction type

INTRODUCTION

Leprosy is commonly known as Hansen disease. Leprosy is a persistent inflammatory infection typically caused by Mycobacterium leprae & Mycobacterium lepromatosis. These bacteria mainly target the skin & peripheral nerves.[1] The pattern of disease in leprosy is decided by host cellular response to mycobacterium leprae.[2]

The skin lesions of leprosy consist of all the cell types found in normal skin, as well as those that enter the lesions from the peripheral blood as part of the granulomatous process. The role of cells typically seen in the dermis in the development & advancement of granuloma is unclear. There has been less focus on mast cells in the context of leprosy in recent times. However, there is data suggesting a connection between mast cells & the development of delayed hypersensitivity reactions.[3] This raises the idea that mast cells may have some significance in leprosy lesions. Mast cells exhibit heterogeneity in terms of their appearance, biochemistry, & function.[4-6] The variations between them seem to be contingent on the specific tissue & species they originate from, making it impossible to confidently extrapolate the findings of mast cell distribution or function investigations from one location or animal to another.

Type 1 reversal reactions are believed to indicate an elevated immune response to Mycobacterium leprae, resulting in significant nerve damage in people with borderline leprosy.5 There is inflammation present in both the skin lesions & the afflicted nerves, resulting in discomfort, swelling, & tenderness when touched.[7] From a histological perspective, there is a notable presence of intercellular edema, an increase in the number of lymphocytes, clear evidence of fibroblast proliferation, & the occurrence of giant cell development.[8] Patients classified on the Ridley-Jopling scale have varying levels of vulnerability to reversal reactions.

Mast cells have been widely recognized as playing a crucial role in the development of edema, which is a characteristic symptom of a reversal reaction. Chowdhury & Ghosh explored the potential contribution of mast cells to the development of these reactions.[9] They discovered that tuberculoid lesions in reaction had a decreased concentration of mast cells compared to non-reactive tuberculoid lesions. Evidence of mast cell degranulation has been observed in erythema nodosum leprosum (ENL), which is a type II leprosy reaction.[10,11]

Several recent investigations have also established a correlation between mast cells & the development of delayed hypersensitivity reactions. This resulted in the connection between them & their potential involvement in the formation of leprosy sores on the skin. Given this context the present study was done to assess Mast Cell Density in tissues of leprosy & compare with clinic-pathological parameters.

MATERIAL & METHODS

The retrospective study was conducted at Tertiary care hospital hospital Hi tech medical College, among case of skin leprosy patients during the study period of one year. Ethical clearance was taken from institutional ethics committee before commencement of study.

Through consecutive sampling a total of 100 cases of leprosy were selected on the basis of inclusion & exclusion criteria.

Inclusion criteria

1. Skin specimens with tissue blocks & Haematoxylin & Eosin & Fite-Faraco for acid fast bacilli slides representing skin leprosy
2. Cases with adequate clinical & demographics data of the patient.

Exclusion criteria

1. Situations where the skin tissue is not sufficiently represented in sections.
2. Situations where tissue slices are insufficiently large to count mast cells.
3. The study did not include cases with insufficient clinical & demographic information.

The Ridley & Joping classification was used to categorise the patients. At the time of diagnosis, a skin sample was taken from each case; some people also experienced type I or type II leprosy reactions at the same time. A second biopsy was then taken, perhaps at the same time as the reaction, which could have happened more than once. In order to identify acid-fast bacilli, the biopsy specimens were preserved in formalin, embedded in paraffin, & the slices were stained with Fite-Faraco & haematoxylin & eosin. Metachromatic purple-staining granules in the cytoplasm in

the Fites stain region were used to identify mast cells. Quantification of mast cells was carried out separately using a light microscope set to 400X magnification. Ten non-overlapping fields were inspected in each specimen, & the number of mast cells in each compartment was recorded. They calculated the average number of mast cells per high power field.

Statistical Analysis

The collected data was entered into a Microsoft Excel document. Accordingly, IBM SPSS (Statistical Package for Social Science) Statistics for Windows, version 25 (IBM Crop., Armonk, NY), was used to perform descriptive & inferential statistics. A significant result was defined as a P-value of less than 0.05.

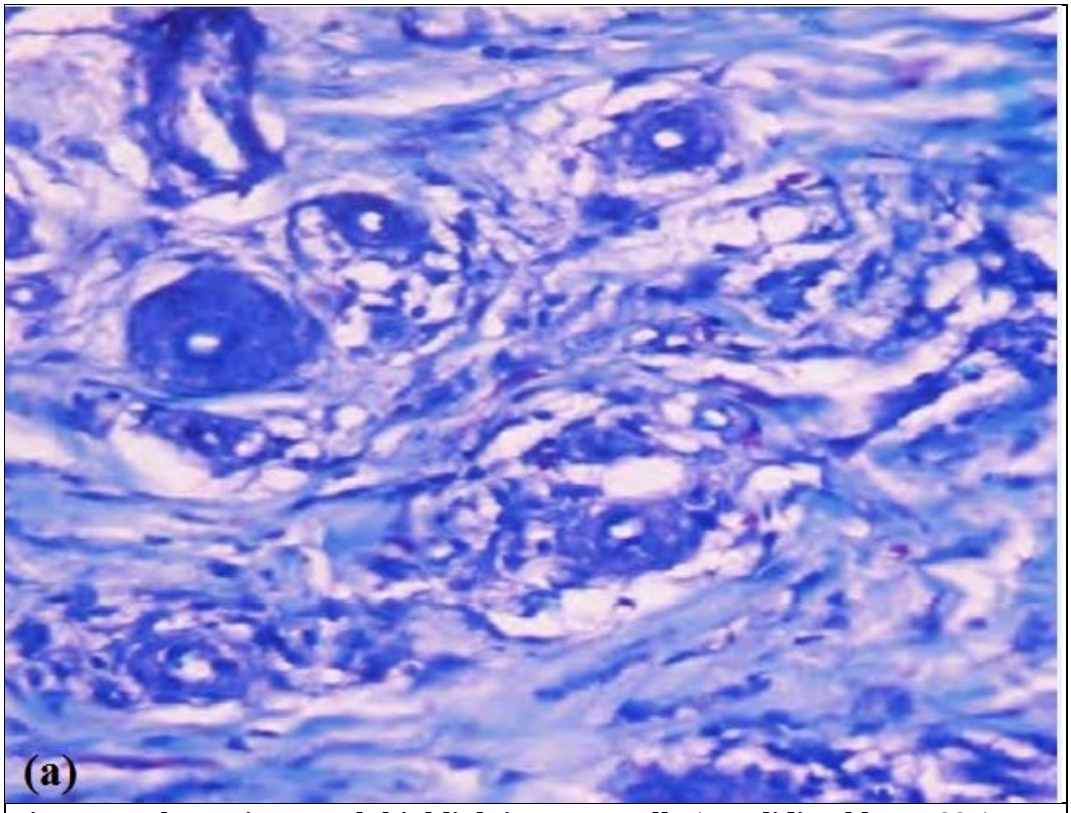

Figure a: Photomicrograph highlighting mast cells (Toulidine blue; 100x)

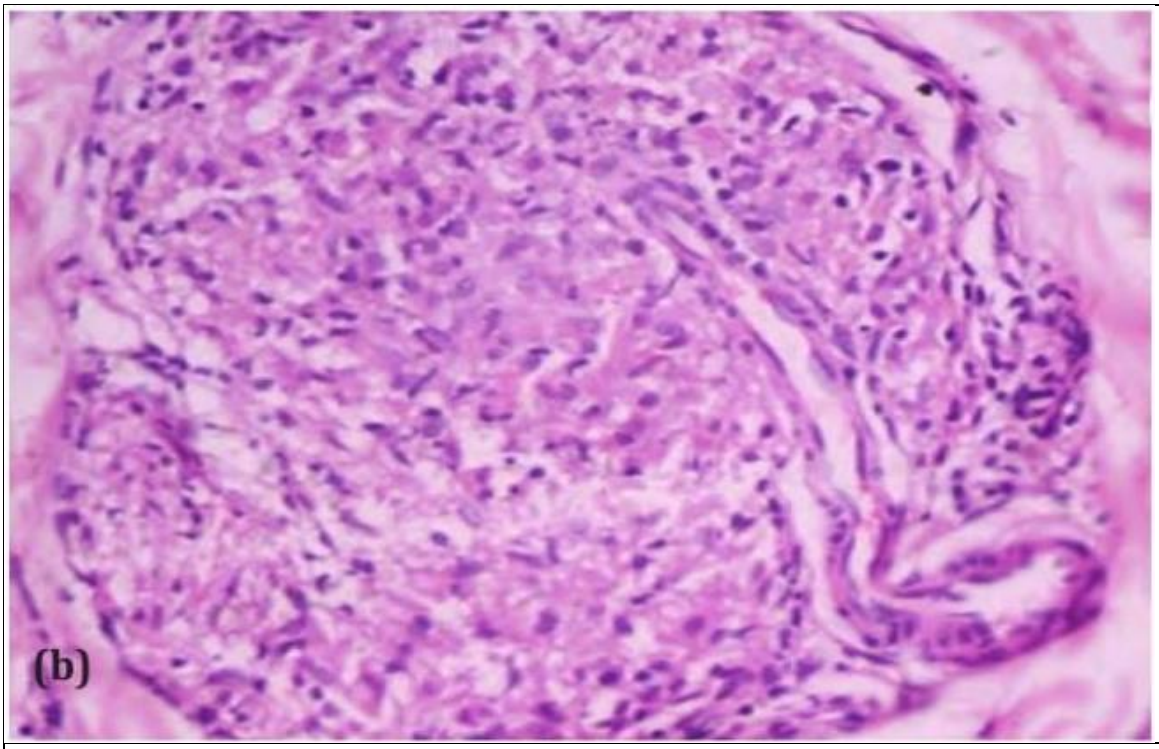

Figure b: Photomicrograph showing mixed inflammatory infiltrate pattern composed of lymphocytes , histiocytes & mast cells (H &E;40x)

RESULTS

The mean age of patients was 43.5±3.8 years. Out of 100 cases; 76% were male & 24% were female (figure 1). The most common type of leprosy seen were paucibacillary (60) & mutibacillary (40) (figure 1). No reaction was found in 64 cases whereas type I reaction was seen 26 cases & type II reaction was seen in 10 cases (figure 1). The number of mean mast cell found in no reaction was 22.3±10.1, in Type I reaction was 10.4±3.9 & in Type II reaction was 12.7±4.1 (figure 2).

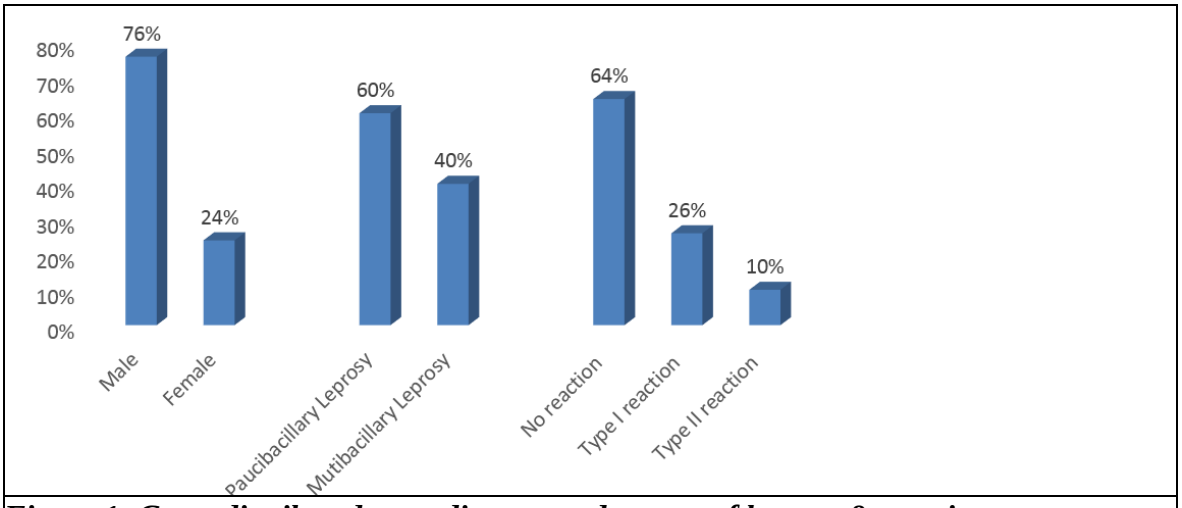

Figure 1: Cases distributed according to gender, type of leprosy & reaction

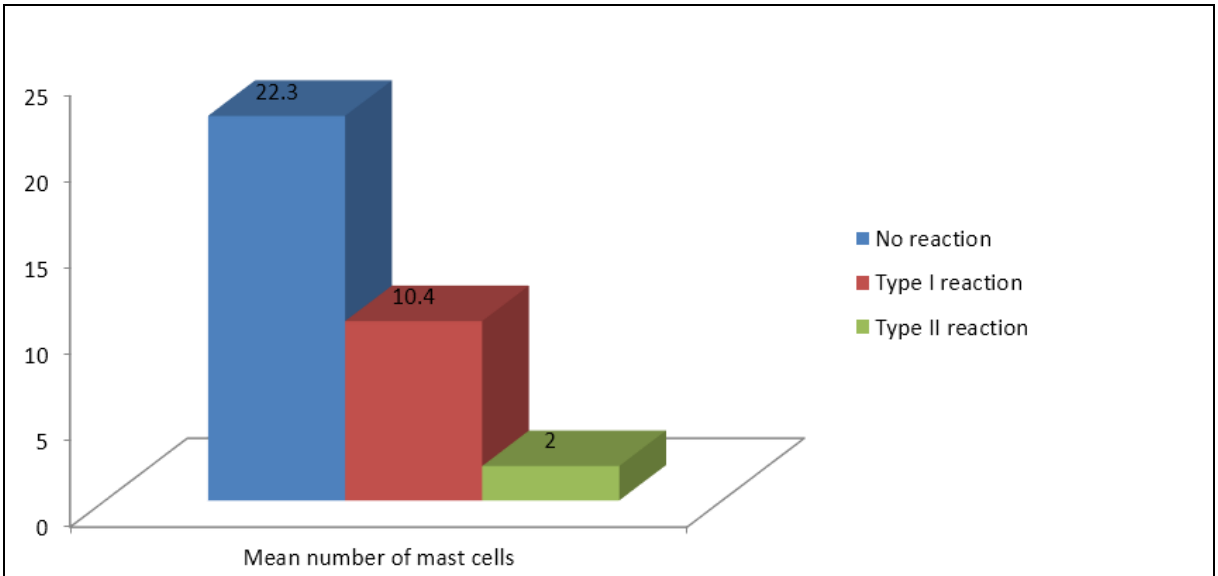

Figure 2: Mean number of mast cell in each reaction type

The histopathological slides shows tuberculoid in 14% cases, borderline tuberculoid in 12% cases , borderline in 20% cases, borderline lepromatous in 22% cases, lepromatous in 16% cases, indeterminate leprosy in 12% cases & histoid leprosy in 4% cases as shown in table 1.

Histopathological type	**Frequency (%)**
TT	14 (14)
BT	12 (12)
BB	20 (20)
BL	22 (22)
LL	16 (16)
Indeterminate leprosy	12 (12)
Histoid leprosy	4 (4)

Table 1: Histo-Pathological distribution of cases

Significant association ($p<0.05$) between number of mast cell in each area (central, peripheral, interstitial) by leprosy reaction type (no reaction, type I reaction, type II reaction) was seen as shown in table 2.

Leprosy reaction type	**Central**	**Peripheral**	**Interstitial**	**P value**
No reaction	4.9±0.5	8.9±0.3	22.3±10.1	<0.001*
Type I reaction	4.5±0.3	3.4±0.5	10.4±3.9	0.001*
Type II reaction	4.0±0.2	3.7±0.7	12.7±4.1	0.002*

Table 2: Association of number of mast cell in each area by leprosy reaction type

*: statistically significant

DISCUSSION

Leprosy is a chronic disease caused by Mycobacterium leprae, exhibiting a broad spectrum of clinical symptoms. Leprosy is a predominant cause of irreversible physical impairments among communicable diseases. Prompt diagnosis & intervention are therefore critical to minimize morbidity. [13]

Leprosy is a complicated disease, & the degree of cellular immune response to M. leprae determines its clinical & histological features. The immunopathogenesis is yet not fully understood. Many cell types, including mast cells, are thought to be involved. In both immediate & delayed hypersensitivity reactions, mast cells play a crucial part. [14]

In the present study; 100 cases of leprosy were taken, the average age of patients 43.5 years & number of male patients were higher in number as compared to female patients. The number of mean mast cell found in no reaction was 22.3±10.1, in Type I reaction was 10.4±3.9 & in Type II reaction was 12.7±4.1. The most common type of leprosy was paucibacillary (60) while most common histologically was Borderline lepromatous (BL) (44). Cases were distributed on the basis of no reaction (64) & type I (26) & type II reaction (10) as there was significant association found between type of reaction & number of mast cell in each area ($p<0.05$).

According to a study by Kamra HT et al. [15], the control females had a higher mast cell density, which may have been caused by the action of female sex hormones. With 35 cells per square millimetre, borderline lepromatous leprosy has the highest mean MCD. Polar tuberculoid & indeterminate leprosy groups in the current study showed lower values than the control group, whereas all other categories showed higher values than the controls. KR Comparable outcomes were also obtained by Chatura et al. [16]. At the tuberculoid pole, the average number of mast cells per 2 mm is highest in BT at 14.23 & lowest in TT at 7.9. In BL, it has peaked at 9.21 at the lepromatous end, while in LL, it is 8.23. Overall, the borderline categories have shown the highest frequencies. The study found a negative but not statistically significant correlation between mast cell count & histological diagnosis, with a correlation coefficient of -0.17. To measure mast cells in leprosy lesions, Magalhaes Gde O et al. [17] used an antitryptase antibody in a quantitative & morphometric examination of tryptase-positive mast cells. Of the three groups under investigation, they found that the lepromatous leprosy group had the lowest dermal mast cell density values. Furthermore, lepromatous leprosy biopsies have a much larger mean cross-sectional area of mast cells than borderline & tuberculoid leprosy biopsies, suggesting functional differences between the groups. It is believed that the increased mast cell density in the borderline & tuberculoid groups is indirect proof that mast cells contribute to the heightened immune response to Mycobacterium leprae infection. From the lepromatous to the tuberculoid end of the spectrum, there is a trend for the mast cell count to decrease, according to Rav et al. [18]. When compared to lepromatous lesions, Aroni et al. [19] found that the tuberculoid group had less mast cells. Mysorekar et al [20] reported that lepromatous patients exhibit significantly higher mast cell density compared to those with tuberculoid Hansen disease. The indeterminate group has also demonstrated a significantly high mast cell density. Bagwan et al [21] identified normal mast cell numbers in indeterminate & tuberculoid leprosy, demonstrating an increase in counts corresponding to the immunological spectrum from borderline tuberculoid to lepromatous leprosy. Naik et al [22] are the sole researchers to have noted a high mast cell level in tuberculoid patients & the lowest count in indeterminate leprosy. The rise in mast cell density corresponds with the typical density variations reported in different histological forms of leprosy & is contingent upon physiological changes in mast cell count.

According to the literature, the higher mast cell count in lepromatous Hansen's disease as opposed to tuberculoid leprosy can be explained by the T-helper cell type 2 (TH-2) response, which is more prevalent in lepromatous leprosy & encourages the release of anti-inflammatory cytokines, whereas the T-helper cell type 1 (TH-1) response is more prevalent in tuberculoid leprosy & facilitates the release of pro-inflammatory cytokines. TNF-alpha, IL-1, IL-4, IL-5, IL-6, & GM-CSF are among the several cytokines that are believed to be associated with mast cells; most of these are a component of the TH-2 response. The increased mast cell count seen in lepromatous leprosy is explained by the TH-2 response's predominance in this state [15]. Interleukin-6 (IL-6), which stimulates the production of acute phase proteins, is recognized as an additional chemical that promotes the adherence of mast cells to extracellular matrix (ECM) components by the upregulation of integrin mRNA & protein expression in these cells. The discovery of the IL-6 receptor on HMC-1 cells & the reduction of HMC-1 binding to fibronectin, & to a lesser extent to vitronectin, by antibodies targeting α1, α5, & α4α5 integrins highlight the specificity of this impact. In another study, ECM adhesion in murine mast cells was not enhanced by platelet-derived growth factors, TNF-α, IFN-γ, IL-1, IL-4, & endothelial growth factors α & β. On the other hand, it has been shown

that IL-1 increases the adherence of human mast cells in the uterus to endothelial cells by a factor of five. [23]

According to the main findings about Mast Cell Analysis, the difference in the mean number of Mast Cells between Non-Reactional Leprosy & Leprosy with Reaction may indicate the pivotal role that Mast Cells play in the dynamic changes of the Cell Mediated Immune Response in Leprosy & its reactions. Another possible explanation, assuming that most connective tissue cells are descended from a common stem cell, is that when proliferation in one cell lineage is initiated, it is also triggered in other cell lineages. Both the disease process & the host's immune response may produce changes in mast cells in leprosy, which is caused by M. leprae.[24]

CONCLUSION

The current study shows significantly increased mast cell density in skin leprosy lesions, proving that infiltrating mast cells play a role in lesion progression. MCD was significantly higher in border line leprosy cases with interstitial region. Therefore, it can be concluded that increased numbers of mast cells point towards better disease prognosis in skin leprosy lesions.

REFERENCES

1. Singh P, Benjak A, Schuenemann VJ, Herbig A, Avanzi C, Busso P, Nieselt K, Krause J, Vera-Cabrera L, Cole ST. Insight into the evolution & origin of leprosy bacilli from the genome sequence of Mycobacterium lepromatosis. Proc Natl Acad Sci U S A. 2015 Apr 07;112(14):4459-64.
2. Ridley DS. Histological classification & the immunological spectrum of leprosy. Buli WHO. 1 974; 51: 45 1 -465.
3. Galli SJ, Dvorak AM. What do mast cells have to do with delayed hypersensitivity? Lab Invest 1984;50:365-8.
4. Barrett KE, Metcalfe DD. Mast cell heterogeneity: evidence & implications. J Clin Immunol 1984;4:253-61.
5. Friedman MM, Kaliner M. In situ degranulation of human nasal mucosal mast cells: ultrastructural features & cellcell associations. J Allergy Clin Immunol 1985;76:70-82.
6. Friedman MM, Metcalfe DD, Kaliner M. Electron microscopic comparison of human nasal & lung mast cell degranulation. In: Befus AD, Bienenstock J, Denburg JA, eds. Mast cell differentiation & heterogeneity. New York: Raven Press, 1986:367-78.
7. Ridlcy DS. The pathology of leprosy. In: Hastings RC, ed. Leprosy. Edinburgh: Churchill Livingstone, 1985:100-33.
8. Pfaltzgraff REP, Bryceson A. Clinical leprosy. In: Hastings RC, ed. Leprosy. Edinburgh: Churchill Livingstone, 1985:1 34-76.
9. Ridley DS, Radia KB. The histological course of reactions in borderline leprosy & their outcome. Int J Lepr Other Mvcobact Dis 1981;49:383-91
10. Chowdhury SK, Ghosh S. Distribution of tissue mast cells in "reaction in tuberculoid leprosy". Bull Calcuitta School Trop Med. 1968;16:13-14.
11. Mabalav MC, Helwig EB, -Tolentino JG, Binford CH. The histopathology & histochemistry of Erythema Nodosum Leprosum. Int . Lepr Other Mycobact Dis. 1965;33:28-49.
12. Ridley DS. Erythema nodosum leprosum. In: Ridley DS, ed. Pathogenesis of leprosy & related diseases. London: Wright. 1988:123-34.
13. Anderson's Pathology "Bacterial Diseases", edited by Washington C & John C Kissane, 10th ed, Mosby year book, 854-61, 1996.
14. Galli SJ, Dvorak AM. What do mast cells have to do with delayed hypersensitivity? Lab lnvest. 1 984; 50: 365.
15. Kamra HT, Munde SL, Gangane N, Sharma SM, Gulia A, Agarwal R. Significance of mast cell density & distribution in various histopathological lesions of leprosy. JKIMSU. 2014 Jan 1;3(1):57-63.

16. Chatura KR, Sangeetha S. Utility of Fite-Faraco stains for both mast cell count & bacillary index in skin biopsies of leprosy patients. Indian J Lepr 2012; 84(3):209- 215.
17. Magalhães Gde O, Valentim Vda C, Pereira MJ, Nery JA, Illarramendi X, Antunes SL. A quantitative & morphometric study of tryptase-positive mast cells in cutaneous leprosy lesions. Acta Trop 2008; 105(1):62-66.
18. Rav SD, Pratap VK, Sharma NK, Dayal SS. Mast cell in leprosy. Indian J Lepr 1990; 62(4):467-472.
19. Aroni K, Kontochristopoulos G, Liossi A, Panteleos D. An investigation of mast cells in two basic leprosy groups. Int J Lepr Other Mycobact Dis 1993; 61(4):634- 635.
20. Mysorekar VV, Dandekar CP, Rao SG. Mast cells in leprosy skin lesions. Lepr Rev 2001; 72(1):29-34
21. Bagwan IN, Khandekar MM, Kadam P, Jadhav MV, Deshmukh SD. A study of mast cells in granulomatous lesions of skin, with special emphasis on leprosy. Indian J Lepr 2004; 76(1):31-37.
22. Naik R, Pai MR, Bantwal PB, Shankarnarayana, Nayak KS, Gandhi A. Study of mast cells in non-neoplastic skin lesions. Indian J Pathol Microbiol 2003; 46(2):173- 175.
23. Schoeler D, Grützkau A, Henz BM, Küchler J, KrügerKrasagakis S. Interleukin-6 enhances whereas tumor necrosis factor alpha & interferons inhibit integrin expression & adhesion of human mast cells to extracellular matrix proteins. J Invest Dermatol 2003; 120(5):795-801.
24. Mohit, Dixit S, Sharma R, Sharma P, Kumar P. Evaluation of Mast Cell Profile in the Skin Lesions of Leprosy. Ann. Int. Med. Den. Res. 2018; 4(6): PT05-PT08.