

## ANALYSIS OF MAST CELL COUNTS IN ORAL LEUKOPLAKIA

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### Abstract

Mast cells are regarded as one of the important cells of the immune system. They are generally believed to have different roles in human health and disease. Present study aimed to evaluate the total mast cell count, the distribution of degranulated mast cells and the possible role played by them in Oral Leukoplakia (OLK). Total mast cell count and the count of degranulated mast cells were analyzed separately in 40 histopathologically confirmed cases of OLK after staining the sections with 1% Toluidine blue. These values were compared with sections taken from clinically healthy papillary gingival in 10 normal subjects. The count of degranulated mast cells was also separately evaluated in the three zones viz. Sub-epithelial, Intermediate and Deeper zones of the connective tissue in all the OLK cases. There was significant increase in the total mast cell count and the count of degranulated mast cells in Oral Leukoplakia as compared to normal oral mucosa ( $p < 0.001$ ). The count of degranulated cells was found to be the highest in the Deep zone in all the OLK cases studied. The present study revealed that the total count of mast cells and the count of degranulated mast cells were significantly increased in oral leukoplakia. This may be attributed to their pro-inflammatory and pro-angiogenic role in oral leukoplakia and may actually play a significant role in its progression to invasive carcinoma.

Keywords : Mast cells, Leukoplakia, Degranulation

### Introduction

Paul Ehrlich in 1877 discovered a granular connective tissue cell and named it 'Mastzellen' meaning 'well fed cell'<sup>1</sup>. It was nearly sixty years later that the presence of these cells was reported in the gingiva<sup>2</sup>. Today these cells are regarded as complex and multifunctional, playing a significant role in immunopathology.

Mast cells are thought to be bone-marrow derived, widely distributed in the connective tissues especially beneath the skin, in the respiratory tract, GI tract and adjacent to the blood vessels and peripheral nerves<sup>3</sup>. They are small cells, round to ovoid in shape with a diameter of 12 – 15 microns. The numerous cytoplasmic granules in the mast cells bind to basic dyes such as Toluidine blue and exhibit the property of metachromasia<sup>4</sup>. The pre-formed mediators like histamine, heparine, leukotrienes, cytokines etc. are released from these granules by a process called degranulation<sup>5</sup>.

The role played by mast cells has been extensively studied in conditions like asthma and allergy, Odontogenic cysts, Oral Lichen planus (OLP) and Oral Submucous Fibrosis (OSF)<sup>6-8</sup>. Not many studies have been done to assess the presence and role of mast cells in Oral Leukoplakia (OLK), a common potentially malignant lesion. The present study was therefore carried out to estimate the total mast cell count and the count of degranulated mast cells, thereby determining the probable role played by these cells in the pathogenesis of OLK.

### Materials and Methods

Forty cases of previously diagnosed OLK were retrieved from the archives of the Departments of Oral Pathology & Microbiology, Yenepoya Dental College, Mangalore and Amrita School of Dentistry, Kochi. Ten specimens were taken from clinically normal and healthy papillary gingiva of patients who had come to the dental clinics for

orthodontic extractions. Two sections, each of 5 microns thickness, were taken from each paraffin block and one stained with routine Hematoxylin & Eosin, the other stained with 1% Standard Toluidine Blue Stain for demonstrating mast cells. Mast cell counting was done using a 10x eye piece fitted with a 1cm<sup>2</sup> graticule, and 40x objectives. The area encompassed by the graticule ie., 1cm<sup>2</sup> was taken as one Microscopic Field (MF), and the mast cells within this area were counted. In each MF, the intact and degranulated mast cells were identified based on the criteria given by Dyson and Luke<sup>9)</sup>. Those mast cells, which showed no disruption of their surface membranes were counted as intact cells (Fig. 1) and those which showed partial or complete discontinuity of their cell membranes with one or more extruded purple staining granule(s) were counted as degranulated mast cells (Fig. 2). In all the sections, mast cells were also counted separately in 3 zones – Zone I or the sub-epithelial zone, Zone II or the intermediate zone and Zone III or the Deep Zone. Starting from the basement membrane, each zone was identified by moving the graticule two MFs down at a time.

The obtained values were tabulated and statistical analysis done. This included the estimation of value of significance using the Mann Whitney U test for inter-group comparisons between OLK and normal oral mucosa and Wilcoxon's signed – rank sum test for intra group comparisons between the 3 zones in OLK.

**Results**

The mean total mast cell count in OLK was 8.2 as compared to 3.6 in the normal oral mucosa and this difference was found to be highly significant [*p* < 0.001] (Table 1). The mean count of degranulated mast cells in OLK was 5.3 as compared to 1.8 in the normal oral mucosa; this difference was highly significant. [*p* < 0.001]. (Table 2).

When the counts of degranulated mast cells were compared among the three connective tissue zones in OLK, zone III showed the highest number of degranulated mast cells and this was significantly higher than in zone I and zone II [*p* < 0.001]. (Table 3).

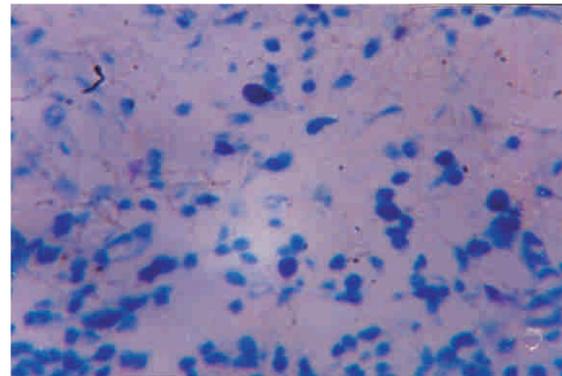


Fig 1: Photomicrograph showing an intact mast cell in oral leukoplakia – Toluidine blue stain, 40x. Note the elliptical purple stained cell with continuous cell membrane.

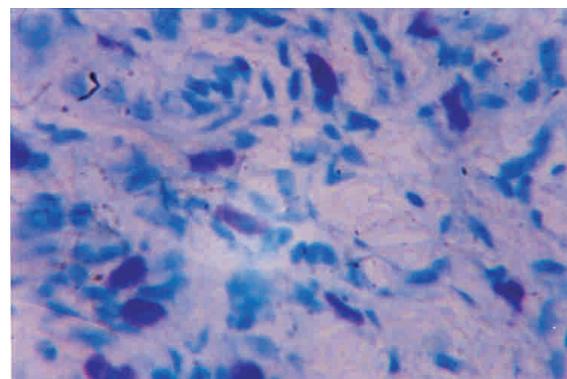


Fig. 2. Photomicrograph showing degranulating mast cells in oral leukoplakia - Toluidine blue stain, 40x. Note the discontinuous cell outlines of the elliptical cell (at the centre) indicating the degranulation process.

Table 1. Comparison of the mast cell counts in the two study groups.

Group	Total number of *MFs counted	Maximum count of mast cells per *MF	Mean total mast cell count
Oral leukoplakia	625	10	8.2
Normal oral mucosa	625	4	3.6

\*MF = Microscopic Field

The count of mast cells was tabulated in 625 microscopic fields in both groups and the maximum count recorded in each group was noted, to calculate the mean total mast cell count.

Table 2. Comparison of the counts of degranulated mast cells in the two study groups.

Group	Total number of *MFs counted	Maximum count of degranulated cells per *MF	Mean count of degranulated mast cells
Oral leukoplakia	625	6	5.3
Normal oral mucosa	625	3	1.8

\*MF = Microscopic Field

The count of degranulated mast cells was tabulated in 625 microscopic fields in both groups and the maximum count recorded in each group was noted, to calculate the mean count of degranulated mast cells.

Table 3: Intra-zone comparison of degranulated mast cells in oral leukoplakia

Zone	Total number of *MFs counted	Maximum cells per *MF	Mean count of degranulated mast cells
Zone 1 - Superficial	208	1	0.4
Zone 2 - Intermediate	208	2	1.8
Zone 3 - Deep	208	6	4.3

\*MF = Microscopic field

The count of degranulated mast cells was tabulated in 208 microscopic fields in each of the 3 zones in oral leukoplakia and the maximum count recorded in each zone was noted, to calculate the mean count of degranulated mast cells.

## Discussion

Mast cells are normally distributed in the connective tissue, and possess metachromatic granules in their cytoplasm<sup>10</sup>.

In the skin and oral mucosa, they are generally observed about the microvascular bed in close proximity to the vascular endothelial cells and nerves. They are known to exert their influence on the tissues by releasing many potent mediators through degranulation, which play an important role in both physiologic and pathologic conditions<sup>11</sup>. The role of mast cells has been explained in many oral lesions. In OLP they have been thought to be instrumental in recruiting T-lymphocytes to the sub-epithelial zone<sup>12</sup>, while the interleukin-1 from mast cells is thought to cause increased fibroblastic response, thereby attributing to the increased fibrosis in OSF<sup>13</sup>.

In the absence of many extensive studies on the mast cell count and their probable role in OLK, our study assessed the counts of mast cells in OLK. Compared to the normal mucosa, the OLK showed a significant increase in the total mast cell count. Though one of the earlier studies has reported no significant difference in the mast cell counts in OLK and normal oral mucosa<sup>14</sup>, other previous studies in this regard have shown an increase in the total mast cell count in OLK, as was the case with our study<sup>15,16</sup>. The increase in mast cell count in OLK has been associated with the chronic inflammatory reaction seen in these cases<sup>17</sup>. In our study, all 40 cases of OLK showed a moderate inflammatory infiltrate in the

connective tissue. The pharmacologically active agents in the mast cell granules probably contribute to the inflammatory reaction seen in OLK. The mast cell degranulation releases interleukin-1, which may cause increased epithelial proliferation that is observed in OLK<sup>18</sup>, and also histamine, that may be responsible for the increased mucosal permeability thereby facilitating increased access of the antigen to the connective tissue<sup>19</sup>.

Mast cell degranulation has been studied and reported in periapical pathologies, odontogenic cysts, OLP and OSF<sup>20,6-8</sup>. As part of the present study, the count of degranulated mast cells was also analyzed in OLK and compared with that of normal oral mucosa. Degranulated mast cells were significantly increased in Oral Leukoplakia in comparison with normal oral mucosa. Among the three connective tissue zones in OLK cases where the counts of degranulated mast cells were analyzed, the zone III (deep zone) showed a significant increase in the cell count when compared to the other two zones.

Previous studies have shown a direct correlation between mast cell activity (degranulation) and the state of vascularity and inflammation of the tissue<sup>21-23</sup>. Mast cells are thought to constitute the initial step in neo-vascularization and further inflammatory changes. Degranulation of mast cells probably activates the endothelium through the TNF- $\alpha$  released from their granules, and this may be critical to the elicitation phase of inflammation<sup>24</sup>. The release of potent pro-angiogenic and angiogenic factors by mast cell degranulation may actually favor the progression of OLK through squamous cell carcinoma by bringing about tumor angiogenesis<sup>25,26</sup>. The increased number of degranulated mast cells observed in OLK cases in this study probably strengthens the role of mast cells in this regard. The increased inflammatory infiltrate in the deep zone of the connective tissue in our cases may be responsible for the increased count of the degranulated mast cells in this zone. Mast cells may therefore have a regulatory effect on the inflammatory reaction and angiogenesis, thereby playing a pivotal role in the

transformation of OLK to invasive carcinoma. Further studies need to be initiated in this regard to analyze the correlation between mast cell count and vasculature in OLK and squamous cell carcinoma cases.

### Conclusion

Mast cell counts are increased in oral leukoplakia when compared to the normal oral mucosa. The counts of degranulated mast cells are also significantly higher in oral leukoplakia, and these are maximum in the deep zone of the connective tissue. Our observations indicate that mast cells, with their regulatory role on angiogenesis and inflammation, brought about by the release of their mediators, may well play an important role in the tumor progression, facilitating the transformation of oral leukoplakia into invasive carcinoma. A study to correlate the vasculature and mast cell count needs to be done for a better understanding of their role in these lesions.

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