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ANALYSIS OF MAST CELL DENSITY IN NORMAL ORAL MUCOSA VS DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA

*[1]Dr. Sandeep Gupta, [2]Dr. Rajat Misurya, [3]Dr. Archana Misurya, [4]Dr. Harkanwal Preet Singh

ABSTRACT-

BACKGROUND-*Mast cells display a diversity of roles in extracellular matrix degradation, angiogenesis and innate and purchased immune responses, thanks to their ability to release a variety of pre-formed mediators, including cytokines, vasoactive amines, and enzymes on activation. Angiogenesis or neovascularization is crucial for development and progression of malignant tumors. This biologic process is regulated by angiogenic factors, like basic fibroblast protein (bFGF), vascular endothelial protein (VEGF), tumor necrosis factor-α (TNF-α), and angiogenic inhibitors, like angiostatin, platelet coagulation factor (PF4) and thrombospondin-1 (TSP-1).2,3 These factors are released by tumoral and stromal cells.****MATERIALS AND METHODS-****Formalin-fixed, paraffin-embedded tissue specimens of 60 cases of OSCC were retrieved and divided into group 1-3 (20 poorly differentiated OSCC, 20 moderately differentiated OSCC, and 20 well differentiated OSCC). Twenty normal oral tissues of patients without any habits were included in the study, and they were selected as controls. Along with the H&E stained sections, another set of the same tissues were processed and stained with 1%Toluidine blue. 5 µm sections of formalin-fixed, paraffin blocks were deparaffinized with xylene and they were rehydrated with graded alcohols.* ***RESULTS-****Mean Mast Cell Density in Different Histological Grades of OSCC and Normal Mucosa were calculated. Group1 shows the mean Average Mast Cell (4.15±1.46), Group2 shows the mean Average Mast Cell (7.10±2.29), Group 3 shows the mean Average Mast Cell (9.35±1.72****), Group 4 shows the mean Average Mast Cell (3.35±1.69****).CONCLUSION-****The effect of mast cells on prognosis could not be assessed in this study as time lapse between biopsy and treatment and further follow up was too less to quantify. However larger samples and large scale multi-institutional studies could provide a baseline data of MCD in different grades of OSCC. Along with this,*

[1] Professor, Department of Dentistry, GMC Ambikapur, Chhattisgarh
[2] Professor Department of Dentistry, GMC Jhansi, U.P
[3] Associate professor, Department of Dentistry GMC Jhansi, U.P.
[4] Associate Professor, Department of Oral Pathology and Microbiology, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India

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recurrent cases and follow up studies over long periods of time like 1 – 10 years are required to validate the role and quantification of the MCD in high risk and low risk individuals, role in prognosis predilection, in planning and development of various adjuvant therapeutics strategies.

KEYWORDS- *Mast cell, Oral Squamous Cell Carcinoma, Matrix Degradation, angiogenesis, neovascularization*

I. INTRODUCTION-

Oral squamous cell carcinoma [OSCC] is that the most frequent malignancy within the mouth like the 95% of all oral malignant lesions. It remains a heavy problem of oral health worldwide. Globally, oral epithelial cell carcinoma incorporates a complex biological behaviour and despite the advances within the treatment modalities, the 5-year survival rates of those patients have improved only slightly. This led to an interest in predicting its possible future behaviour, in order that alternative therapeutic strategies may be tailored to treat the severity of the tumour.[1]Mast cells display a diversity of roles in extracellular matrix degradation, angiogenesis and innate and purchased immune responses, thanks to their ability to release a variety of pre-formed mediators, including cytokines, vasoactive amines, and enzymes on activation. Angiogenesis or neovascularization is crucial for development and progression of malignant tumors. This biologic process is regulated by angiogenic factors, like basic fibroblast protein (bFGF), vascular endothelial protein (VEGF), tumor necrosis factor-α (TNF-α), and angiogenic inhibitors, like angiostatin, platelet coagulation factor (PF4) and thrombospondin-1 (TSP-1).[2,3] These factors are released by tumoral and stromal cells.[4] Among the host immune cells, some roles are implicated for mast cells (MCs) in tumor progression via promoting angiogenesis, mitogenic effects and degradation of extracellular matrix in some malignant tumors. Mast cells originate from bone marrow and migrate to the peripheral tissues where they mature in-situ. Several angiogenic factors mentioned above, and a few other factors like tryptase and chymase are found in MCs.[5] Mast cells are phylogenetically old, highly granulated cells, already known by their key role in type I hypersensitivity. they're the most effector cells in Ig E associated disorders but also seem to play important roles in acquired or innate host reactions. The activation of mast cells has been proved to possess many biological consequences like mitogenesis, extracellular matrix degradation, angiogenesis and augmentation of microvascular hyperpermeability and recruitment of inflammatory cells including macrophages. it's already known that neoangiogenesis is required for the expansion and spread of tumors.[3] Mast cells are studied for many years thanks to their prominent role in normal homeostasis and various pathologies. There are volumes of literature on the proposed role of mast cells within the pathogenesis of assorted pathological conditions but few on the evaluation of their role in oral epithelial cell carcinoma. Hence we wanted to histologically evaluate the amount of mast cells in tissue sections of normal mucosa and OSCC.[6] Thus the current study analysed for the expression of mast cells quantity with relevancy normal mucosa and important clinical pathological variables in OSCC

II. MATERIALS AND METHODS-

The present retrospective study is performed to determine the mast cell density [MCD] in different grades of Oral squamous cell carcinoma.It was carried out on a total of 60 biopsy tissues retrieved from the archives of Department of Oral and Maxillofacial Pathology of a private dental college. Formalin-fixed, paraffin-embedded tissue specimens of 60 cases of OSCC (20 poorly differentiated OSCC, 20 moderately differentiated OSCC, and 20 well differentiated OSCC) were retrieved. Twenty normal oral tissues of patients without any habits were included in the studyand they were selected as controls. The sample were categorized into 4 groups (table-1). Along with the H&E stained sections, another set of the same tissues were processed and stained with 1%Toluidine blue. 5 μm sections of formalin-fixed, paraffin blocks were deparaffinized with xylene and they were rehydrated with graded alcohols. The slides were stained with 1% Toluidine blue, mounted with DPX and observed under Olympus 2Li microscope. These slides showed Mast cells as violet/red purple with blue background. Mast cells are found in the connective tissue and their cytoplasm contains granules (metachromatic) composed of heparin and histamine. Toluidine blue should stain mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). Metachromasia, tissue elements staining a different color from the dye solution, is due to the pH, dye concentration and temperature of the basic dye. Blue or violet dyes will show a red color shift, and red dyes will show a yellow color shift with metachromatic tissue elements.eliminate an inter observer variation.

Quantification ofMast Cell Density[7]: Generally accepted criteria for determining density of mast cell. by identifying the area by scanning the section under scanner view (x40)(Fig 1). The area of highest vascularisation was identified and four high power fields were selected. The mast cell density was expressed as no of cells per sqmm. Toluidine blue staining revealed mast cells as large, purple,oval and highly granulated cells. Mast cells were observed in the lamina propria in highly populating areas around the tumor margins. Mast cells were also observed at the tumor periphery and at the stem of the lesion when it existed. Increased numbers of mast cells were also found in areas of high vascularization (namely the ‘hot spots’).All data were tabulated and statistical tests were performed for obtaining significance s value using ANOVA and POST HOC TEST. P values of < 0.00 were regarded as significant. MCs were characterized by blue nuclei and purple cytoplasm, when stained with acidified toluidine blue technique.

III. RESULTS:-

The present study is a retrospective study done to determine Mast celldensity[MCD] on total of 80 cases(Graph-1).

These samples were retrieved from the archives of the department of the Oral pathology and microbiology. There is a complete availability of record with relevant details of these cases.The total no. of subjects are 80 and were divided into four groups.

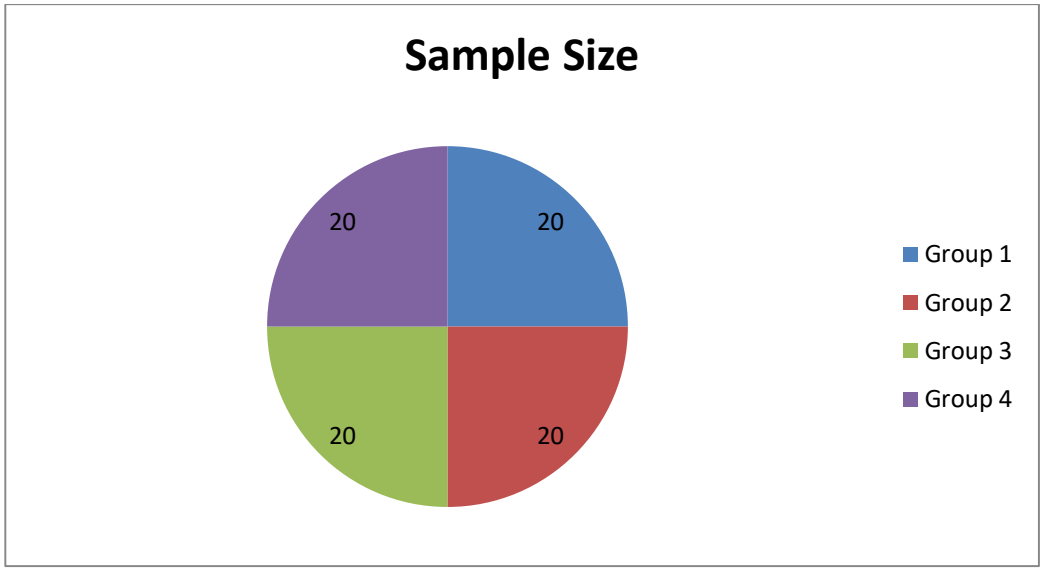
By using the independent ‘t’ test the mast cell density (MCD) was found to be significantly higher IN WDOSCC (7.1±2.29) than in the oral mucosa (4.15±1.46 /NORMAL). The MCD was found to be higher in

moderately differentiated oral squamous cell carcinoma (9.35±1.72) as compared to well differentiated oral squamous cell carcinoma (7.10±2.29). However MCD is lower in poorly differentiated oral squamous cell carcinoma (3.35±1.69) as compared to moderately differentiated oral squamous cell carcinoma (table-2, 3)

Table 1: Histopathological Grading of OSCC in Study Group and Normal Mucosa

Histopathological Diagnosis	No. of Cases
Normal	20
Well Differentiated Squamous Cell Carcinoma	20
Moderately Differentiated Squamous Cell Carcinoma	20
Poorly Differentiated Squamous Cell carcinoma	20
Total	80

Table1 shows the that total 80 samples have been taken and categorized into 4 groups, out of them 20 were normal mucosa (Group 1), 20 WD0SCC (Group 20), 20 MDOSCC(Group 3) and 20 PDOSCC (group 4).



Graph 1: Sample Size in Different Groups

Table 2: Average No. of Mast Cells in Different Histological Grades of OSCC and Normal Mucosa

Histopathological Diagnosis	**Mast Cell Density (MCD) (Mean±SD)**
Normal (Group 1)	4.15±**1.46**
Well Differentiated Squamous Cell Carcinoma (Group 2)	7.10±**2.29**
Moderately Differentiated Squamous Cell Carcinoma (Group 3)	9.35±**1.72**
Poorly Differentiated Squamous Cell carcinoma (Group 4)	3.35±**1.69**

Table 2 shows the Mean Mast Cell Density in Different Histological Grades of OSCC and Normal Mucosa. Group1 shows the mean Average Mast Cell (4.15±**1.46)**, Group2 shows the mean Average Mast Cell (7.10±**2.29)**, Group 3 shows the mean Average Mast Cell (9.35±**1.72)**, Group 4 shows the mean Average Mast Cell (3.35±**1.69).**

Graph 2: Mean No. of Mast Cells in Different Histological Grades / Different Groups.

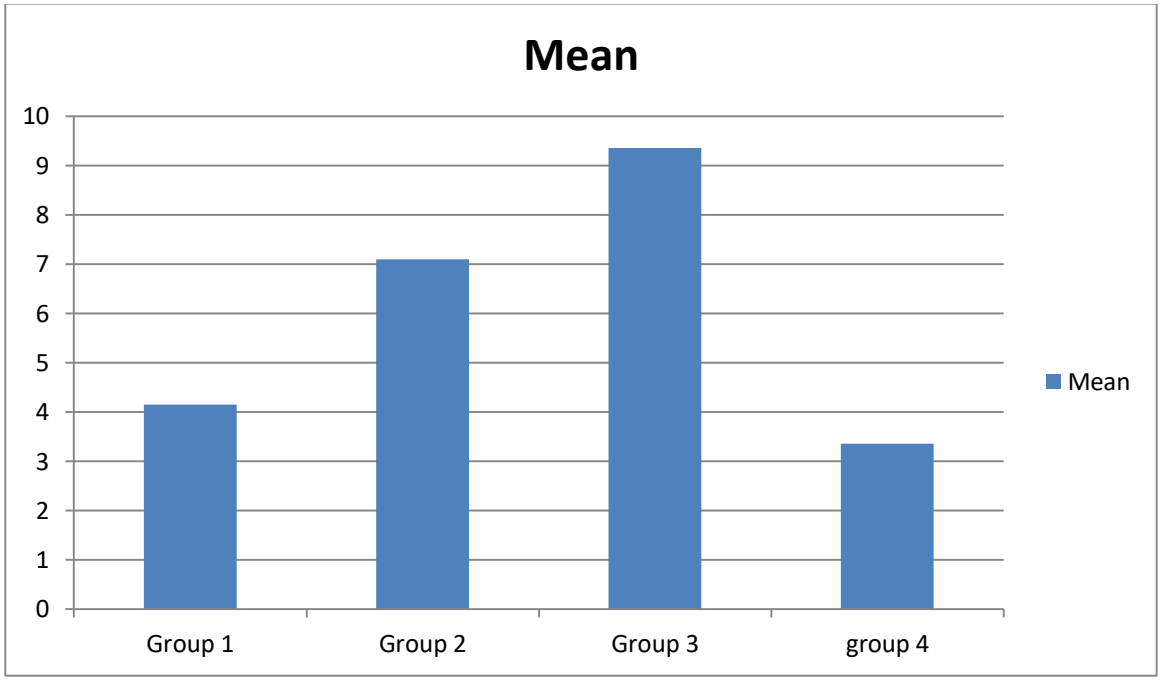


Table 3; Comparison of Average No. of Mast Cells in Different Histological Grades of OSCC

WDSCC	**MDSCC**	**PDSCC**	**P- Value**

N=20			N=20			N=20			<0.000
Mean	±	SD	Mean	±	SD	Mean	±	SD	
7.10	±	2.29	9.35	±	1.72	3.35	±	1.69	

Table 3 shows Inter Group Comparison of AverageN.o of Mast Cells in Different Histological Grades of OSCC (*Group 2, Group 3 and Group 4) Which is showing Statistically significant using ANOVA t Test *(P-* <0.000) Highly Significant comparative relationship in seen all three study groups.

Table 4:Comparison of Average number of mast cells between any two grades of OSCC

WDSCC			**MDSCC**			**PDSCC**			**P- Value**
N=20			N=20			N=20			
Mean	±	SD	Mean	±	SD	Mean	±	SD	<0.000
7.10	±	2.29	9.35	±	1.72				<0.001
7.10	±	2.29				3.35	±	1.69	<0.001
			9.35	±	1.72	3.35	±	1.69	<0.001

Table 4 shows the inter GroupComparison of Average number of mast cells between any two grades of OSCC. Group 1 and Group 2 Showing significant relationship *(P<0.001*). Group 1 and Group 3 showing Significant relationship, Group 2 and Group 3 Showing significant relationship *(P<0.001*).and Group 1 and Group 3 Showing significant relationship *(P<0.001*).

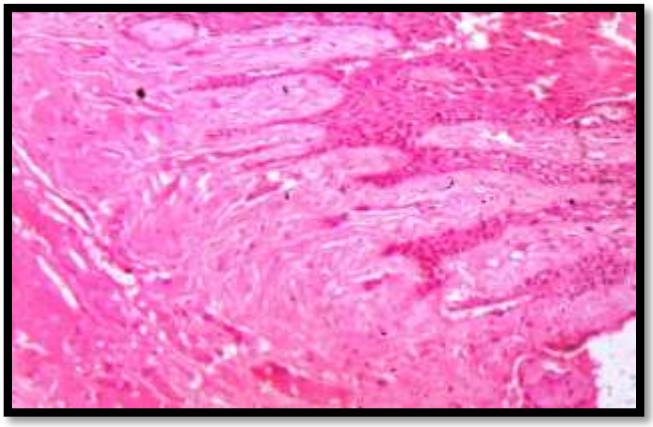


Fig. (1) Microphotograph showing H and E staining in normal mucosa.[10x]

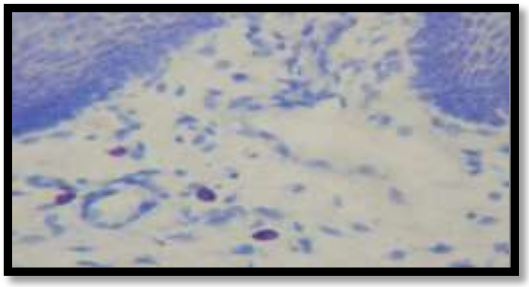


Fig. (2). Mast cells in normal buccal mucosa (x100) with 1%Toluidine blue.

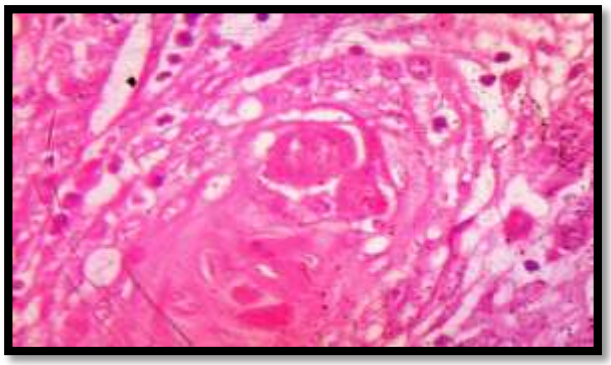


Fig-3. Microphotograph showing well differentiated OSCCH & E staining.

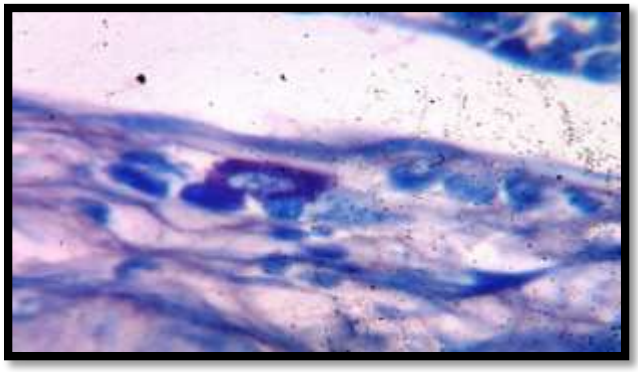


Fig. (4) Microphotograph showing Mast cells stained with1% toluidine blue in well differentiated SCC (x100).

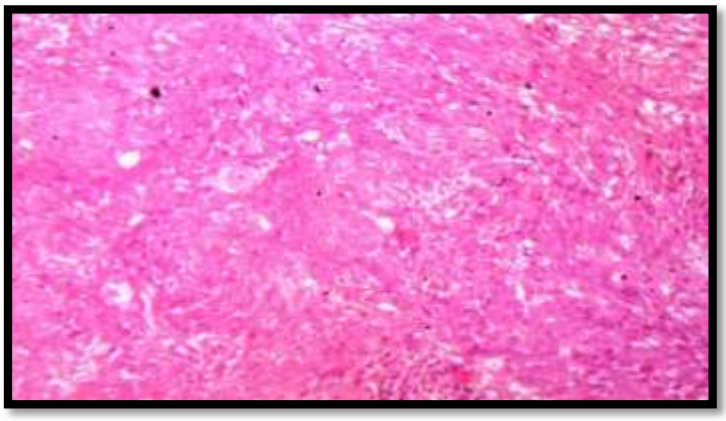


Fig-5 Microphotograph show moderately differentiated OSCC in H&E staining (10x)

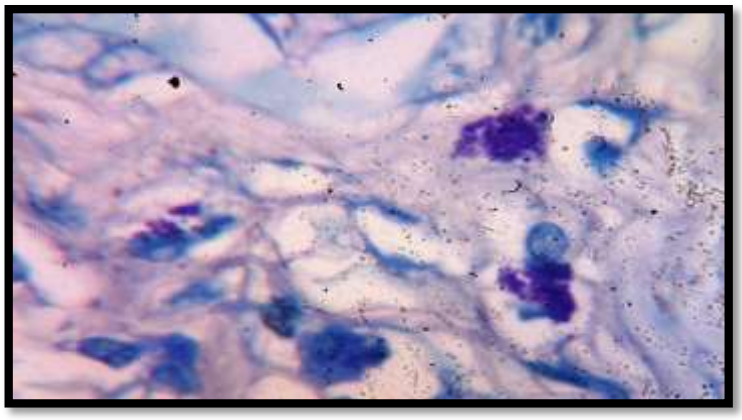


Fig.(6) Microphotograph showing Mast cells stained with1% toluidine blue in moderately differentiated OSCC (x100).

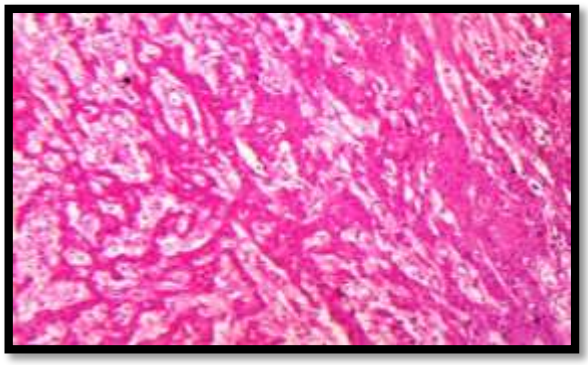


Fig-7 Microphotograph showing poorly differentiated OSCC in H&E staining.

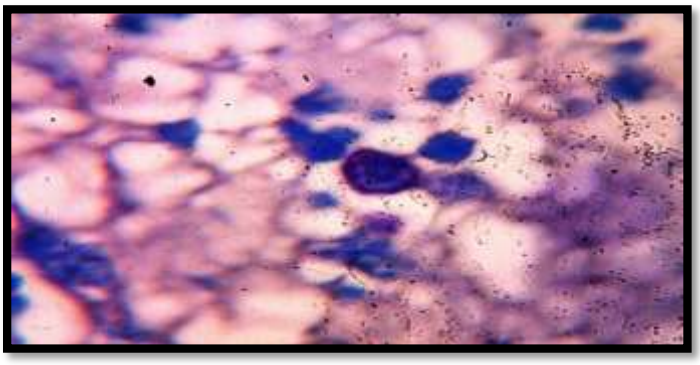


Fig. (8).Microphotograph showing Mast cells stained with1% toluidine blue in poorly differentiated OSCC (x100).

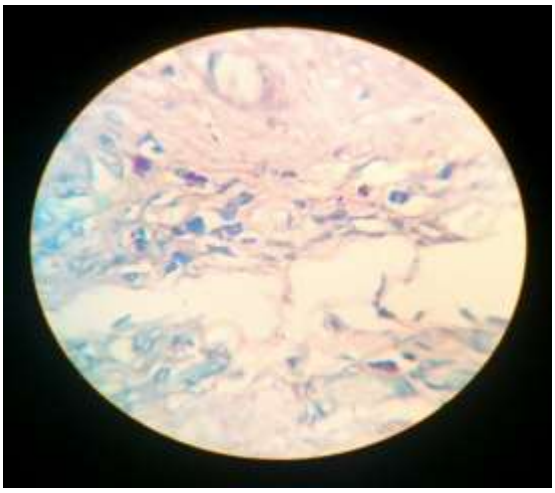


Fig. 9 Microphotograph showing mast cell in 1% toluidine blue staining x100.in normal mucosa.

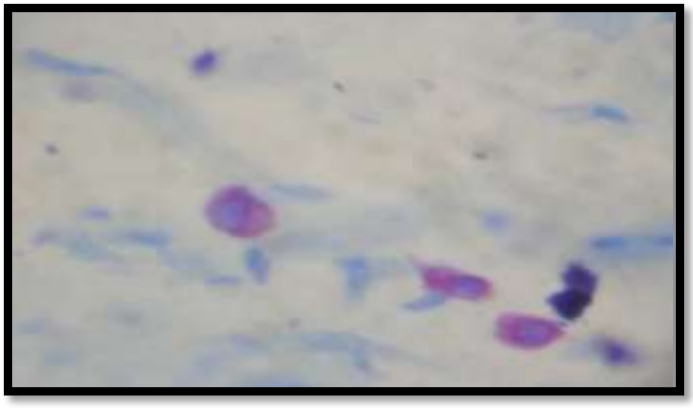


Fig. (10) Degranulating Mast cells in Oral Squamous Cell Carcinoma (x100).

IV. DISCUSSION-

On comparative analysis by using the independent 't' test, MCD was found to be significantly higher in WDOSCC (7.10=+_2.29) than in the normal oral mucosa (4.15+-1.46). The MCD was found to be higher in moderately differentiated oral squamous cell carcinoma (9.35+-1.72) as compared to well differentiated oral squamous cell carcinoma (7.10+2.29). However MCD was found to be lower in poorly differentiated oral squamous cell carcinoma(3.35+-1.69) as compared to moderately differentiated oral squamous cell carcinoma (table-2, 3). An interpretation can be thus drawn that MCD showed a linear increase from normal mucosa to well differentiated and moderately differentiated oral squamous cell carcinoma but a decrease of same in poorly differentiated oral squamous cell carcinoma.

Our results were similar to another study[8] where they observed a linear increase in MCD from normal oral mucosa, hyperkeratosis, premalignant dysplasia to squamouscell carcinoma suggesting that this finding could be due to possible role of mast cells in upregulation of angiogenic process by promoting tumour secreting several potent angiogenic factors. Similarly in other study observers were observed that MCD and microvessel density did increase significantly between normal oral mucosa and oral leukoplakia without dysplasia and oral leukoplakia with mild, moderate or severe dysplasia.[9] They concluded that an angiogenic switch seemed to be turned on in the later stages of dysplasia indicating a transformation into malignancy. Also a possible role of mast cells during the progression from normal oral tissue to oral epithelial dysplasia and subsequently to oral squamous cell carcinoma was elucidated. In contrast to our studies. A decrease in mast cell numbers in premalignant and malignant oral lesions which was attributed to the failure of mast cell migration.[10]

Our study showed an increase in MCD from normal oral mucosa to well differentiated and moderately differentiated oral squamous cell carcinoma (Table 1and 2). A hypothesis can be suggested that hypoxia during tumor formation and enlargement might induce tumour cells to release angiogenic factors which in turn could chemoattract mast cells to migrate into the hypoxic areas of the tumour

MCD in our study showed a positive linear correlation in normal oral mucosa and oral squamous cell carcinoma of well and moderate types only. Reduced MCD in OSCCs with poor prognosis may be suggested by changes reflected in tumour microenvironment. This makes it more permissive to neoplastic cell proliferation[11]. However, a statistically significant increase of mast cells was shown in SCC of the mouth in the study done by correlation between them with an increase in MCD was observed only in oral epithelial dysplasia and SCC. Their results were similar to our study which showed progressive increase of mast cells from normal oral mucosa to well differentiated and moderately differentiated SCC.

However, once invasion is established as in oral squamous cell carcinoma, the role of mast cells isprobably shifted from angiogenesis to furtherpromotion in invasion of tumor by degradation of adjacent connective tissue matrix. This shift is related to their involvement in invasion now.

Results of our present study are similar to another study who also found low MCD in advanced stage of OSCC[12] and also a poor prognosis and adjuvant therapy requirement. Thus MCD at the invading front might be seen as a prognostic marker. They observed that the stroma in poorly-differentiated carcinoma was devoid of mast cells. They suggested that the angiogenic regulation in squamous carcinogenesis was biphasic. In the early phases, angiogenic activators are released via the mast cell degranulation and as the neoplastic progression proceeds, the angiogenic growth factor gene expression is upregulated in the cancer cells, wherein the tumor cells control the angiogenic phenotype directly, instead of depending upon the manipulation of the inflammatory cells to indirectly affect the neovascularization and angiogenesis[1].

The progressive depletion of the mast cells in the present study from well and moderately differentiated OSCC to poorly differentiated OSCC could be probably also because the mast cells may have been degranulated as the disease progressed. Hence, the lack of mast cell granules in the advanced disease states may have resulted in the negative staining with Toluidine blue. The significantly higher densities of the mast cells in well and moderately-differentiated OSCC as compared to those in poorly-differentiated OSCC strongly suggests that MCD may be used as an indicator for the disease progression in the oral carcinogenesis. This finding has a clinical significance in helping in delineating a risk population, which might benefit from adjuvant therapeutic strategies e.g. the mast cell degranulation blocking therapy[1].

V. CONCLUSION-

According to the findings of the present study, mast cell density (MCD) was found to show significant linear correlation of increase from normal mucosa to well and moderately differentiated state of OSCC. However poorly differentiated OSCC showed significantly less quantity of mast cells. The reason for same could be that tumor initiation and progression which occurs in well differentiated OSCC and moderately differentiated OSCC probably is the function of the mast cell which provides mitogenic stimulation and angiogenesis through the release of the various mediators which is the hallmark of the tumor growth and metastasis. However, poorly differentiated OSCC shows statistically significant less MCD. In poorly differentiated OSCC, it is hypothesized that due to the

massive degranulation of the mast cells, identification of the same becomes difficult. Another reason put forward for the same is that in poorly differentiated OSCC, mast cells reduce astumor cells are not dependent on mast cells for angiogenesis but concentrate more on extracellular matrix degeneration.The effect of mast cells on prognosis could not be assessed in this study as time lapse between biopsy and treatment and further follow up was too less to quantify. However larger samples and large scale multi-institutional studies could provide a baseline data of MCD in different grades of OSCC. Along with this, recurrent cases and follow up studies over long periods of time like 1 – 10 years are required to validate the role and quantification of the MCD in high risk and low risk individuals, role in prognosis predilection, in planning and development of various adjuvant therapeutics strategies.

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