

Evaluation of Mast Cell Profile in the Skin Lesions of Leprosy.

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Received: September 2018

Accepted: September 2018

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ABSTRACT

Background: Aim of the present study was to evaluate the role of Mast cells in the Histo-Pathogenesis of Leprosy by assessing incidence of various Histo-Pathological types of Leprosy, Correlating of Mast cell profile (Mast cell Density & Morphology) in different Histological types of Leprosy lesions, by correlating with Reactional & Non-Reactional groups of Leprosy cases and by correlation of Mast cell profile in doubtful cases as in Indeterminate type. Lepromatous Leprosy was observed in higher age group as compared to Tuberculoid leprosy. Mast Cell Density was found increasing from Polar Tuberculoid to Polar Lepromatous spectrum of disease. In every type of Leprosy, mast Cell Density was reduced significantly in the Reactional Group as compared to Non-Reactional Group. The aim of the present study was to evaluate the role of Mast cells in the Histo-Pathogenesis of Leprosy by assessing incidence of various Histo-Pathological types of Leprosy. **Methods:** A cross sectional, observational study was conducted among 200. Correlation of mast cell profile (Mast cell Density & Morphology) in different Histological types of Leprosy lesions was done, by correlating with Reactional & Non-Reactional groups of Leprosy cases and by correlation of Mast cell profile in doubtful cases as in Indeterminate type. Lepromatous Leprosy was observed in higher age group as compared to Tuberculoid leprosy. **Results:** Mast Cell Density was found increasing from Polar Tuberculoid to Polar Lepromatous spectrum of disease. In every type of Leprosy, mast Cell Density was reduced significantly in the Reactional Group as compared to Non-Reactional Group. **Conclusion:** Larger such studies of Leprosy employing special staining methods viz. pH dependent Toluidine Blue staining, studies employing Immuno-Fluorescence on Tissue biopsy specimens, as well as studies on experimental animals, to help us achieve a better understanding of the pathogenesis, outcome and prognosis of Leprosy.

Keywords: Tuberculoid leprosy, Mast Cell Profile, Lepromatous Disease.

INTRODUCTION

The pattern of disease in leprosy is decided by host cellular response to mycobacterium leprae.^[1] In the last few years a number of studies conducted, linking the important role of mast cells in development of delayed hypersensitivity reaction.^[2] This led to link their possible role in development of leprosy lesion in skin. With this background we therefore aim to assess the density and distribution of mast cells and to assess the lesions of leprosed skin.

MATERIALS AND METHODS

A cross sectional, observational study was conducted

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among 200 cases taken from patients attending Skin OPD and also in-patients admitted to Skin & V.D. ward of M.L.B. Medical College, Jhansi (U.P.). All leprosy cases were subjected to Incisional skin biopsy.^[3] Biopsy sample were collected and fixed in 10% buffered Formalin. Tissues were processed for Paraffin embedding techniques. Sections were cut on Microtome at 4 µm. thickness. The sections thus prepared were subjected to routine Hematoxylin & Eosin staining (Clayden 1971).^[4] The Special staining procedures were Acid fast staining for AFB (Clyden 1971), and Toluidine Blue for Mast cells (Clayden 1971).^[4]

A. Acid fast staining for AFB^[5]

After staining Mycobacterium Leprae were RED in appearance while nuclei were BLUE. The Bacterial Index (BI) was helpful in classifying the type of Leprosy and in appreciating not only the effectiveness of the Drug therapy but also the development of resistance to the drug or relapse of the disease. The standard enumeration of Leprosy

bacilli in lesions (BI) followed Ridley's Logarithmic Scale.^[6]

BI 0 -> No Bacilli observed

BI 1 -> 1-10 Bacilli in 10-100 Oil Immersion Fields

BI 2 -> 1-10 Bacilli in 1-10 Oil Immersion Fields

BI 3 -> 1-10 Bacilli per Oil Immersion Field

BI 4 -> 10-100 Bacilli per Oil Immersion Field

BI 5 -> 100-1000 Bacilli per Oil Immersion Field

BI 6 -> more than 1000 Bacilli per Oil Immersion Field

Patients with no Bacilli detectable in lesions were termed Paucibacillary, those with some or many bacilli were termed Multibacillary.

The Morphological Index (MI) was determined after assessing BI, the slide is reviewed to obtain the MI, which measures percentage of Bacilli that are of normal size and shape and stained uniformly (Solid Staining).^[1] Solid staining Bacilli indicate that organisms are capable of Multiplication. Fragmented Bacilli indicate that organisms were dead. The MI correlates with the viability of the organism because when the MI becomes zero, the bacilli can no longer be grown routinely in the Mouse Foot Pad system⁷. The MI is very sensitive measure of the effectiveness of the drug therapy and helps to signal Drug Resistance and Relapse of the Disease.

B. Toluidine Blue for Mast cells^[8]

After staining Mast cells appeared Dark Blue or Purple, while other tissues were Lightish Blue as background.

Evaluation of Mast Cell Profile:

Mast Cell Profile was evaluated on the basis of Mast Cell Density (Cell count per 20HPF) at the following 3 components of skin lesion (Cree, Coghill, Swanson Beck 19899, and Mysorekar et. al. 200010): -

1. The Granulomas
2. The Appendages
3. The Intervening Dermis

The Mast Cell Density was graded by the method described by Revel et.al.

GRADE	Average number of Mast Cells per HPF (20 non-overlapping fields counted)
+	1 – 5
++	6 – 10
+++	More than 10

Table 2: Mast Cell distribution was observed as follows

S. No.	Histo-Pathological Type	Nil (No Mast Cells)	Grade (+) (1-5 Mast cells/ hpf)	Grade (++) (6-10 Mast Cells/ hpf)	Grade (+++) (>10 Mast Cells/hpf)
1	Tuberculoid leprosy	66.7%	25%	8.3%	0%
2	Borderline tuberculoid leprosy	40%	60%	0%	0%
3	Borderline leprosy	40%	46.7%	13.3%	0%
4	Borderline lepromatous leprosy	6.3%	50%	31.2%	12.5%
5	Lepromatous leprosy	0%	7.1%	35.7%	57.2%
6	Histoid leprosy	0%	0%	0%	100%
7	Indeterminate leprosy	0%	9.1%	72.7%	18.2%

The Mast Cell morphology was assessed by following method (Cree, Coghill, Swanson Beck 1989)9:

1. **Active Mast Cell:** - with large ovoid nuclei, prominent nucleoli, and extensive cytoplasm with many granules.
2. **Intermediate Fusiform Mast Cell:** - with elongated dark staining fusiform nuclei and little cytoplasm.
3. **Resting Mast Cell:** - with small dark nuclei, scanty cytoplasm and few granules.

The Clinical and Pathological data was recorded on Pre-set Proforma for further analysis and evaluation.

RESULTS

Following observations were made during the course of study. 53.75% subjects were Male and 46.25% were Female. 17.5% were from Urban population and 82.5% were from Rural population. Age-wise distribution shows 1.25% in 0-10 years age group, 10% on 11-20 years age group, 26.25% in 21-30 years age group, 27.5% in 31-40 years age group, 15% in 41-50 years age group, 11.25% in 51-60 years age group, and 8.75% in more than 60 years age group. Chief presenting complaints were distributed as; HYPOPIGMENTED/ ERYTHEMATOUS ANAESTHETIC PATCH in 45% cases, NUMBNESS/ PARESTHESIA in 17.5% cases, NODULAR LESION OVER FACE/ EXTREMITIES/ TRUNK in 28.75% cases, LOSS OF EYEBROWS/ EYE LASHES (MADAROSIS) in 5% cases, FEVER/ ERYTHEMATOUS TENDER NODULE WITH BODY ACHE in 6.25% cases, THICKENED NERVES in 21.25% cases, and 78.75% cases presented with Combination of symptoms.

Table 1: Histo-Pathological distribution of cases was as follows.

S. No.	Histo-Pathological type	% of cases
1	Tuberculoid (TT)	15.0
2	Borderline Tuberculoid (BT)	12.5
3	Borderline (BB)	18.7
4	Borderline Lepromatous (BL)	20.0
5	Lepromatous (LL)	17.5
6	Indeterminate Leprosy	13.8
7	Histoid Leprosy	2.5

Most common age-wise distribution of different Histo-Pathological types was observed as, TUBERCULOID type shows 33.3% cases in 31-40 years age group, and 25% cases in 21-30 years age group, BORDERLINE TUBERCULOID shows 50% cases in 21-30 years age group, and 20% each in 11-20 and 31-40 years age group, BORDERLINE Leprosy shows 40% in 31-40 years age group, BORDERLINE LEPROMATOUS type shows 43.8% in 31-40 years age group, and 31.3% in 21-30 years age group, in LEPROMATOUS type 26% were in 41-50 years age group, and 21.1% in 51-60 years age group, in INDETERMINATE Leprosy 45.5% were in 21-30 years age group, while in HISTOID type 50% each were in 31-40 and 61-70 years age group.

Grading of Mast Cell Density in Reactive & Non-Reactive types of Leprosy was observed as, TUBERCULOID type shows 33.3% REACTIVE and 66.7% NON-REACTIVE, BORDERLINE TUBERCULOID shows 20% REACTIVE and 80% NON-REACTIVE, BOREDERLINE type shows 26.7% REACTIVE and 73.3% NON-REACTIVE, BORDERLINE LEPROMATOUS type shows 18.8% REACTIVE and 81.2% NON-REACTIVE, while LEPROMATOUS Leprosy shows 7.1% REACTIVE and 92.9% NON-REACTIVE.

DISCUSSION

Leprosy is a chronic disease caused by M. Leprae, having wide range of clinical manifestations. Among communicable diseases,^[1] Leprosy is a leading cause of permanent physical disabilities. Timely diagnosis and treatment is thus of utmost importance to reduce morbidity.

Leprosy is a spectral disease; the clinical and histopathological features depend upon the level of cellular immune response to M. Leprae. The immuno-pathogenesis has not been clarified completely. It is believed that several cell populations including Mast Cells may participate. Mast cells play important role in the Immediate Hypersensitivity as well as Delayed Hypersensitivity reactions.^[2]

Upon detailed analysis of observations made during the course of study, apart from demographic distribution, gender prevalence, histo-pathological variants, the key observations on Mast Cell Analysis point that the change in average Mast Cell number in Non-Reactional Leprosy and Leprosy with Reaction may indicate the important role of Mast Cells in dynamic change in the Cell Mediated Immune Response in Leprosy and Leprosy reactions.

Another plausible interpretation, assuming that most of the connective tissue cells have a common stem cell, is that induction of proliferation in any one cell series is simultaneously accompanied by proliferation in other cell system. Since Leprosy is caused by M. Leprae, the changes in Mast Cells may

be due to either disease process or as an outcome of the immune response of the host.^[12]

CONCLUSION

The present study is summarized and concluded in following points

- Male to Female ratio is 1.16:1.
- Rural to Urban patient ratio is 4.7:1.
- Most common age group affected (or reported in hospitals) is 31-40 years.
- Most common presenting complaints/ signs/ symptoms are Hypopigmented or erythematous hypoaesthetic patches, thickened nerves, nodular lesions, loss of eyebrows, and fever.
- The ratio of LL type and TT type is 1.16:1.
- Lepromatous Leprosy was observed in higher age group as compared to Tuberculoid leprosy.
- Mast Cell Density was found INCREASING from Polar Tuberculoid to Polar Lepromatous spectrum of disease.
- In Indeterminate Leprosy 90.9% cases showed Grade (++) & (+++) Mast Cell Density.
- In every type of Leprosy, mast Cell Density was reduced significantly in the Reactional Group as compared to Non-Reactional Group.
- Finally, it was stressed to undertake larger such studies of Leprosy employing special staining methods viz. pH dependent Toluidine Blue staining, studies employing Immuno-Fluorescence on Tissue biopsy specimens, as well as studies on experimental animals, to help us achieve a better understanding of the pathogenesis, outcome and prognosis of Leprosy.

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How to cite this article: 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.

Source of Support: Nil, **Conflict of Interest:** None declared