ORIGINAL ARTICLE

Significance of Mast Cell Density and Distribution in Various Histopathological Lesions of Leprosy

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Abstract:
Background: Leprosy also known as Hansen's disease (HD), is a chronic infection caused by the Mycobacterium leprae. Mast cells in leprosy have been investigated in the recent past and are being examined as a basis for future studies. *Materials and Methods:* 119 leprosy cases and 14 control biopsies stained with toluidine blue were assessed for density and distribution of mast cells. 38 cases had undergone treatment. *Results:* Significantly higher mast cell count was obtained in the skin lesions of borderline lepromatous leprosy. On comparison with controls lower counts were observed in polar tuberculoid and indeterminate leprosy whereas higher values were observed in all other groups. The mast cell count in leprosy is probably determined by the pattern of cytokines released by the T lymphocytes. Mast cell density assessed after treatment was reduced in borderline tuberculoid and borderline lepromatous cases however in indeterminate group, the counts were increased. *Conclusion*: The distribution of mast cells in leprosy is a variable feature and there is no constant site or predilection for a particular type of leprosy.

Keywords: cytokines, T helper cells, immune response.

Introduction:

Mast cells can no longer be regarded simply as cells that initiate acute allergic reaction [1]. Paul Ehrlich who coined the term "Mastzellen" also proposed that mast cells help in maintaining nutrition of connective tissue [2]. They can orchestrate the infiltration of leucocytes into sites of mast cell activation, an effect that develops over a period of several hours and is at least partially cytokine dependent [1].

Mast cells have received little attention in leprosy but evidence linking them with delayed type hypersensitivity reaction raises the possibility that they may be of some importance in leprosy in both nonreactional and reactional states [3]. In murine leprosy, in early stages mastocytosis was observed and there was disintegration, disruption and degranulation of mast cell number and release of histamine, heparin and 5-Ht, thus permitting an afflux of immune factors into affected areas [4].

Naik et al 2003 [5] suggested that periodic follow up of indeterminate and borderline lesions for mast cell count might help in predicting stability of lesions. According to Bagwan et al 2004 [6], the study of mast cells provides a very cost effective way of having insight into immunological aspects of leprosy and tuberculosis and has a diagnostic application. Montagna NA et al 2005 [7] have related increase in tryptase rich mast cells to epineuria collagenization in leprosy through their tryptase secretion.

The present study was conducted to ascertain the density and distribution of mast cells in leprosy and to correlate the differences in mast cell density in lesions relapsing after therapy.

Materials and Methods:

The present study was conducted in Department of Pathology, Mahatma Gandhi Institute of Medical Sciences Wardha, Maharashtra on 119 histopathologically confirmed cases of leprosy irrespective of their ages, sex and histological type and 14 biopsies from normal skin as controls. In each case punch biopsy of 6mm was taken from the margin of the lesion with subcutaneous tissue and fixed in 10% buffered for-

malin. Tissue was processed and serial sections were stained with hematoxylin and eosin for histomorphological study of skin, Fite Feroco for acid fast bacillus and toluidine blue stain for mast cells. Leprosy cases were classified into five groups according to Ridley and Jopling [8] classification as Lepromatous leprosy (LL), borderline lepromatous leprosy (BL), mid borderline leprosy (BB), Borderline tuberculoid leprosy (BT) and Tuberculoid leprosy (TT). Cases where there were one or two small hypopigmented macules on the skin with a slight sensory impairment clinically and where the skin biopsy histologically showed a nonspecific lymphocyte infiltrate around dermal nerve twigs with occasional acid fast bacillus were labeled as indeterminate leprosy. In each case the mast cell density was calculated in toluidine blue stained sections using Olympus CH30 model biological microscope in all the cases using EA100 oil immersion objective and NCWHK 10x eyepiece. With these specifications and the magnification of 1000X and field of view diameter of 0.18 mm., the radius r of a circle was calculated as = 3.14 x 0.09 x 0.09 sq.mm = 0.0254 sq.mm

Thus, for counting in 1 sq.mm area we added the cell count in 1/0.0254 =39.37 fields or rounded upto 40 fields. Therefore, 40 non overlapping fields were counted to obtain mast cell count per sq.mm. area and their distribution was further studied subepidermally, in granuloma, intervening dermis, skin appendages, blood vessels and nerves.

Results:

Out of the 119 leprosy cases in the present study there were 8TT, 42BT, 19BL, 9LL, 34. Indeterminate and 7 Erythema nodosum leprosum cases. Maximum number of patients were in the age groups of 21-30 years. Male to female ratio was 2:1. The mean density of mast cells in the 14 control biopsies was 29.28 sq.mm. The density of mast cells in the skin lesions in 119 cases is given in (Table 1).

On comparison with controls, it was observed that lower counts were observed in polar tuberculoid and indeterminate leprosy, whereas higher values were observed in all other groups. The highest values were seen in borderline lepromatous lesions (Mean of 35 cells per sq.mm). However, on statistical analysis the differences between mean mast cell counts in cases and control were not found to be significant ($p>0.05$). The total mean mast cell count was analysed in different age groups in the skin biopsies of controls and was maximum in the age group 41-50 years that is 60 cells/sq.mm and females showed slightly higher mast cell density when compared to males.

Table 1: Density of Mast Cells in the Skin Lesions of Various Types of Leprosy

Sr. No.	Type of leprosy	Mean mast cell density per sq.mm	
		Mean	Std. Deviation
1	Tuberculoid leprosy	21.25	05.17
2	Borderline Tuberculoid leprosy	30.35	15.43
3	Borderline Lepromatous leprosy	35.00	14.71
4	Lepromatous leprosy	31.66	17.85
5	Indeterminate leprosy	27.94	13.93
6	Erythema nodosum leprosum	30.00	15.54
7	Control	29.28	14.91

The distribution of mast cells was more commonly found in granulomas in skin biopsies of Erythema nodosum leprosum (40% cases), Lepromatous leprosy (35.71% cases) and in Borderline lepromatous leprosy (33.33% cases) whereas in Indeterminate leprosy mast cells were predominantly located around the skin appendages, in tuberculoid group they were preferentially located in the intervening dermis. The mast cells were more commonly observed in subepidermal location in the control group.

Active mast cell morphology was commonly seen in controls as well as in patients of tuberculoid leprosy, borderline tuberculoid leprosy and borderline lepromatous leprosy. However, the fusiform morphology was commonly observed in lepromatous leprosy, indeterminate leprosy and erythema nodosum leprosum. Active degranulation was observed in only three cases.

Mast cell density was correlated with previous skin biopsies on treatment and in 38 patients who relapsed after treatment and in patients who developed new active lesions.

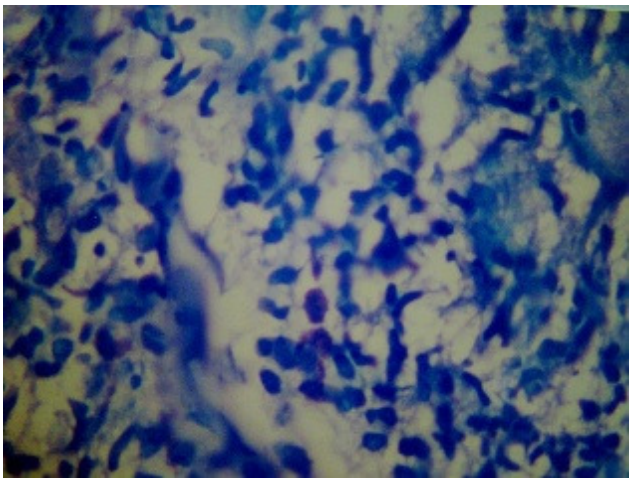
It was observed that the mean mast cell density per sq. mm was decreased in both borderline tuberculoid leprosy and borderline lepromatous groups; however in indeterminate groups the counts increased after treatment. However on statistical analysis the differences were not found to be significant. Morphological examination of mast cells in these cases revealed that fusiform mast cells were more commonly seen in all three groups after relapse as compared to predominant active morphology in same groups before treatment.

Discussion:

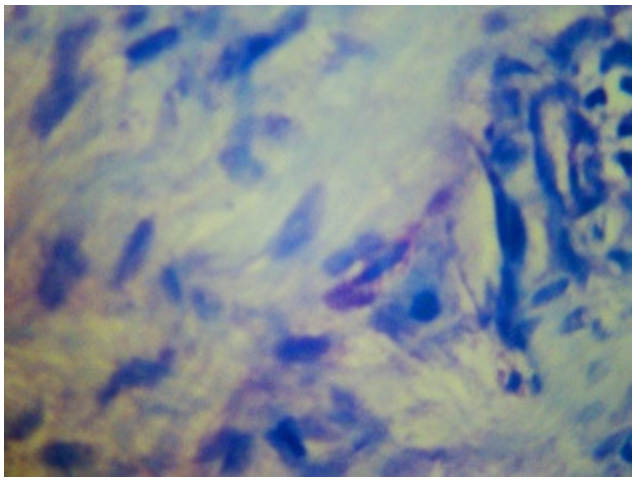
Arvy (1956) [9] has demonstrated an increase of mast

Table 2: Comparison of Mast Cell Density in the Patients Before and After Leprosy Treatment.

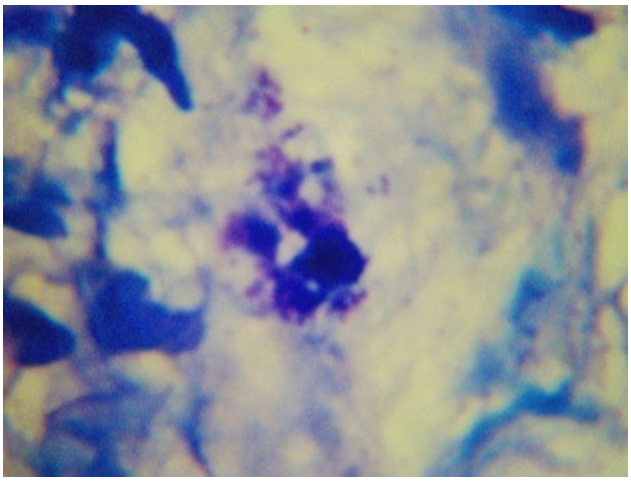
Sr. No.	Type of leprosy	Mean mast cell density per sq.mm	
		Before Treatment	After Treatment
1	Borderline Tuberculoid leprosy	32.59(18.20)	26.33(07.43)
2	Borderline Lepromatous leprosy	37.50(14.63)	33.18(15.21)
3	Indeterminate leprosy	26.36(13.10)	30.80(15.49)



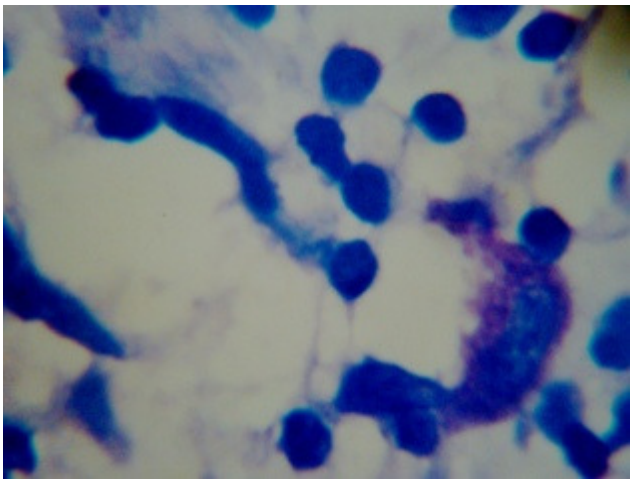
Microphotograph 1: Shows Presence of Mast Cells in Midst of Foamy Macrophages in Lepromatous Leprosy. (Toludine blue stain)



Microphotograph 2: Shows Presence of Mast Cells in Intervening Dermis. (Toludine blue stain)



Microphotograph 3: Showing Active Degranulation of Mast Cell in Intervening Dermis. (Toludine blue stain)



Microphotograph 4: Showing Active Morphology of Mast Cell in Indeterminate Leprosy. (Toludine blue stain)

cell in most organs of mouse following estrogen administration. Other authors have also observed that mast cells are present in significantly higher numbers in females than males. However with advancing age these values decreased to the same level in both the sexes. A higher mast cell density was observed in the present study in the control females and this could be probably attributed to the effect of female sex hormones. The mean mast cell density has been highest in borderline lepromatous leprosy (35 cells/sq.mm). Lower values than control have been observed in polar tuberculoid and indeterminate leprosy where as all other groups have shown higher values than controls in the present study. KR Chatura et al 2012 [10] also have had similar results. The mean mast cell count per^2 mm at the tuberculoid pole has been lowest in TT 7.9 and highest in BT 14.23. At the lepromatous end, it has been highest in BL 9.21, while in LL it has been 8.23. Highest counts have been seen in the borderline types overall. In their study also the correlation coefficient between histopathological diagnosis and mast cell count has been found to be -0.17, which is a negative correlation but not to a significant degree. Magalhaes Gde O et al 2007 [11] in a quantitative and morphometric study of tryptase positive mast cells using antitryptase antibody in order to quantify mast cells in leprosy lesions have also observed that lepromatous leprosy group has had the lowest dermal mast cell density values among the three groups. Furthermore, the average mast cell cross-sectional area has been significantly higher in lepromatous leprosy in comparison to the borderline borderline leprosy and tuberculoid leprosy biopsies suggesting mast cell functional differences within the groups. The higher mast cell density in the tuberculoid and borderline groups is considered as an indirect evidence of role of mast cells in the activated immune response to *Mycobacterium leprae* infection. Rav et al 1990 [12] have also observed that there is a tendancy for decreasing mast cell count from the lepromatous to the tuberculoid end of the spectrum. Aroni et al 1993 [13] have found that tuberculoid group has shown lower mast cell count than lepromatous lesions. Mysorekar et al 2001 [14] have observed that very high mast cell density is seen in lepromatous patients as compared to tuberculoid Hansen disease. The indeterminate group also has shown very high mast cell density. Bagwan et al 2004 [6] have observed normal mast cell counts in indeterminate and tuberculoid leprosy and have shown a rise in counts according to

immunological spectrum from borderline tuberculoid to lepromatous leprosy. We have also observed that cells count tend to be higher in lepromatous Hansen's disease as compared to tuberculoid Hansen's disease as described by other authors. Only Naik et al 2003 [5] have observed high mast cell count in tuberculoid cases and lowest in indeterminate leprosy. Increase in density of mast cell has also corresponded with normal increase in density observed in various histological types of leprosy and depends on the physiological changes in the mast cell count which are observed in control groups of the present study.

The raised mast cell count in lepromatous Hansen disease over that of tuberculoid leprosy can be explained on the predominance of T-helper cell type 2 (TH-2) response of anti-inflammatory cytokine liberation in lepromatous leprosy as against T helper cell type 1 (TH-1) response of proinflammatory cytokine liberation in tuberculoid leprosy. Mast cells are believed to be associated with a variety of cytokines such as TNF-alpha, IL-1, IL-4, IL-5, IL-6 and GM-CSF most of which belong to the TH-2 response. Predominance of TH-2 response in lepromatous leprosy thus explains the presence of raised mast cell count in it [14]. IL-6, an interleukin that induces acute phase proteins, is identified here as another molecule that enhances adhesion of mast cells to ECM molecules by an up regulation of integrin mRNA and protein expression of these cells. Specificity of this effect is underlined by blockage of HMC-1 binding to fibronectin, less so to vitronectin, by antibodies against α 1, α 5 and $\alpha 4\alpha 5$ integrins, and by demonstration of the IL-6 receptor on HMC-1 cells. In another study, the platelet-derived growth factors, TNF-α, IFN-γ, IL-1, IL-4, and endothelial growth factors α and β have failed to induce murine mast cell ECM adhesion. In contrast, IL-1 has been reported to cause a 5-fold increase of adherence of uterine human mast cells to endothelial cells. [15]

We have also observed higher mast cell counts in borderline lepromatous leprosy than in polar lepromatous leprosy. This may also indicate that the (TH-2) response is higher in borderline lepromatous leprosy than in polar lepromatous disease. The mast cell density is known to differ between different anatomical locations in the normal skin. Aroni et al 1993 [13] have not found any difference between distribution of mast cells in tissues of tuberculoid and lepromatous leprosy. Mysorekar et al 2001 [14] and Naik et al 2003 [5] also have not found any difference in distribution of mast cell in different lesions of leprosy. Cree et al 1990 [15] have observed that the density of mast cells has been greatest in granuloma and least in intervening dermis in both paucibacillary and multibacillary type. In the granulomas mast cell density is observed to be reduced in multibacillary as compared with paucibacillary patients whereas Mahasavariya et al 2000 [3] have observed that the greatest number of mast cells have been in interstitium and not in granuloma. The findings of the present study suggest that the distribution of mast cells in leprosy lesions is a variable feature and there is no constant site or predilection for a particular type of leprosy.

Cree et al 1990 [16] have suggested that density within granulomata is determined by systemic response to infection by M. leprae and not by local factors. However we have observed mast cell density to be high in granulomas in patients of erythema nodosum leprosum. In the absence of sequential studies of biopsies during and after the development of reactional stage the clinical importance of these differences is not clear. But the fact that such a difference exists in mast cell density might be related to a tendancy to develop reactions and it can be hypothesized that these patients with higher mast cell density in granulomas might be susceptible to more damaging reversal reactions due to the release of mediators from the mast cells seems to be an attractive hypothesis and is worth further study. Cree et al 1990 [16] have also observed that the appearance of mast cells in leprosy lesions is very variable. They have observed several morphologically distinct variants-the active mast cells, fusi-

form mast cells or resting mast cells. They have also observed that the fusiform mast cells are present more commonly in the inflammatory foci. Most of the authors agree with Cree et al 1990 [16] that in histology there are too many mast cells of indeterminate morphology that do not allow the typing of mast cells easily and they cannot be counted separately on morphological grounds alone and therefore on routine histological examinations it is not possible to differentiate or assign any morphological subtype designation for all mast cells.

Mast cell density has also been correlated with previous skin biopsies in 38 patients who relapsed after treatment or who developed new active lesions. Mast cell density is reduced in borderline tuberculoid and borderline lepromatous leprosy lesions after treatment but in indeterminate group the counts show increase. Active morphology of mast cells has been observed before treatment and fusiform morphology after treatment. Van Hale et al 1984 [17] have observed the presence of mast cells to be much more common before treatment than in those undergoing therapy. The change in mast cell density after treatment is possible because most of the lesions upgrade to become more of tuberculoid type with changes in the cytokine pattern. Antunes et al 2003 [18] have observed a relative increase of MC tryptase in the inflammatory infiltrate in reactional biopsies. Also, the total number of mast cells and the MC tryptase/MC tryptase, chymase ratio in inflammatory infiltrate has been significantly higher than in intervening dermis. Moubasher et al 1998 [19] have observed significant reduction in serum cytokines after one year of treatment in paucibacillary forms than in multibacillary patients. They have observed significant reduction in serum cytokines after one year of treatment in paucibacillary forms than in multibacillary forms even touching the levels seen in healthy controls. This suggests that mast cell morphology may show significant alteration as a response to therapy or in response to cytokine pattern leading to the development of new active lesions.

However, before labeling this cell as a master cell of immune response we need to carry out studies which take into account the normal biological variations in the number and morphology of the mast cells.

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