Incidence of Mast Cells in Oral Leukoplakia, Oral Lichen Planus, and Oral Squamous Cell Carcinoma: A Retrospective Study

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ABSTRACT

Introduction: Oral leukoplakia (OL), oral lichen planus (OLP), and oral squamous cell carcinoma (OSCC) are the most commonly occurring oral lesions associated with chronic inflammation at some stage of the disease process. Mast cells (MC) release a variety of chemical mediators through degranulation. In several malignancies also, mast cell density (MCD) has been found to correlate with angiogenesis, increased risk of metastasis, and poor prognosis.

Materials and methods: A retrospective study was carried out on 40 patients, 10 cases each of normal oral mucosa (NOM), OL, OLP, and OSCC, which were previously histologically confirmed at the Department of Oral Pathology and Microbiology, Hazaribagh Dental College and Hospital, Hazaribagh, India. These sections were then stained using 1% toluidine blue. The MCD/sq.mm was assessed in all the cases.

Results: An increase in MC count was observed in all the three above-mentioned oral lesions. The maximum mean MCD was reported in OLP. The MCD/sq.mm in OL, OLP, and OSCC are as follows: 33.10, 69.00, and 66.50.

Conclusion: A significant MC hyperplasia was reported in all the above considered lesions. This suggests that the use of MC stabilizers may give new hope in the treatment of these lesions.

Keywords: Mast cells, Oral leukoplakia, Oral lichen planus, Oral squamous cell carcinoma, Toluidine blue stain.


Source of support: Nil

Conflict of interest: None

INTRODUCTION

Mast cell (MC) was first reported by Paul Ehrlich in 1878, which he named as “Mastzellan.” Mast cells originate from the bone marrow as immature progenitor cells which migrate to the peripheral tissues and mature in situ where they can act locally and systemically by releasing variety of potent mediators through degranulation. Mast cells are widely distributed throughout the body and their increased numbers have been reported in lesions of hypersensitivity and inflammation. They are also known as “unicellular endocrine glands” due to their ability to release a variety of preformed secretory mediators like histamine, serotonin acid hydrolases, heparin, beta-fibroblast growth factors (FGF), vascular endothelial growth factors (VEGF), proteases (tryptase, chymase, and cathepsin G), and cytokines like tumor necrosis factor (TNF) alpha, and interleukin (IL)-16. Mast cells have also been reported to play a role in tumor progression and metastasis by promoting angiogenesis.

Few commonly occurring oral diseases like oral leukoplakia (OL), oral lichen planus (OLP), and oral squamous cell carcinoma (OSCC) are commonly associated with chronic inflammation. In addition, autoimmunity is strongly associated with OLP and angiogenesis with proliferation and metastasis of cancer.

The present study was carried out to evaluate the functional significance of MCs in disease progression in premalignant and malignant lesions.

MATERIALS AND METHODS

The present study was a retrospective study carried out at the Department of Oral Pathology and Microbiology, Hazaribagh Dental College & Hospital, Hazaribagh. Ten cases each of OL, OLP, and OSCC were retrieved from the department archives and from the archives of a histopathology diagnostic center Shreya Diagnostics. Ten biopsies of normal oral mucosa (NOM) constituted the control group which was obtained from the patients undergoing extraction for orthodontic treatment, with their consent. From all the blocks, two sections were cut each of about 3 to 4 µm thickness. The sections were standardized by maintaining the thickness at 3 to 4 microns. One section was stained by hematoxylin and eosin (H&E), the others were stained with 1% toluidine blue stain for identification of MCs. Mast cells are easily identifiable by light microscopy, but it is difficult to differentiate them from fibroblasts at H&E level. Henceforth, 1% toluidine
blue stain is used which stains the numerous MC granules that fill the cytoplasm metachromatically and the nuclei appear sky blue in color.\textsuperscript{7,8}

All the stained slides were examined under binocular light microscope. Mast cells were counted using an oculometer grid in five high power fields (40×) in a stepladder fashion. The MC count was then expressed as the number of MCs per grid field and the MC count was expressed per square millimeter using the following formula. The radius of one field under the high power objective (40×) was 0.24 mm, measured with the oculomicrometer. Hence the area of the field was $\frac{22}{7}r^2$, that is, $\frac{22}{7} \times (0.24)^2$ were approximately 0.2 sq.mm, which was later multiplied by five to get the number of MCs/sq.mm.\textsuperscript{9}

The results thus obtained by the above method were subjected to statistical analysis for obtaining significance value using Student’s t-test.

**RESULTS**

The toluidine blue-stained, MCs were easily identifiable in all the three groups, by their metachromatically stained granules. The mean and standard deviation was calculated in all the groups of NOM, OL, OLP, and OSCC cases. All the values were expressed in terms of mean standard deviation per sq.mm, and the maximum mast cell density (MCD) was reported in OLP (Table 1, Figs 1A to D).

The present study reported a significant increase in the number of MCs in all the above considered lesions, with the highest MC count in OLP (69.00 ± 2.67). The reported increase in the MCD in OL, OLP, and OSCC on comparing with NOM which formed the control group was statistically significant (*p < 0.001) (Table 2, Graph 1).

Further, a positive correlation was observed on comparing the MCD of OL with OLP and OSCC and the results were also significant with p-value < 0.001. In contrast, on comparing the MCD/sq.mm of OLP with OSCC elicited, no statistical significance was found with p-value = 0.027.

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**Table 1: Mean and standard deviation among all study groups**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Total number of cases</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal OM</td>
<td>10</td>
<td>10.80 ± 1.23</td>
</tr>
<tr>
<td>OL</td>
<td>10</td>
<td>33.10 ± 1.60</td>
</tr>
<tr>
<td>OLP</td>
<td>10</td>
<td>69.00 ± 2.67</td>
</tr>
<tr>
<td>OSCC</td>
<td>10</td>
<td>66.50 ± 1.90</td>
</tr>
</tbody>
</table>

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**Figs 1A to D:** (A) Hematoxylin and eosin-stained section of OSCC (10×). Toluidine blue stained sections of MCs; and (B) OSCC (10×); (C) OL; and (D) OLP (40×)
DISCUSSION

Mast cells normally reside in the connective tissue structures near vessels and nerves. Because of their location, plasticity, and the various mediators they produce, MCs are important immune effector and modulatory cells. In most histological sections, MCs appear as round or elongated cells with a diameter ranging between 8 and 20 µm. They are easily recognized by light microscopy and are stained by toluidine blue due to acidophilic metachromatic granules present in the cytoplasm. Activation of MCs results in the release of its granules to the cell exterior in the form of either massive or limited degranulation. The former is called exocytosis which occurs in type I allergic reactions and the later piecemeal degranulation which is seen in chronic inflammatory settings, such as cancers for instance. 

Mast cells have been studied in various oral inflammatory lesions: OL, OLP, oral submucous fibrosis, and oral cancers. The multifunction of MCs in the field of tumor growth has always attracted the attention of various researchers in this field. Mast cells can release numerous proinflammatory, immunoregulatory, and angiogenic molecules through selective stimulation pathways. There is increasing evidence to support the view that angiogenesis and inflammation are mutually dependent and increased angiogenesis and metastasis has been associated with neoplastic progression, as seen in several previously reported studies. The role played by the MC mediators in tumor progression is still intriguing. Therefore, the present study was undertaken to evaluate and compare the MCD in potentially malignant disorders and malignant lesions of the oral cavity which constituted OL, OLP, and OSCC; NOM was kept as control.

In the present study, an increase in MCD/sq.mm was observed in all the three above mentioned lesions. The mean MCD and standard deviation in OL, OLP, and OSCC were reported as 33.10 ± 1.60, 69.00 ± 2.67, and 66.50 ± 1.90 respectively. The results were in accordance to several previously reported studies.

A study was conducted evaluating the MC count and the number of degranulated MCs. In 40 cases of OL comparing with normal gingival, a significant rise of MCs was seen in leukoplakia. This is possibly attributed to the proinflammatory and proangiogenic role played by MC in OL, which may actually help in progressing to invasive carcinoma. In another similar study on OL, the authors concluded that the biologically and pharmacologically active agents released by the MCs might contribute to inflammatory reaction in leukoplakia. Interleukin-1 causes increased epithelial proliferation and the release of proangiogenic and angiogenic factors, such as histamine, heparin, chymase, beta-FGF, and VEGF by MCs may lead to increase in microvessels density significantly in leukoplakia and OSCC.

Lichen planus is a mucocutaneous disease of uncertain etiology; many genetic, psychogenetic, traumatic, and immunological factors have been implicated in the etiology of lichen planus. Current opinion suggests that lichen planus is a cell-mediated process bearing resemblance to delayed type of hypersensitivity reaction where MCs plays an integral role in the pathogenesis of this disease.

In the present study, highest MCD was observed in OLP 69.00/sq.mm. Most of the previous studies has reported an increase in the MC count in OLP, when compared to normal mucosa, suggesting their possible involvement in the pathogenesis of OLP. Products of MC have been implicated to bring about structural changes in the epithelium and connective tissue in lesions of lichen planus. Mast cells have been reported to lie in close proximity to external environment. In OLP, they are observed along the basement membrane as a response to external agents or antigenic stimuli, to release histamine, which helps in trafficking of lymphocytes and submucosal edema. The TNF-alpha released by these cells ultimately leads to necrosis and liquefactive
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degeneration of basal cell keratinocytes, thus attributing
to the chronicity of this lesion.18

The mean MCD in OSCC in the present study was
reported as 66.50/sq.mm and a positive correlation
was observed on comparing MCD of OL with OSCC.
These findings were in agreement with the studies of
various authors.19 Recent evidence suggests that MCs
are implicated in promoting tumorogenesis through four
different mechanisms. (1) Immunosuppression; histamine
released by activated MC leads to immunosuppression
of the cell immune system. (2) angiogenesis; MCs are
the source of several angiogenic factors, such as tryptase
that stimulates the proliferation of endothelial cells, and VEGF
induces the release of other angiogenic factors through the
endothelial wall of the matrix, while proteases facilitate in
the migration of the endothelial cells. Moreover, heparin
and proteoglycan contribute in vascular invasion and
metastases. (3) extracellular matrix degradation; MCs
contain various preformed matrix metalloproteinases
(MMPs), e.g., MMP-2 and MMP-9, and tissue inhibitor
metalloproteinases, which enable MCs in modulating
extracellular matrix degradation that further helps the
tumor to disseminate. (4) mitogenesis; numerous cytokines
are produced by MC, including FGF-2 and IL-8, TNF-
alpha, and transforming growth factor (TGF)-beta, which
have been related to tumor-associated angiogenesis.20-22

In contrast to the above findings, few authors have
failed to find any association of MC in tumor progression.
Few studies have reported a considerably low MC count
in OSCC, relating it to failure in migration of these cells.
This possibly highlights an important aspect of these cell
that mediators in MCs are known to vary with variation
in microenvironment in various diseases.23,24

CONCLUSION
The present data converge on indicating MCs playing a
crucial role in processes like tissue remodeling, angiogen-
sis, tumor development, and disease progression.
The potential implication of MC in human health and
diseases has stimulated the researchers to carry out the
experimental procedures leading to MC depletion or
inhibition of its secretion. In future studies, larger cohorts
need to be done to enlighten the fact that depletion in MCs
might act as an adjunct in the treatment of these diseases.

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