CHRONIC INFLAMMATION IN LEUKOPLAKIA AND ORAL SUBMUCOUS FIBROSIS UNMASKED WITH THE HELP OF INCREASED NUMBER OF MAST CELL

Dr. Rohit Singh¹, Dr. Supriya Singh², Dr. Abhishek Kumar³, Dr. Gaurav Kumar⁴, Dr. Jazib Nazeer⁵* and Dr. Vaibhav Kamal⁶

¹Lecturer, Department of Prosthodontic Crown Bridge and Implantology, Patna Dental College & Hospital, Patna, Bihar, India.
²Lecturer, Department of Oral Medicine and Radiology.
³Private Consultant, Department of Oral Pathology, Maharastra, India.
⁴Senior Lecture, Department of Oral Pathology, Shree Bankey Bihari Dental College & Research Centre, Uttar Pradesh. India.
⁵Lecturer, Department of Oral Pathology, Patna Dental College & Hospital, Patna, Bihar, India.
⁶Senior Lecturer, Department of Pedodontics and Preventive Dentistry, Dr B R Ambedkar Institute of Dental Sciences, Patna, India.

ABSTRACT

Introduction: Oral leukoplakia and submucous fibrosis (OSMF) are the commonly occurring oral diseases, with characteristic clinical and histological features. These diseases at some stage are associated with chronic inflammation in adjacent connective tissue. Mast cells are the local residents of the connective tissue and are said to be pro-inflammatory, immunoamplifying in action and producing mitogenic cytokines. These functions of mast cells may play a significant role in the pathogenesis of other oral diseases. Aims: This study was done to histologically evaluate the number of mast cells in tissue sections of oral leukoplakia and submucous fibrosis. Materials and Methods: ten cases each of normal oral mucosa, oral leukoplakia and oral submucous fibrosis were studied for mast cell number using 1% Toluidine blue. Results: Increase in mast cell number were seen in both the above mentioned oral diseases, The mast cell number/sq.mm in oral leukoplakia, submucous fibrosis were; 59.50, 48.25 respectively.
Conclusion: As compared to normal oral mucosa, increase in the mast cell number was noted in both conditions. Mast cell hyperplasia in oral leukoplakia and OSMF suggests their probable role in the pathogenesis of these diseases.

KEYWORDS: Mast cells, oral leukoplakia, oral submucous fibrosis.

INTRODUCTION
Mast cells and their granules have captured the interest of investigators from variety of scientific disciplines over the last century.\(^1\) Mast cells which reside in connective tissue matrices and epithelial surfaces are “effector cells” that initiate inflammatory responses.\(^2\) A mast cell (mastocyte) is a resident cell of areolar connective tissue (loose connective tissue) that contains many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing and defense against pathogens.\(^3\) The development of staining techniques for histologic sections led to the initial definitive description of mast cells by a medical student named Paul Ehrlich over a 100 years ago.\(^4\)

Mast cells have a diameter of about 12 microns; they are heterogeneous in shape; round, oval or spindle-shaped and are packed with 50-100 granules. They have a life span of weeks to months.\(^5,6\) Mast cells release preformed secretory mediators like histamine, heparin, tryptase; lipid derived mediators like leukotrienes B4 (LTB4), LTC4, LTD4 and LTE4; pro inflammatory cytokines like TNF-alpha, IL-1; mitogenic cytokines: IL-3, IL-5 and immunomodulatory cytokines like IL-4, IL-10.\(^7\) Therefore mast cells have been studied in various conditions like wound healing, chronic inflammation, keloid, pulmonary fibrosis and angiogenesis.\(^8\)

The commonly occurring oral diseases like oral leukoplakia, submucous fibrosis, lichen planus, squamous cell carcinoma have chronic inflammation in common. In addition, autoimmunity is strongly associated with OLP and angiogenesis is associated with the proliferation of carcinoma. Therefore the role of mast cells was evaluated in these diseases. The present study was carried out to estimate and compare mast cell number in oral leukoplakia and OSMF.
MATERIALS AND METHODS
Ten cases each of oral leukoplakia and OSMF were retrieved from the archives of the Department of Oral Pathology and Microbiology, Patna dental college and hospital, Patna. Biopsies of normal oral mucosa were obtained from adult patients undergoing extraction for orthodontic treatment. Two sections each of 5 microns thickness were cut; one section was stained with Hematoxylin and Eosin; the other was stained with 1% toluidine Blue at about pH 4 for mast cells. Toluidine blue stains the mast cell granules metachromatically due to its reaction with sulphated mucopolysaccharides.\(^9\)

Mast cells were counted using an oculometer grid in 30 grid fields under a magnification of x400 under Motic microscope with a magnification. Mast cell count was expressed as the number of mast cells per grid field and the number of mast cells per square millimeter.

Criteria to identify the mast cells
Mast cells are spindle to oval-shaped and have the same staining characteristics as the fibroblasts with hematoxylin and eosin stain. Therefore, they are difficult to differentiate from fibroblasts. Selective stain of 1% toluidine blue is used for mast cells. Mast cell granules are purplish red and the nuclei of mast cells appear sky blue in color.

RESULTS
The results of the study showed a maximum mast cell count in leukoplakia of 59.50/ sq.mm. In OSMF 48.25/ sq.mm respectively as compared to 25.50/ sq.mm of mast cell count in normal oral mucosa [Table 1].

Table 1: Mast cell count in normal oral mucosa, leukoplakia and OSMF

<table>
<thead>
<tr>
<th>Oral disease</th>
<th>No. of cases</th>
<th>number of Average number of mast cells/grid</th>
<th>Average field mast cells/sq.mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal oral mucosa</td>
<td>10</td>
<td>1.02</td>
<td>25.50</td>
</tr>
<tr>
<td>Oral leukoplakia</td>
<td>10</td>
<td>2.38</td>
<td>59.50</td>
</tr>
<tr>
<td>Oral submucous fibrosis</td>
<td>10</td>
<td>1.93</td>
<td>48.25</td>
</tr>
</tbody>
</table>

DISCUSSION
Mast cells are the local residents of the connective tissue. The role played by the mast cell mediators and their interaction with other inflammatory cells has been intriguing. Mast cells have been studied in normal gingiva, chronic inflammatory gingivitis, desquamative
gingivitis, lichen planus, OSMF and oral cancer.\textsuperscript{10,11} Mast cells exhibit phenotypic plasticity.\textsuperscript{12} There is variation in the mast cell mediators with the change in the microenvironment, which makes the study of this cell in various diseases interesting. Therefore, the present study was done to evaluate the mast cell number in 10 cases each of normal oral mucosa, oral leukoplakia and submucous fibrosis. 1% toluidine blue was used as a selective stain for mast cells. Mast cell count was done using an oculometer grid in 30 grid fields.

The results obtained showed an increased mast cell number in oral leukoplakia. The observations by Biviji et al.\textsuperscript{10} showed a mean increase in the number of mast cells /unit microscopic field in oral leukoplakia compared to normal oral mucosa. It can be concluded that the biologically and pharmacologically active agents in the mast cells might contribute to inflammatory reaction seen in leukoplakia. These stimulated mast cells may release interleukin-1, which causes increased epithelial proliferation\textsuperscript{13} that is seen in leukoplakia. Histamine may cause increased mucosal permeability, which could facilitate increased access for the antigen to the connective tissue\textsuperscript{7} (Table 2).

**Table 2: Illustrating the probable effect of mast cell mediators in oral leukoplakia leading to the following clinical and histopathological changes**

<table>
<thead>
<tr>
<th>Mast cell mediators</th>
<th>Histopathological features</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interleukin-1 and TNF</strong></td>
<td>Increased thickness of the epithelium</td>
<td>White patch or a plaque.</td>
</tr>
<tr>
<td>. Increased epithelial cell proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Histamine</strong></td>
<td>Increased mucosal permeability despite hyperkeratosis</td>
<td>Chronicity of the lesion.</td>
</tr>
<tr>
<td>. Enhances permeability across the epithelial surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heparin</strong></td>
<td>Increased vascularity of the stroma and ulceration</td>
<td>Erosive leukoplakia.</td>
</tr>
<tr>
<td>. Causes endothelial cell proliferation and migration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10 cases of oral submucous fibrosis were considered based on the clinical and histopathological features.\textsuperscript{14,15} Mast cell count of 48.25/sq.mm was noted which is in accordance with the Studies on mast cells in OSMF done by Bhatt and Dholakia.\textsuperscript{16} They noted abundant mast cells in Grade I and Grade II of OSMF i.e., 4.5 and 4.9 respectively as compared to 1.02 in normal buccal mucosa. It has been attributed that vesicle formation and symptoms of itching sensation to histamine released from the mast cells and suggested the concept of mast cell histamine chain. The mast cell hyperplasia could probably attribute to some of the signs and symptoms of OSMF. Mast cell mediators like prostaglandins and
leukotrienes are potent secretagogues for the serous and mucous cells. This could attribute to the increased salivation seen in OSMF.\(^7\)

**Table 3: Illustrating the probable effect of mast cell mediators in oral submucous fibrosis leading to the following clinical and histopathological changes**

<table>
<thead>
<tr>
<th>Mast cell mediators</th>
<th>Histopathological features</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chymase</strong>&lt;br&gt;A potent secretagogue.</td>
<td>-</td>
<td>Excessive salivation.</td>
</tr>
<tr>
<td><strong>Prostaglandins and leukotreines</strong>&lt;br&gt;Increase the mucous gland secretion</td>
<td>-</td>
<td>Excessive salivation.</td>
</tr>
<tr>
<td><strong>Heparin</strong>&lt;br&gt;Causes vasoproliferation</td>
<td>-</td>
<td>Petechiae</td>
</tr>
<tr>
<td><strong>Interleukin-1</strong>&lt;br&gt;Stimulates fibroblastic proliferation.</td>
<td>Increased collagen fiber bundles.</td>
<td>Decreased mouth opening.</td>
</tr>
</tbody>
</table>

In this study, mast cell hyperplasia was observed in all the oral diseases considered. The mediators in mast cells are known to vary with the variation in microenvironment in various diseases. Thus it is probable that mast cells play a key role in mediating the cross talks between the external antigenic agent and the local immunologic factors.

**CONCLUSION**

Mast cells serve a critical role in the development of inflammation in the oral mucosa and the dental pulp, both in the early, vaso-inductive events and in the transition from acute to chronic inflammation. Because of the unique properties of mast cells, these cells are ideally poised to serve as "gatekeepers" of the microvasculature in the oral cavity. An appreciation of the multiple interactions among mast cells, endothelial cells, nerves, and other immune system provides a basis for therapies for targeting mast cell responses. Therefore more studies are needs to be carried out in greater number of cases. The tissue level and the type of mediators should be analyzed in the various diseases considered.

**REFERENCES**