Indian Journal of Pathology: Research and Practice 8(2) March-April 2019
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.8219.10

IJPRP

*Original Research Article*

# Histopathological Study of Mast Cells in Leprosy

**Prabhu Mural H.[1], Aditya Vikram Singh[2], Sateesh S Jigjini[3], Niharika Shahi[4]**

[1]Associate Professor [2]Post Graduate [3]Professor [4]Senior Resident, Department of Pathology, S. Nijalingappa Medical College, Bagalkot, Karnataka 587102, India.

## Abstract

*Context:* Mast cells have a multifunctional capacity. Their role in immunity is well known. Mast cells act as local immune modulators with a capacity to co-ordinate between pro and anti-inflammatory response. Leprosy is classified on the basis of immune status of patients. Mast cells in leprosy have been examined in recent past and are being examined as a basis for future studies. Staining of mast cells by Toluidine blue is well documented. Use of Fite Faraco's stain can have an additional advantage of staining bacilli and mast cells.

*Aims: 1.* To study average number of Mast cells in various types of leprosy. 2. To study distribution of mast cells with reference to Acid Fast Bacilli.

*Methods and Material:* Study included 65 skin biopsies of histopathologically diagnosed cases of leprosy. Sections were stained by Fite Faraco's stain, viewed under 400X and mast cells were counted per mm square. Their distribution in various types of leprosy was recorded.

*Results:* Among the patients studied the males outnumbered females by a ratio of 1.6 : 1. Significantly higher Mean Mast Cell Density (27.94) was obtained in Polar Tuberculoid leprosy (TT), lowest was found in Polar Lepromatous leprosy (LL). Maximum numbers of mast cells were distributed around granulomas and glands. However, finding both mast cells and bacilli in same structure was not a constant finding.

*Conclusions:* The higher mast cell density in the immunologically strong group of patients is evidence of the role of mast cells in the activated immune response to M. leprae infections.

**Keywords:** Leprosy; Ridley-Jopling Classification; Mast Cells; Fite Faraco's Stain; Mean Mast Cell Density; Immunity.

**Corresponding Author:**
**Aditya Vikram Singh**, Post Graduate, Department of Pathology, S. Nijalingappa Medical College, Navanagar, Bagalkot, Karnataka 587102, India.

**E-mail:** adi.knight01@gmail.com

**Received on** 31.12.2018,

**Accepted on** 04.02.2019

**How to cite this article:**

Prabhu Mural H., Aditya Vikram Singh, Sateesh S Jigjini et al. Histopathological Study of Mast Cells in Leprosy. Indian J Pathol Res Pract. 2019;8(2):189-194.

## Introduction

Mast cells are dynamic cells and they play an important role in inflammatory allergic reactions and diverse pathological processes [1].

These cells are found all levels of skin including dermo-epidermal junction, dermis, around blood vessels, nerves, appendages and also in subcutaneous tissue [2].

They are involved in various immune inflammatory responses by producing a number of inflammatory mediators. These mediators act on various sites like vasculature, smooth muscle, connective tissue, mucous glands and other inflammatory cells. Hence, by virtue of these mediators they help in immunity [3].

In leprosy, the bacillus triggers the disease. The other major part is played by host cellular immune response level to M. leprae, which determines the pattern of disease. The cellular immune response gives rise to a spectrum of clinical and histopathological features. At one end there is lepromatous leprosy where there is failure of cellular immunity. At the other end is the tuberculoid leprosy, where there is good immune response [4].

However, mast cells have not received much attention in leprosy. There are various evidences which link them to development of delayed hypersensitivity reactions, raise the possibility that they are of importance in leprosy lesions. There are conflicting reports of number of mast cells in either poles of leprosy [1-8].

## Materials and Methods

A study of mast cell number and distribution by Fite Faraco's staining in biopsy specimens of leprosy was conducted in the Department of Pathology, S. Nijalingappa Medical College and HSK Hospital & Research centre, Bagalkot during the period of two years. Data collection was done from December 2016 to May 2018.

*Type of Study:* Case series study.

Total of 65 cases were studied. Specimens included skin biopsy from clinically diagnosed leprosy patients. In each case, the clinical history and findings were recorded, directly from the patients.

A 4 mm skin punch biopsy was obtained, in Department of Dermatology, under local anaesthesia with 2% lignocaine from suspected leprosy patients. Commonly, skin biopsies were taken from the representative sites of lesion. The skin biopsy specimens were fixed with 10% formalin for 10-12 hours. After the fixation of sample, the skin biopsies were processed using conventional histopathological technique.

Hematoxylin and Eosine stain was done and sections were studied for confirmation of leprosy. The skipped serial sections from histopathologically diagnosed cases were further subjected to Fite Faraco's Stain.

*Inclusion criteria*

1. Sections from biopsies reported histopathologically as leprosy.
2. Biopsies where morphological characters are preserved.
3. Biopsies which show any one of vessels, nerves and adnexae.

*Exclusion Criteria*

1. Biopsies where skipped serial sections are not obtained.
2. Allergic skin conditions.
3. Biopsies with any other lesions
4. Biopsies where staining characters are poor.
5. All complicated leprosy cases- including secondary infection of ulcers and amyloidosis

*Mast Cells Quantification*

1. Stained sections were studied using Olympus CH20i microscope with field of view number 18.

2. High power field area was calculated as:

- Diameter of microscope field = Field of view number/Initial magnitude of high power objective = 18/40 = 0.45mm.

- High power field area (A)= $\pi r^2 = 0.152mm^2$ .

3. Hence, if one high power field (hpf) is $0.152mm^2$, 10 hpf areas are $1.52\ mm^2$ .

4. So, if 'X' is value of cells observed in 10 hpfs; 'X'*10/152 is the number of cells observed per $mm^2$.

Mean Mast cell density was counted. Also the numbers of mast cells were counted around adnexae, granulomas and grenz zone.

## Results

Total of 65 skin biopsies were studied from December 2016 to May 2018 and results are as follows:

Among the total number of 65 patients studied, male patients were 40 forming 62 % and female patients were 25 which form 38%. Male patients outnumbered female by a ratio of 1.6:1.

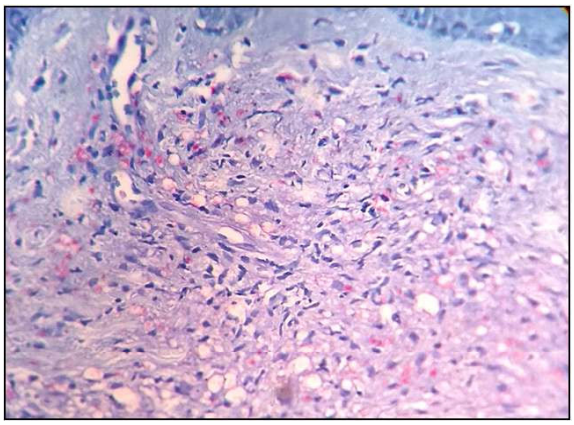

**Fig. 1:** LL showing Globi of Mycobacterium leprae (FF Stain; 40X) (Scant mast cells).

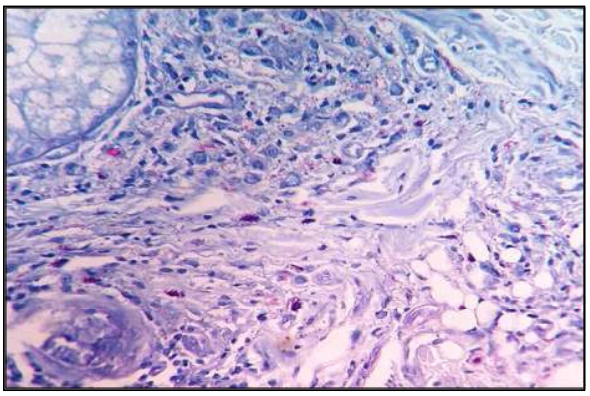

**Fig.2:** BL showing bacilli and mast cells. (FF Stain; 40X)

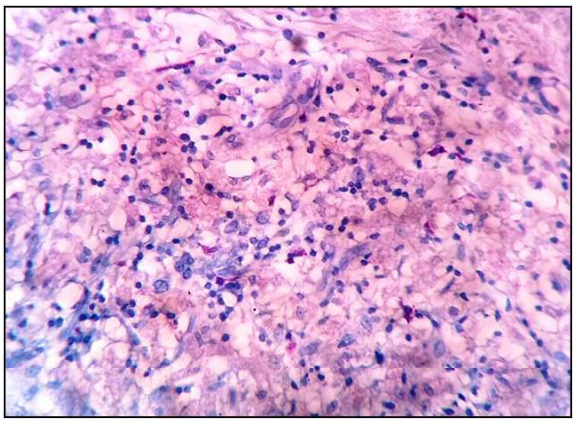

**Fig. 3:** TT showing no bacilli and numerous mast cells in granuloma (FF stain; 40X)

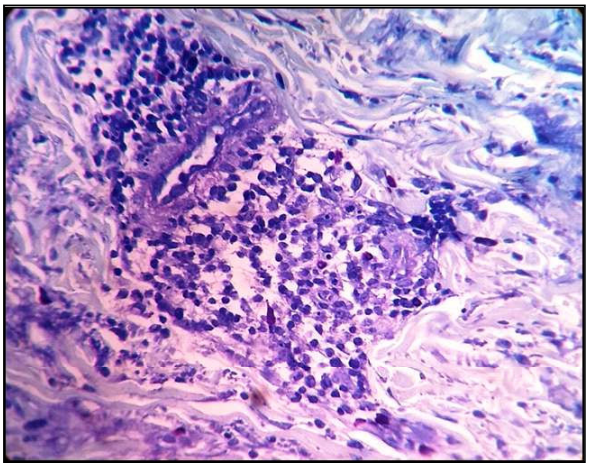

**Fig. 4:** BT showing mast cells around a blood vessel (FF stain; 40X)

**Table 1:** Mean Mast Cell Density in various leprosy

| Type of Leprosy | Cases | Mean mast Cell Density (per mm2) |
|---|---|---|
| LL | 07 | 14.00 |
| BL | 03 | 14.43 |
| BB | 26 | 25.67 |
| BT | 12 | 27.25 |
| TT | 17 | 27.94 |
| Total | 65 | - |

*Abbreviations:* LL- Lepromatous Leprosy, BL- Borderline Leprosy, BB- Borderline Leprosy, BT- Borderline Tuberculoid Leprosy, TT- Tuberculoid Leprosy

It was observed that mean mast cell density was lowest (14.00) in Lepromatous leprosy (LL) (Fig. 1; Fig. 2) , whereas highest (27.94) in Polar tuberculoid leprosy (TT) (Fig. 3; Fig. 4). The trend of Mean Mast Cell Density followed the immune spectrum of leprosy (Table 1).

**Table 2:** Mean Mast Cell Density at various structures

| Structure | Mean Mast Cell Density (per mm2) |
|---|---|
| Granulomas | 7.41 |
| Glands | 6.35 |
| Blood Vessels | 5.38 |
| Hair Follicles | 5.18 |
| Nerve | 2.89 |
| Muscle | 2.56 |
| Grenz Zone | 2.00 |

Mean Mast Cell Density was counted along the different structures of skin like Grenz zone, hair follicles, nerves, glands, muscles, blood vessels and also in the granulomas. It was observed that mast cells were seen irrespective of the presence or absence of bacilli. They had a high predilection for the adnexae. The granulomas were showing the

highest number of mast cell. This was followed by glands and blood vessels (Table 2).

The mast cells and the bacilli were seen in relation to the structures, mostly involved in leprosy. Isolated mast cells and bacilli were seen scattered in the dermis. Although, both mast cells and bacilli have a predilection for the adnexal structures, yet it was not a constant finding to get both at the same site.

## Discussion

An enriched literature can be found as far as disease leprosy is concerned. Similar scenario is seen with immunity and mast cells. Various other cells concerned with immunity such as lymphocytes, plasma cells, macrophages and fibroblasts have been described to play essential immunological role in leprosy infection. Yet, another connective tissue cell, the mast cell, which forms the centre of this study, has received less attention in leprosy. There are different evidences linking them to delayed hypersensitivity reactions and raises the possibility of their importance in clinical outcome. The change of number and density of mast cells in various types of leprosy indicates the important role of mast cells in cell mediated immune response in leprosy and its reactions.

**Table 3:** Comparison of mean mast cell density in various leprosy

| Type of leprosy | Giselle et al. (2007) [1] | Chatura et al. (2012) [4] | Meenakshyee et al. (2013) [2] | Hemlata et al. (2014) [7] | Navdeep et al. (2017) [8] | Shivani et al. (2017) [3] | Present study |
|---|---|---|---|---|---|---|---|
| LL | -- | 8.2 | 120 | 31.66 | 0.5 | 47 | 14.00 |
| BL | 50.43 | 9.2 | 100 | 35.00 | 0.357 | 40 | 14.43 |
| BB | 73.64 | 13.2 | 70 | 27.94 | 0.428 | 38 | 25.67 |
| BT | 65.06 | 14.2 | 105.56 | 30.35 | 0.0317 | 37 | 27.25 |
| TT | -- | 7.9 | 93.33 | 21.25 | 0.25 | 38 | 27.94 |

In the current study the numbers of mast cells were highest in TT. This was followed by BT and BB. However the difference between TT and BT was not much (Table 3). Also it was observed that mast cell density was significantly higher in borderline and tuberculoid leprosy when compared to borderline lepromatous and polar lepromatous forms. Similar findings were observed by Giselle et al. [1] and Chatura et al. [4]

Since, there is a paucity of literature regarding mast cells density in leprosy, various authors have concluded different results. Joshi et al. [2], Hemlata et al. [7], Navdeep et al. [8] and Shivani et al. [3], have concluded mast cell density is highest in borderline lepromatous and polar lepromatous forms. The variation in different studies may be due to difference in number of cases and different criteria used to select the cases. Other factors that must have influenced the observation can be differences in sample size, biopsy site, age of lesion, immunological and treatment status of patient at the time of biopsy [4]. The correlation was better at lepromatous than the tuberculoid pole in most of the studies.

The predominantly peripheral position of mast cells in the TT, BT and BB infiltrates could possibly be related to their interactions with the fibroblasts located in intervening dermis, either by direct contact or through the release of soluble factors. This apparently preferred position on the part of mast cells was not seen in the BL and LL group owing to the lack of intervening dermis in lepromatous lesions as they tend to have a macro-nodular configuration [1].

The higher density of mast cells in the BB, BT and TT groups favours the hypothesis that mast cells somehow influence the higher cell mediated immunity which is found in the T type of leprosy. This is consistent with the reported participation of mast cells in the immune response by direct or soluble factors mediating cross talking with T cells [1]. Increasing evidence indicates that mast cells are critical for the pathogenesis of inflammatory disease, apart from allergic conditions. The only way to explain mast cell involvement in non allergic processes would be through differential or selective secretion of mediators.

Conversely, however, the lower number of mast cells in BL and LL group is not conducive to attributing the higher collagen deposits and prominent angiogenic pattern of lepromatous lesions to mast cells [1].

The role of mast cells in the T cell mediated delayed hypersensitivity response is indirectly evidenced in many aspects. Firstly, morphological evidence: mast cells proliferate at the site of many cellular immune responses. Secondly, mast cells are able to secrete an array of proinflammatory and anti-inflammatory mediators, which play an important role in T cell mediated immune response

[9]. In lepromatous leprosy there is an unlimited growth of bacilli in skin tissue macrophages, nerves and mucous membranes due to selective unresponsiveness of the T lymphocytes to the bacilli. Possibly, the most important consequence of mast cell activation, in context of host defence, is the local release of mast cell mediators that aid in the rapid recruitment of effector cells [10].

In allergic diseases, mast cells are seen as harmful triggers of chronic inflammation, and mast cell stabilizing agents and inhibitors are frequently used. However, emerging data suggests that mast cells are crucial in protecting the host from many infections [11].

Various methods were used by different authors to stain mast cells in leprosy [1,2,3,4,5,6,7,8]. The most common method of staining the mast cells is by Toluidine blue. Here we have used the Fite Faraco's stain for mast cells to be stained metachromatically purple. Similar methodology was used by Chatura et al. [4]. Fite Faraco's stain provides excellent histological results. Therefore it can be used to demonstrate both, acid fast bacilli as well as mast cells [12].

**Table 4:** Comparison of mean mast cell density at various structures

| Structure | Cree et al.(1989) [5] | Present study |
|---|---|---|
| Granulomas | 114.7 | 7.41 |
| Glands | 77.3 | 6.35 |
| Blood Vessels | -- | 5.38 |
| Hair Follicles | 77.3 | 5.18 |
| Nerve | -- | 2.89 |
| Muscle | -- | 2.56 |
| Grenz Zone | 10.3 | 2.00 |

The overall mast cell density in biopsy specimens showed varied distribution in various structures. In current study the highest mast cell density was found in granulomas followed by glands. Least number of mast cells were found in and around Grenz zone. The observations were comparable to that done by various other authors [3,5]. Hemlata et al. [7] has also observed that mast cells are more concentrated in and around granulomas, followed by papillary dermis (Table 4).

The T cell response in cell mediated immunity, shows, that ratio of T cells in granulomas varies from 1.2 to 5 in tuberculoid leprosy, while it is between 0.2 to 1 in lepromatous leprosy. It is well documented that in TT and BT, the CD8+ cells are present in a ring like fashion around the granuloma while CD4+ cells are scattered inside it. Generation of suppressor T cells (CD8+) by the host has been suggested as one of the mechanisms of immunosuppression in LL. Hence, the mast cells are most commonly found in and around granulomas [10].

In the present study, the mast cell density has showed progressive increase over immunological spectrum of leprosy, i.e., ranging from polar lepromatous to polar tuberculoid form. Overall, the study demonstrates that TT, BT and BB group had higher mast cell level than that of the BL and LL group. Thus, indicating a possible role of mast cells in the more active immune response of the patients having BB, BT and TT leprosy. The mast cells could play a decisive role in the configuration of the Ridley-Jopling immunological leprosy spectrum. In addition, an unequivocal influence of mast cells on the configuration of the bipolar spectrum has not yet been proved definitively. Therefore, in attempting to answer these questions, further studies of mast cells distribution should be done on leprosy patients.

## Conclusion

- Fite Faraco's stain is suitable to study Lepra bacilli and Mast cells together.
- This study reflects the role of mast cells in immunological phenomena occurring in leprosy.
- Their elevated concentration in TT and around the granulomas provides an important footmark for its role in T cell mediated immune response.
- The density distribution of mast cells is independent of number of bacilli. Nonetheless their presence at various appendageal structures, complement to their participation in process of inflammation caused by leprosy bacilli that mainly inhabits these structures.
- The functional activity of mast cells in the pathogenesis of leprosy is at best speculative and needs to be evaluated further.

*Source(s) of support:* NIL

*Presentation at a meeting:* NIL

*Conflicting Interest*

*(If present, give more details):* NIL.

## References


1. Magalhães GDO, Valentim VDC, Pereira MJDS, Nery JADC, Illarramendi X, Antunes SLG. A quantitative and morphometric study of tryptase-positive mast cells in cutaneous leprosy lesions. Acta Tropica. 2008;105(1):62-6.
2. Joshi MM, Panicker NK, Buch AC, Chandenwale SS. Mast Cells in Non-neoplastic Skin Lesions. Indian Med Gaz. 2013;145(January):8–13.
3. Jindal S, Manjari M, Girdhar M. Role of Mast Cells in Leprosy- a Study of 62 Cases. J Evol Med Dent Sci. 2017;6(75):5328–31.
4. Chatura KR, Sangeetha S. Utility of Fite-Faraco stain for both mast cell count and bacillary index in skin biopsies of leprosy patients. Indian J Lepr. 2012;84(3):209–15.
5. Cree IA, Coghill G, Swanson BJ. Mast cells in leprosy skin lesions. J Clin Pathol. 1990;43(3):196–200.
6. 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):634-5.
7. Kamra HT, Munde SL, Gangane N, Sharma SM, Gulia A, Agarwal R. Significance of mast cell density and distribution in various histopathological lesions of leprosy. J Krishna Inst Med Sci Univ. 2014;3(1):57–63.
8. Kaur N, Hiremath SS, Suresh KK. Mast cells in leprosy. 2017;(March):139–45.
9. Mahaisavariya P, Jiamton S, Khemngern S, Manonukul J. Mast cells in leprosy and leprosy reaction. 2000;274–7.
10. Sacchidanand S. Leprosy: Immunology. In: IADVL Texbook of Dermatology. Fourth. Sengupta U, editor. Mumbai: Bhalani Book Depot; 2016.pp. 2027-29.
11. Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B,et al. Mast cells and inflammation. Biochim Biophys Acta - Mol Basis Dis. 2012;1822(1):21–33.
12. Sengupta V. Leprosy: Immunology. In: valia RG, Valia AR, editors, Textbook and Atlas of Dermatology. 2nd ed, Mumbai: Bhalani Publishing House; 2001.pp.1573-76.