

Mast Cells Association in Different Grades Dysplasia & Oral Squamous Cell Carcinoma: A Histochemical Study

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ABSTRACT:

Introduction : Angiogenesis is a complex process mediated by angiogenic factors which get produced by cancer cells mast cells play a key role in promoting angiogenesis thus they are related with progression of tumor and metastasis. Tryptase found as a potent angiogenic factor.

Material and Method: To evaluate mast cells count using 1% standard Toluidine Blue stain, 15 tissue sample of normal epithelial mucosa, 10 samples of each of low to high grades of dysplasia along with 10 samples each of low to high grades OSCC were taken for the study. Followed by comparison of mast cell count in these following groups.

Result: On comparing the mast cell counts in different grades of epithelial dysplasia with normal oral mucosa we found that there is increase in number of mast cells from mild to severe dysplasia on the contrary the mast cells count in well differentiated OSCC was higher while comparing to the moderately differentiated OSCC and poorly differentiated OSCC. When comparison was analysed between low to high grades of dysplasia and OSCC it was demonstrated that more number of mast cells were found in epithelial dysplasia as compare to OSCC.

Conclusion: It was concluded that mast cells can be used as an indicator to evaluate increased angiogenesis which is correlated with the increased progression of potentially malignant disorder into malignant lesions.

Keywords: Oral Leukoplakia, Oral Squamous Cell Carcinoma, Mast cells.

INTRODUCTION:

Mast cells are granule-rich granules that are abundant in histamine and heparin. They are resident cells of many different types of tissues. The oral mucosa's micro-vascular endothelial cells are the site where mast cells are distributed preferentially. The cell count fluctuates in a variety of disorders, including epithelial dysplasia and oral squamous cell carcinoma, because these cells are crucial to the inflammatory process (**Kamal R et al, 2015**). Because of their capacity to release a range of distinct pre-formed inflammatory mediators, including vasoactive cytokines, amines, and various enzymes upon activation, mast cells play a varied role in extracellular matrix breakdown, angiogenesis, and innate and acquired immunological responses (**Spoorthi BR et al, 2013**).

Neovascularization is the term used to describe the process of producing new microvessels from the preexisting vasculature. Unless a tumour creates its own network of new microvessels, it cannot increase in size beyond 1-2 mm (**Astekar M et al, 2012**). Therefore, it has been determined that the functioning of mast cells results in a variety of biological effects, including angiogenesis, extracellular matrix degradation, mitogenesis, an increase in microvascular permeability, and the recruitment of inflammatory cells, including the macrophage cell cycle (**Michailidou EZ, 2008**). Mast cells are drawn to the tumour microenvironment by stem cell factor (SCF), which is secreted by the tumour cells. SCF also produces a number of angiogenic factors and matrix metalloproteinases (MMPs), which, respectively, increase tumour vascularization and invasiveness (**Belgaumi UI, 2017**). MCs were found at areas of intense vascularization (called as "hot spots"), near areas of new capillary development and around the tumor margins. It shows that the accumulation of mast cells in sites of the tumor margins and secretion of potent angiogenic and pro-angiogenic factors may simulate a tumor-host interaction which possibly favors tumor progression (**Cheema VS, 2012**). Mast cells leads to have a correlation with malignant transformation (**Flier JS, 1993**). Various studies have been done in correlation of mast cells with OSCC and dysplasia but our study mainly focus on comparison of mast cell count among different grades of OSCC and epithelial dysplasia.

MATERIAL AND METHOD:

All the tissue material for the present histological study were achieved from the Department of Oral and Maxillofacial Pathology and Oral Microbiology, Santosh Dental College and Hospital after receiving an informed consent. As a control group, a sample of 15 clinically normal gingival tissues or oral mucosa tissue were preserved from people who had undergone surgical removal of impacted tooth. Whereas 10 tissue samples, each histologically graded as mild dysplasia, moderate dysplasia and severe dysplasia as well as histopathological grades of OSCC tissue samples into well differentiated, moderately differentiated were further collected. For poorly differentiated 5 tissue samples were considered. So, total 70 paraffin tissue blocks of the selected cases were procured for the study. Consideration was given only

to clinical cases of oral leukoplakia presenting epithelial dysplasia and proved cases of OSCC on its histological examination.

Staining and Observation

Furthermore, all the tissue collected tissue blocks were cut thin sections of 4 microns thickness and slides were stained with 1% Standard Toluidine Blue Stain for demonstration of mast cells. An Olympus CX41 microscope with an Olympus oculometer grid was used to evaluate the number of mast cells. Density of mast cell was assessed in connective tissue at the hot spots in zigzag manner using a light microscope at 400X magnification. These characteristic cells were mainly dispersed around micro vessels which were purple oval or spindle shaped cells exhibiting reddish granular cytoplasm with single centred nucleus. Density of mast cells was analysed in relation of mild to moderate to severe dysplasia cases and similarly with different histological grades of OSCC cases. (Figure 1 & 2) Followed by comparison with normal oral epithelium. (Figure 3) SPSS (Statistical Package for Social Sciences) version 16.0 using Chi square test were used for the statistical analysis and the results were tabulated. (Table 1)

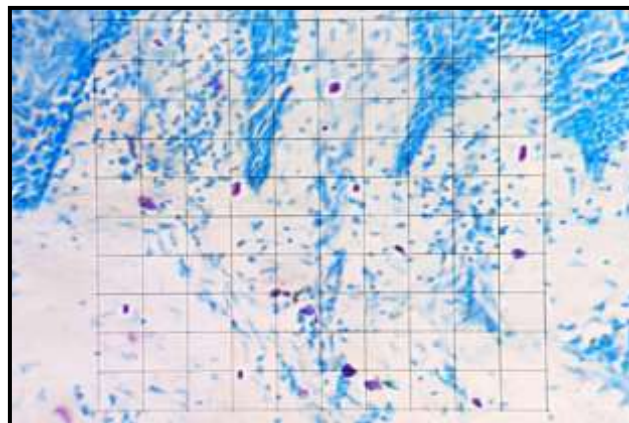


Fig 1: Photomicrograph showing mast cells of dysplasia (40X toluidine blue)

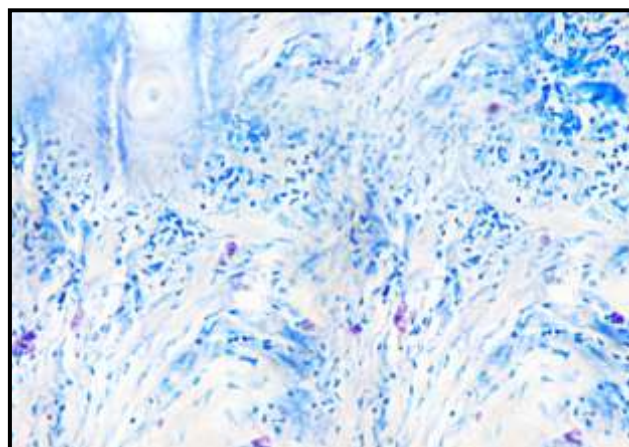


Fig 2: Photomicrograph showing mast cells of OSCC (40X toluidine blue)

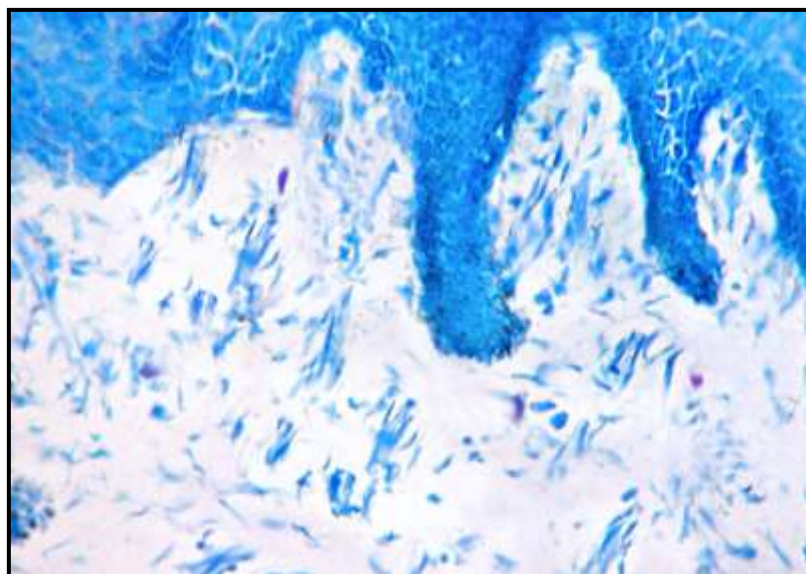


Fig 3: Photomicrograph showing few mast cells of normal oral mucosa (40X toluidine blue)

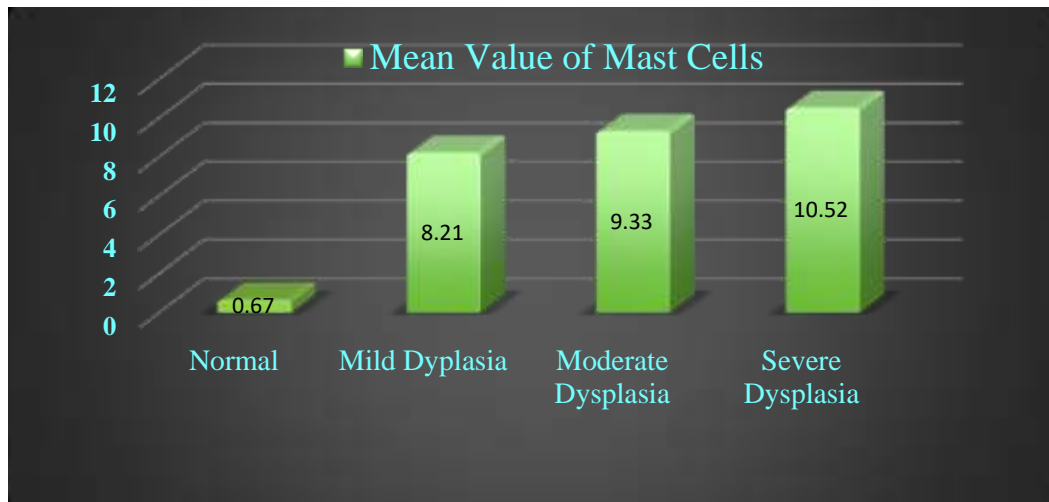
Table 1: Evaluation of mean number of mast cell in Control group, Leucoplakia and OSCC.

	Groups	No. of Cases	Mean	SD	P Value
	Normal mucosal tissue	15	0.67	1.75	
Grade of Epithelial dysplasia	Mild	10	8.21	0.61	0.0001*
	Moderate	10	9.33	0.88	
	Severe	10	10.52	1.16	
Grade of OSCC	Well differentiated	10	7.54	0.81	0.0010*
	Moderately differentiated	10	7.04	0.75	
	Poorly differentiated	5	6.11	0.36	

Statistical significance set at 0.05; SD: Standard deviation; OSCC; Oral squamous cell carcinoma

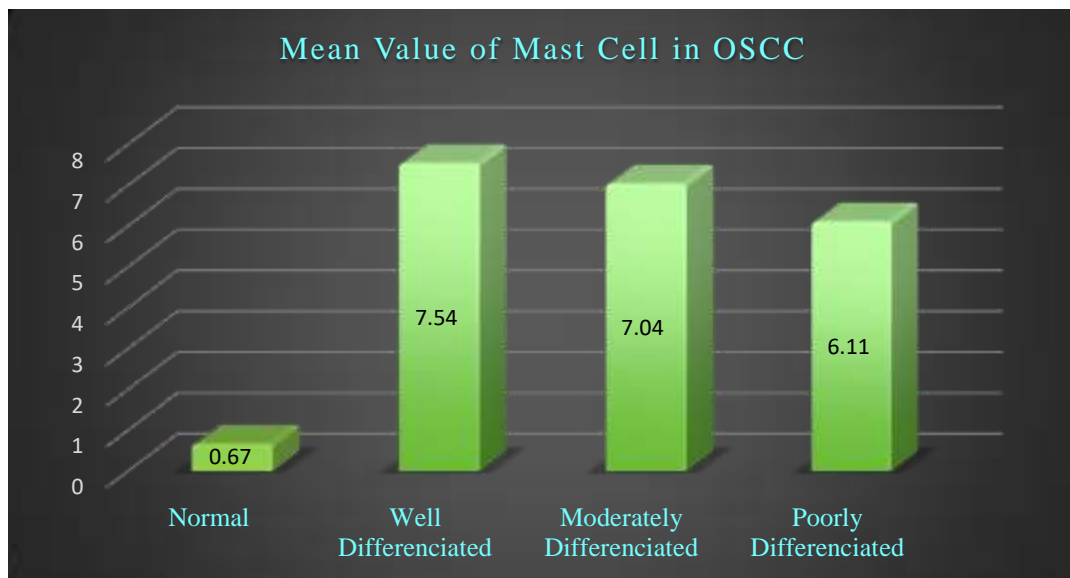
RESULTS:

When the number of mast cell count was compared between control group as Oral mucosa and different grades dysplasia using using Chi square test. It was found that is gradually rise in the number of mast cell count from mild to moderate and from moderate to severe dysplasia which was highly significant with p-value 0.0001. (Graph 1)



Graph 1: Showing increasing MC count in dysplasia & comparison with control group.

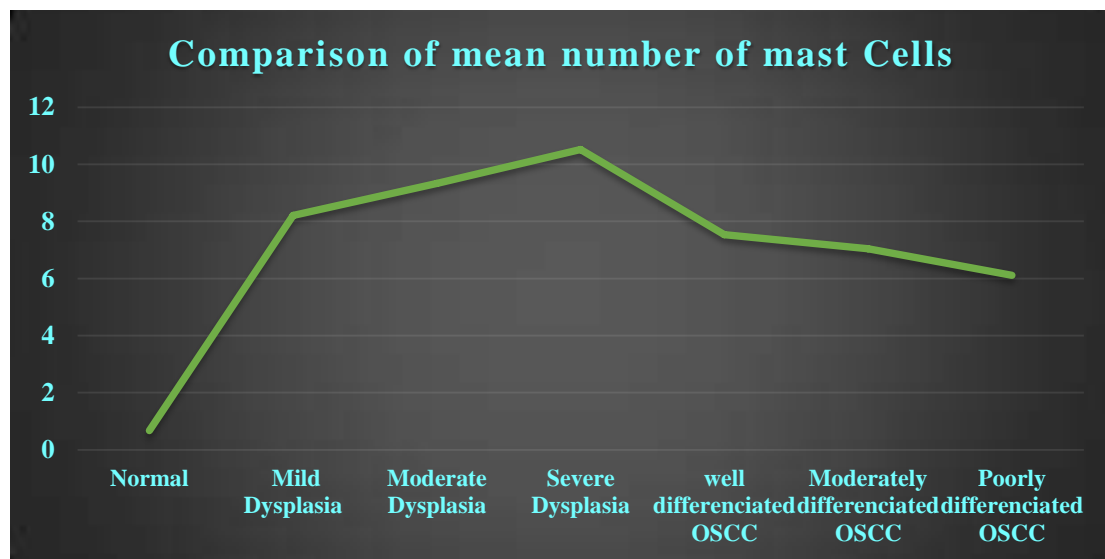
Similarly, when the number of mast cell count was compared between control group and different histological grading of OSCC. It was analysed that there is considerably declining in number of mast cells from well differentiated to moderately differentiated as well as from moderately differentiated to poorly differentiated OSCC cases. This result was highly significant with p-value 0.0010. (Graph 2)



Graph 2: Showing decreasing MC count in OSCC & comparison with control group.

Interestingly, on comparison of mast cell count in low to high grades of OSCC and oral epithelial dysplasia.

There was significantly rise in number of mast cells in different grades of oral epithelial dysplasia but, there was slight decline when seen in histological grades of OSCC as shown in graph. (Graph 3)



Graph 3: Showing increasing MC count in dysplasia & decreasing in OSCC.

DISCUSSION:

Mast cells live nearby in the connective tissue. Mast cell mediators' function and their interaction with other inflammatory cells have been fascinating to study. (Bone M) Mast cells have also been linked to tumour invasion in a number of malignancies, including melanomas, esophageal, breast, and oral cancers. Angiogenesis has been identified as a poor predictor of survival in many malignancies. (N. Mohtasham et al)

In present study, we analysed and compared the mast cell count in histopathological sections of oral epithelial dysplasia (OED), oral squamous cell carcinoma (OSCC) and normal oral mucosa by using metachromatic stain.

S. Rakesh et al. in 2012 conducted a similar study where he found significant increase in the total mast cell count and the count of degranulated mast cells in oral epithelial dysplasia compared to normal oral mucosa. In our study we found increase in the number of mast cells as the severity of dysplasia increases from mild to moderate. (S. Rakesh et al, 2012)

Similar study was done by H T Chaturvedi et al. in 2021 where they took thirty cases each of OSCC, oral leukoplakia, normal oral mucosa to study mast cell number using 1% Toluidine blue. According to them there was an increase in mast cell numbers in leukoplakia and OSCC as compared to normal mucosa. But in our study no. of mast cells decreased as grading increases in case of OSCC which is in contrast to our study. (Chaturvedi T H et al, 2021)

Sharma B et al. in 2010 compared the density of mast cells (MCD) and microvessels (MVD) in normal oral mucosa (NM) and between various OSCC grades. He discovered that there was strong expression of mast cell tryptase in both normal oral mucosa and oral squamous cell carcinomas, where MVD was assessed immunohistochemically using anti-Factor VIII related von Willebrand factor and MCD using anti-mast cell tryptase. When compared to the

normal oral mucosa, the density of mast cells and micro vessels was greater in OSCC. These results were contradictory to our findings. (Sharma B et al, 2010)

Mohtasham N et al. in 2010 conducted a study to analyze mast cell density and angiogenesis in oral dysplastic epithelium and increasing grade of oral squamous cell carcinoma. They hypothesized that density of mast cells increases in normal and oral dysplasia, normal oral mucosa and OSCC and epithelial dysplasia but no significant difference between low grade and high grade OSCC which was in contrast to our study where we found increased mast cell count in well differentiated SCC with decreased count in moderate to poorly differentiated SCC. (Mohtasham N et al, 2010)

CONCLUSION:

Toluidine blue stain has metachromatic property which can be used to visualize various type of histological cells including mast cells. It enhanced the visualisation of mast cells with great accuracy in low to high grades of Oral leucoplakia and OSCC cases. However, when distinctiveness of mast cell density is appreciated, there was a linear increase in number of mast cell from mild to severe degree of dysplasia followed by slight reduction in different grades of the tumour as compared to normal mucosa.

The present study concluded that mast cell is an indicative of enhanced angiogenesis which is associated with progression of potentially malignant disorder into malignant lesions . This aid in poor prognosis of such malignant epithelial tumor.

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