Indian J Lepr 2012, 84 : 209-215

http://www.ijl.org.in

Original Article

Utility of Fite-Faraco stain for both mast cell count and bacillary index in skin biopsies of leprosy patients

KR Chatura, S Sangeetha

Received : 25.02.2012 Revised : 06.08.2012 Accepted : 08.08.2012

To assess the utility of a single stain for both mast cell count and bacillary index (BI), 50 skin-biopsies of leprosy patients were stained with Fite-Faraco (FF) stain, viewed under oil immersion and BI calculated using the Ridley's logarithmic scale, and mast cells counted as the number of cells per mm^2. Mean mast cell count per mm^2 at the tuberculoid pole was lowest in TT 7.9 and highest in BT 14.23. At the lepromatous end, it was highest in BL 9.21, while in LL it was 8.23. Highest counts were seen in the borderline types overall. The correlation coefficient between histopathological diagnosis and BI is 0.822 which is a positive correlation to a significant degree. The correlation coefficient between histopathological diagnosis and mast cell count was found to be -0.17, which is a negative correlation but not to a significant degree. FF stain was utilised to visualise both bacilli for estimation of BI and mast cells for mast cell count, a seldom attempted feature in literature.

Keywords : Fite-Faraco stain, mast cells, bacillary index

Introduction

The bacillus is only part of the leprosy story. An individual's immune system responds to the infection and accordingly mounts a cell mediated or humoral response. Towards tuberculoid pole (TT) the host macrophages are able to kill *M. leprae* whereas towards the lepromatous pole (LL) *M. leprae* grows abundantly in these macrophages. There is an inverse correlation between the BI, antibody levels and cell mediated immunity (CMI) in the spectral manifestation of the disease. Mast cells in leprosy have been investigated in the recent past, and have been examined as a basis for future studies. (Cree et al 1990). On light microscopy, mast cells have characteristic granules which contain heparin and histamine. In human tissue, formalin fixation is adequate but the sections are stained specially using Toludine blue, Methylene blue, Giemsa, Cresyl violet, Napthol AS-D chloroacetate, Esterase reaction or Azure A.

The granules of mast cells contain high molecular ester sulphuric acid, which has strong affinity for basic aniline dyes. When stained with basic thiazine dyes, a reddish colour, which differs from the normal blue colour of the stain, is produced. This is known as metachromasia. The above stains have been used to study the mast cells, as well as

[1] KR Chatura, M.D, Professor
[2] S Sangeetha, M.B.B.S, Undergraduate Student
Department of Pathology, JJM Medical College, Davangere-577004
Correspondence to : KR Chatura **Email :** chaturakr@yahoo.co.in

other acid fast stains such as Fite-Faraco (FF) stain (Sen Gupta and Ghosh 1963).

Leprosy can resemble many entities clinically like a chameleon. Histologically it ranges from paucibacillary to multibacillary. *M. leprae* requires a modified acid-fast bacilli stain such as FF for visualization. To diagnose leprosy, one must first consider it as a possibility. In endemic areas, a diagnosis may be made without biopsy, but careful scrutiny of FF stained sections can better the correlation between the clinical and histopathological diagnosis. The same sections stained with FF could be used for mast cell count.

There have been conflicting reports on mast cell density from the tuberculoid to the lepromatous pole in leprosy, and more cases need to be studied to ascertain the pattern. Use of FF stain for both the mast cell count and as well as the bacilli would simplify collection of data and provide some key trends in patterns. We would be able to assess the immune status as that reflected by the mast cell count of a patient and gather more information about being able to stimulate the mast cells into fighting the lepra bacillus.

Material and Methods

Skin punch biopsies from 50 consecutive patients of leprosy received in 2 months, July and August 2009, the scheduled study period to conduct accepted STS 2009 project, in the Department of Pathology, JJM Medical College, were included.

38 new cases, 5 cases on treatment, 4 cases in relapse [3 Borderline tuberculoid (BT) and 1 Borderline lepromatous (BL)] and 3 cases in reaction (2 Type 1, 1 Type 2) have been included. Clinically suspected cases but histologically not correlating have been excluded. Controls were not used.

Biopsies were routinely processed and embedded in paraffin wax and sections stained with Haematoxylin and Eosin (H&E) and FF stain separately. The property of acid fastness is related to the carbon chain length of the mycolic acid found in any particular species. 10% sulphuric acid is used as a decolouriser in place of acid/alcohol solution. The sections were also deparaffinised using a mixture of xylene and coconut oil in equal parts, this helps to protect the more delicate waxy coat of the organisms.

Biopsies stained with FF stain, were viewed under oil immersion with a high power objective and BI calculated using the Ridley's logarithmic scale, and mast cells were counted as the number of cells per mm^2. This parameter was used to compare with other studies.

Mast Cell Count

Microscope used was Olympus CH20i with field of view number 18.

High power field area was calculated as follows:

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

High power field (hpf) area = A = r^2 = 0.152 mm^2

Therefore, if one hpf area is 0.152mm^2, ten hpf areas are 1.52 mm^2.

So, if 'x' is the value of cells observed in ten hpfs, 'x'*10/1.52 is the number of cells observed per mm^2.

In other words, number of mast cells in 10 hpf were counted multiplied by 6.578 to get the number of mast cells in 1 mm^2.

Bacillary Index (BI)

Sections stained by FF stain, were graded, similar to slit skin smears, based on the number of bacilli under oil immersion, as it is difficult to visualise bacilli in high power. Approximations of BI for Ridley- Jopling Classification were used as follows:

Tuberculoid (TT) = nil
Borderline Tuberculoid (BT) = 1 to 0+
Borderline Borderline (BB) = 2 to 1+
Borderline Lepromatous (BL) = 3 to 2+
Lepromatous (LL) = 5 to 3+

We have also attempted clinicopathological correlation by Ridley-Jopling classification on H&E stained sections.

Statistical Test used was Pearson product-moment correlation coefficient.

Results

We correlated the BI on FF stain with the Ridley-Jopling classification based on histological diagnosis. The BI ranged from 0-6, with a majority of cases having a BI of 0. BI was highest in LL and low in BT types. Bacilli were always present in BB and numerous in BL and LL. Majority of our cases belong to borderline group.

Two BL cases had BI of 0 and had been receiving treatment for the past one year.

The Pearson correlation coefficient is a measure of the correlation (linear dependence) between two variables X and Y. The correlation coefficient ranges from –1 to 1. A value of 1 implies that a linear equation describes the relationship between X and Y perfectly, with all data points lying on a line for which Y increases as X increases. A value of –1 implies that all data points lie on a line for which Y decreases as X increases.

The correlation coefficient between histo-pathological diagnosis and BI is 0.822 which implies that there is a strong correlation between them and a positive one, that is, BI increases towards the lepromatous but decreases towards the tuberculoid pole.

Table 1 : BI and mean mast cell count across the Ridley-Jopling spectrum of leprosy

Type BI	TT	BT	BB	BL	LL	No. of cases
0	05	21	—	02	—	28
1+	—	01	—	—	—	01
2+	—	01	03	01	—	05
3+	—	—	—	03	01	04
4+	—	—	—	02	—	02
5+	—	01	—	02	03	06
6_+	—	—	—	—	04	04
Total	5	24	3	10	8	50
Mean Mast cell count / mm^2	7.9	14.2	13.2	9.2	8.2	—

Table 2 : Histological diagnoses of different types in our study vs. other studies

Histological diagnosis	Our study (2009)	Kaur et al, (2003)	Moorthy NB et al, (2001)
TT	5 (10%)	2 (0.4%)	26 (6.99%)
BT	24 (48%)	109 (21.8%)	269 (72.31%)
BB	3 (6%)	2 (0.4%)	2 (0.54%)
BL	10 (20%)	8 (17%)	40 (10.72%)
LL	8 (16%)	302 (60.4%)	10 (2.68%)
TOTAL	50	500	372

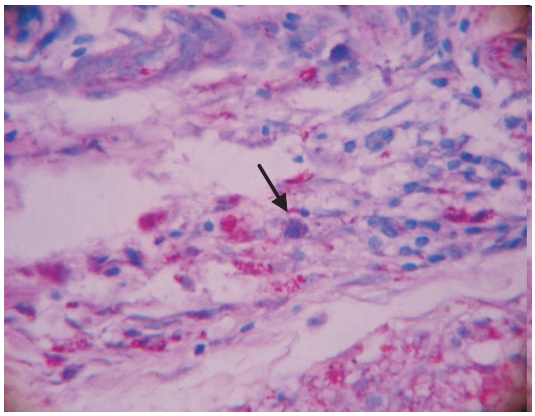

Fig 1 : FF stained sections of LL with BI 4+

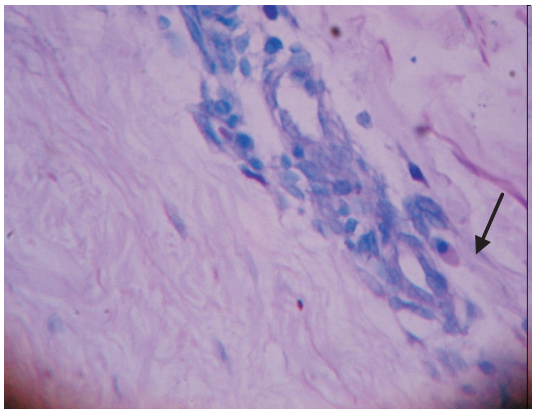

Fig 2 : FF stained sections of BT with BI 0 showing mast cells (arrows)

Table 3 : Histological diagnoses of different types in our study vs. other studies

Histological diagnosis	Our study, 2009	Moorthy NB et al, (2001)	Nadkarni et al,(1999)
TT	0.00%	46.15%	97.20%
BT	70.00%	66.66%	95.00%
BB	66.00%	50.00%	89.00%
BL	70.00%	70.00%	87.00%
LL	62.50%	80.00%	98.20%

Table 4 : Density of mast cells in different types of leprosy

Histological diagnosis	Mast cell count inour study (cells/mm^2)	Mast cell count in Naik et al, 2003 (cells/mm^2)
TT	7.9	51
BT	14.23	37
BB	13.16	36
BL	9.21	46
LL	8.23	49

The clinico-histopathological correlation was found to be the maximum in BT type of leprosy, and lowest with BB. Maximum number of cases belonged to the BT type.

We correlated the mast cell count with the Ridley Jopling Classification of Leprosy, to be able to assess the mast cell density. The mean mast cell count was highest at the borderline tuberculoid and borderline lepromatous groups, as compared to their polar counterparts.

The correlation coefficient between histo-pathological diagnosis and mast cell count is -0.17 which is not a very high correlation but there is a negative relationship between the two, that is,

towards both the poles there is a decrease from the median.

Both (one BT and one BL) the Type 1 reactions cases had no mast cells but the BL type 2 reaction had mast cell count of 6.58/mm^2.

In cases with relapse mast cell count range was 6.58-39.4/mm^2. Cases on treatment had a range of 6.58-19.7/mm^2.

Discussion

There is no independent gold standard for diagnosis of leprosy. The variation in different studies may be due to different criteria used to select the cases and difference in number of cases of each type. Various factors also influence the histopathological diagnosis such as differences in sample size, choosing the biopsy site, age of the lesion, immunological and treatment status of patient at the time of biopsy (Nadkarni and Rege 1999).

Since a sizeable portion of leprosy patients would be in a continuously changing immunological spectrum i.e., BT, BB and BL, majority of cases would be in the borderline group in most of the studies. Though the borderline leprosy group is common and BT in particular is the commonest type of leprosy encountered in the world, many disagree with it. Failure to appreciate this fact, is due to failure to recognize the exact clinical and histological features and the tendency to classify BT as TT.

The correlation was better at lepromatous than the tuberculoid pole in most of the studies.

Ridley and Jopling classification is based on clinical, histopathological and immunological features, which is widely accepted by histo-pathologists and leprologists. Simultaneously clinicians also have adopted Ridley-Jopling nomenclature and at present, the clinical diagnosis is made along the lines of this classification, even when a histopathological examination has not been done and thus this may lead to discordance between clinical and histo-pathological diagnosis.

As there can be some degree of overlapping among different types of leprosy both clinically and histopathologically, correlation with BI appears more useful for accurate typing of leprosy than considering any of the single parameters alone. Hence Ridley and Jopling classification is widely accepted by histo-pathologists and leprologists. This helps the clinician for better care and management of the patients.

In leprosy mast cells were first investigated with FF stain by Sen Gupta and Ghosh (1963). Since then various other stains have been employed to assess mast cell density in leprosy cases. Evidence linking mast cells with delayed hypersensitivity reactions raises the possibility that they might be of some importance in leprosy lesions.

We stained mast cells a metachromatically purple with FF stain. The stain provided excellent histologic results. Therefore, we agree that acid-fast stains are another useful special staining method for distinguishing mast cell granules from other cells.

A close association of lepra bacilli with the mast cells which either accumulated around the bacilli or were lying in relation to the structures severely affected in leprosy i.e. nerves, blood vessels, muscles, hair follicles and glandular elements indicates similar distribution of both (Kumar and Vaidya 1982).

The morphology of the mast cell and its degranulation usually depend upon the type and severity of the disease and hence we see a wide range of observations quoted in literature.

A trend for mast cells to decrease from lepromatous to tuberculoid pole was observed in our study. The predominance of mast cells in lepromatous group may be linked to the increased vascularity and changes observed in

the endothelial cells, which are more obvious in lepromatous leprosy. But at tuberculoid end of spectrum mast cells do not appear to stay long as in lepromatous leprosy (Aroni et al 1993).

In another study, significantly higher mast cell counts were obtained in the skin lesions of indeterminate leprosy ($P<0.01$). The mast cell count in the tuberculoid group was significantly lower than that in the lepromatous group ($P<0.05$). The lepromatous group also showed increased mast cell degranulation and altered morphology (Mysorekar et al 2001).

Mast cell counts using toluidine blue stain were normal in indeterminate and TT leprosy and showed a rise over the immunological spectrum BT to LL, with values in LL being 32.86/mm^2 (28-40/mm^2) (Bagwan et al 2004).

Seventy cases of only tuberculoid leprosy were stained for mast cells with toluidine blue. The mean mast cell count was found to be 26.6/mm^2 outside the granuloma and 15.1/mm^2 inside the granuloma. The difference was statistically significant with $p < 0.001$ (Pilli et al 2005).

51 cutaneous biopsies of leprosy patients were stained with anti-tryptase antibody to quantify mast cells in all the forms of leprosy. It was found that the lepromatous group had the lowest dermal mast cell density values. The higher mast cell density in the tuberculoid and borderline-borderline groups was considered indirect evidence of the role of mast cells in the activated immune response to *M. leprae* infection (Magalhaes et al 2008).

Across the spectrum of leprosy we observed a lower mast cell density in the polar tuberculoid compared to polar lepromatous leprosy. In lepromatous and tuberculoid areas higher counts in borderline cases than polar cases were seen.

Probably reduction of mast cell density occurs when the lesions upgrade to become more tuberculoid type with the changes in cytokine pattern. Extensive degranulation because of increased functional activity may be the cause for lower values in lepromatous leprosy. Probably because of these two reasons the mast cell count decreased, as we moved from BB cases towards either poles.

However, in a study of sixty non-neoplastic skin lesions for mast cells by toluidine blue stain it was observed that in leprosy cases as the lesions moved from indeterminate to both polar tuberculoid and lepromatous, the mast cell count increased (Naik et al 2003).

The mast cell density reflected by our study is comparable to various studies in literature. Hence, an attempt to count the mast cells in the same FF stained sections yields comparable results to other methods of staining for mast cells.

We observed lesser number of mast cells over all sub-types of leprosy than quoted in literature, probably attributable to the staining method. But this needs to be evaluated by staining mast cells using different methods and observing the pattern of density in the sub-types in skin biopsies of leprosy cases as well as healthy individuals who are taken as controls and standardise method of counting.

The change in the average mast cell number in nonreactional leprosy and leprosy reactions may indicate the important role of mast cells in dynamic changes in the cell-mediated immune response in leprosy and leprosy reactions (Mahaisavariya et al 2000).

In our study both (one BT and one BL) the Type 1 reactions cases had no mast cells but the BL type 2 reaction had mast cell count of 6.58/mm^2.

Absence of mast cells was conspicuous in 16.7% LL, 41.7% BB, 40.9% BT, and 68.0% TT lesions. It is suggested that mast cells might play a role in the early stages of the disease and in post-reactional connective tissue proliferation (Rav et al 1990).

Studies of cases with relapse and treatment history ranging from 6 months to 18 months in various types had a mast cell count range from 6.58 to 39.57. We had few cases in each type and hence did not find a difference between treated and relapsed cases.

No significant difference in mast cell density was noted between treated and untreated lepromatous leprosy lesions as the treatment period of 6 months was perhaps too short a period to expect reduction in the study (Jayalakshmi 1995).

Conclusion

We found the FF stain, satisfactory to estimate mast cell count as well as to calculate the BI of a given skin biopsy. Using minimal resources, this process may be carried out routinely by inexpensive techniques in many laboratories. We have assessed mast cell density and BI using FF stain. This study served to confirm the diagnosis of the lesion, detected the presence of bacilli and revealed the presence of the mast cells.

Understanding the mechanisms and scope of the contribution of mast cells to host defense will be crucial in regulating their activity therapeutically. The functional activity of mast cells in the pathogenesis of leprosy is at best speculative and needs to be evaluated further.

Acknowledgement

This research was carried out with sanction from ICMR STS 2009 (ref F.No.21/KA/JJM-33-36/09/BMS)

References

1. Aroni K, Kontochristopoulos G, Liossi A et al (1993). An investigation of mast cells in two basic leprosy groups. *Int J Lepr*. **6:** 634-635.
2. Bagwan IN, Khandekar MM, Kadam P et al (2004). A study of mast cells in granulomatous lesions of the skin, with special emphasis on leprosy. *Indian J Lepr*. **76:** 31-37.
3. Cree IA, Coghill G and Swanson B (1990). Mast cells in leprosy skin lesions. *J Clin Pathol*. **43:** 196-200.
4. Jayalakshmi P (1995). Mast cells in lepromatous leprosy. *Int J Lepr Other Mycobact Dis*. **63:** 291-293.
5. Kumar R and Vaidya MC (1982). Mast cells and *Mycobacterium leprae* in experimental leprosy. *Hansen Int*. **7:** 1-7.
6. Magalhaes Gde O, Valentim Vda C, Pereira MJ et al (2008). A quantitative and morphometric study of tryptase-positive mast cells in cutaneous leprosy lesions. *Acta Tropica*. **105:** 62-66.
7. Mahaisavariya P, Jiamton S, Manonukul J et al (2000). Mast cells in leprosy and leprosy reaction. *Int J Dermatol*. **39:** 274-277.
8. Moorthy NB, Kumar P, Chatura KR et al (2001). Histopathological correlation of skin biopsies in leprosy. *Indian J Dermatol and Venereol Leprol*. **67:** 299-301.
9. Mysorekar VV, Dandekar CP and Rao SG (2001). Mast cells in leprosy skin lesions. *Lepr Rev*. **72:** 29-34.
10. Nadkarni NS and Rege VL (1999). Significance of histopathological classification in leprosy. *Indian J Lepr*. **71:** 325-332.
11. Naik R, Pai MR, Bantwal PB et al (2003). Study of mast cells in non-neoplastic skin lesions. *Indian J Pathol Microbiol*. **46:** 173-175.
12. Pilli GS, Davangeri R and Dhaded AV (2005). Distribution and density of mast cells in tuberculoid leprosy. *Indian J Lepr*. **77:** 58-9.
13. Rav SD, Pratap VK, Sharma NK et al (1990). Mast cell in leprosy. *Indian J Lepr*. **62:** 467-472.
14. Sen Gupta PC and Ghosh S (1963). Tissue mast cells. *Nature*. **197:** 506-507.

How to cite this article : Chatura KR and Sangeetha S (2012). Utility of Fite-Faraco stain for both mast cell count and bacillary index in skin biopsies of leprosy patients. *Indian J Lepr.* **84** : 209-215.