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ORIGINAL ARTICLE

Assessment of Role of Mast Cells in Progression of Oral Squamous Cell Carcinoma.

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ABSTRACT

Oral squamous cell carcinoma (OSCC)is a malignant neoplasm arising from the oral mucosal epithelium which constitutes around 90% of all oral malignancies. Mast cells play a significant part in tumor progression and in metastasis.To investigate and compare the number, morphology, and topographical spread of mast cells in oral squamous cell carcinoma cases and correlate diverse types of mast cells with the inflammatory infiltrate. From each block of 45 paraffin blocks of different grades of OSCC two sections were made. One section was stained by hematoxylin and eosin and another section were stained by toluidine blue stain. The slides were then analyzed under a microscope and mast cells were counted in five different zones in all sections starting from sub epithelial connective tissue using the image analyzer software with the graticule. One way ANOVA was used for statistical analysis. The numbers of mast cells were significantly more in moderately differentiated squamous cell carcinoma as compared to well-differentiated squamous cell carcinoma and control groups ($p<0.05$). An increased number of typical(TMCs), atypical(AMCs) and Granular(GMCs) mast cell in more inflamed areas contribute to the active participation of mast cells in various phases of the inflammatory process manifested by their degranulation. This study reveals that there is a definitive increase in mast cell count when compared to normal mucosa substantiating their contributing role in tumor progression.

Keywords *- Malignant neoplasm, tumor progression, metastasis, inflammatory infiltrate.*

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INTRODUCTION

Oral squamous cell carcinoma remains a major issue of oral health worldwide. Every single multicellular animal and definitely each vertebrate can acquire cancer. The ability to acquire cancer is a feature of all cells that are proficient of reproduction and growth. Hence, the word 'cancer' implies an abnormal, seemingly unrestricted growth of body cells.

Thus, for several solid tumors as well as oral squamous cell carcinoma (OSCC) it is difficult to assess the overall prognosis of patients using conventional clinicopathologic criteria. Regardless of attempts and

methods to interfere in the disease, aiming at identifying the premalignant lesions and OSCC at the initial stage and treating patients with numerous modalities, a marginal improvement can only be appreciated in the overall 5-year survival rate of patients with OSCC.

Solid tumors don't contain neoplastic cells in isolation, rather they include array of cellular types, which frequently outnumber the tumor cell populace. The interaction of cellular proliferation and progressive acquisition of a specialized phenotype shows a notable level of coordination between neoplastic cells and various stromal cell populations of which one significant factor is inflammatory cell infiltrate. The cellular constituents of this host reaction to tumorigenic cells include lymphocytes, macrophages, neutrophils, mast cells, and eosinophils [1].

Paul Ehrlich was the man who postulated the first description of some of the distinctive histochemical features of the cells that he shortly thereafter named "Mastzellen" (or "well-fed cells"), [2] which empower them to respond to multiple specific and non-specific stimuli [3]. In the majority of histological sections, MCs show up as round or elongated cells with a diameter in the range of 8 and 20 μm. They are effortlessly distinguished by light microscopy for their abundant toluidine blue positive, metachromatic granules that fill the cytoplasm [4].

This wide scope of biological function, universal distribution and strategic location near blood vessels, nerves, inflamed tissues and neoplastic foci empower mast cells to play a significant role in a multitude of physiologic, immunologic, and pathologic processes [3]. Lately, apart from their roles in the maintenance of homeostasis and in inflammation, relationship of mast cells with a variety of tumors has been defined in quite a few malignancies and carcinoma.[5]The intricate and diverse function of MCs in the field of tumor development addresses quite possibly the most exciting frontiers in MC biology. The event of a likely causative relationship between MCs, persistent inflammation and cancer has for quite some time been suggested [7].

As most tumors have inflammatory cell infiltrates, which frequently comprise of abundant MCs, the inquiry with regards to the probable involvement of MCs to tumor growth has progressively attracted the concentration of basic and clinical researchers [7]. Coussens *et al* [8] reported that mast cells, "infiltrate hyperplasia, dysplasias, and invasive fronts of carcinomas," and then discharge the mast cell-specific proteases chymase and tryptase—the former acting as a mitogen and the latter being angiogenic to skin fibroblast.

We formulated a hypothesis that there should be an increase in both mast cell count and density with the increase in degree of inflammatory infiltrate in the stroma of the lesion with advancing grade of dysplasia to frank OSCC.

MATERIAL AND METHODS

Source of Data:

A retrospective study on 45 cases was conducted on the tissue specimens embedded in paraffin wax blocks retrieved from the archives of the Department of Oral and Maxillofacial Pathology along with retrieval of available records with relevant details of cases diagnosed as oral squamous cell carcinoma (OSCC).

Method of Collection of Data

Patient Selection:

The stored blocks were retrieved from the archives of the department of Oral and Maxillofacial Pathology along with retrieval of available records with relevant details of these cases.

The control group consisted of the tissue samples taken for third molar extraction purposes. The OSCC cases of moderately and well differentiated squamous cell carcinoma cases was considered with high inflammation.

The groups that were considered are as follows:

Group 1: Control group,15 samples.

Group 2: Comprise of 15 cases of well differentiated squamous cell carcinoma.

Group 3: Comprise of 15 cases of and moderately differentiated squamous cell carcinoma.

Ethical clearance was taken from Rama Dental College, Hospital and Research Centre, Kanpur

Method of analysis:

Two section of 5 micrometre thickness from each tissue implanted in paraffin wax block was prepared. One section was stained with hematoxylin and eosin and another section were stained with toluidine blue stain. The sections were observed under x4, x10 and x40 magnification and the selected areas with high inflammation was captured and the mast cells was counted in five different zones in all sections starting from sub epithelial connective tissue from one end, proceeding along the entire length of sections using

the image analyser software with the graticule. The average of each type of mast cells was taken from five different zones.

The following criteria was established for the morphological study of mast cell:

1. Typical (TMC):When the cytoplasmic membrane, the nucleus and the granules of cytoplasm are clearly seen.
2. Atypical (AMC): When the nucleus and the granules was clearly seen but the cytoplasm was not defined.
3. Granular (GMC): The presence of three or more aggregations of granules resulting from degranulated AMCs without the presence of their nucleus.

Statistical analysis:

All collected data was analysed using SPSS software and appropriate statistical tests was applied. Data was classified based on the objectives mentioned. The mean, standard deviation and range was calculated for all the groups. One way ANOVA was used for statistical analysis. Level of significance was set at $p<0.05$.

RESULTS

It was observed in table 1, that the mean number of mast cellswere higher in the moderately differentiated SCC group (24.8±2.1112) followed with well differentiated SCC group (19.13±2.3258) and control group (3.86±1.1872). This difference in the distribution between the groups was statistically significant (p=0.001).

Table 2 demonstrates the comparison of different mast cells according to groups. Similar pattern of distribution was observed across the groups, which were higher in the moderately differentiated followed with well differentiated SCC groups and control groups. This difference in the distribution between the groups was also statistically significant ($p<0.05$).

In the table 3 we observe that in WDSCC cases with mild, moderate and severe inflammation shows maximum number of typical mast cell than Granular mast cells and minimum no of atypical mast cell respectively. We observe that in MDSCC cases with mild, moderate and severe inflammationall the three mast cells (typical, atypical and granular) are most in severe than in moderate and least in mild.

Table 1.Distribution of mean number of mast cells in the study population.

Groups	Mean	SD	F value	P value
Control	3.86	1.1872	467.835	0.001*
Well differentiated SCC	19.13	2.3258		
Moderately differentiated SCC	24.8	2.1112		

* p<0.05- Statistically Significant, **ANOVA test

Table 2.Comparison of Typical, Atypical and Granular mast cells in three groups.

Mast cells	Groups	Mean	SD	F value	P value
Typical	Control	2.0	0.8452	208.954	0.001*
	Well differentiated SCC	8.4	1.1212		
	Moderately differentiated SCC	10.133	1.4075		
Atypical	Control	0 .467	0.5164	133.941	0.002*
	Well differentiated SCC	4.333	1.1127		
	Moderately differentiated SCC	6.4	1.2421		
Granular	Control	1.4	0.5071	235.212	0.001*
	Well differentiated SCC	6.4	1.1212		
	Moderately differentiated SCC	8.267	1.0998		

* p<0.05- Statistically Significant, **ANOVA

Table 3.Comparison of individual types of mast cells in Well and Moderately differentiated squamous cell carcinoma with inflammatory infiltrate

Groups	Inflammatory Infiltrate	No of Cases	Typical	Atypical	Granular	Total no of mast cells
Well differentiated	Mild	7	7.4	3.4	5.4	17.1
	Moderate	5	8.6	4.6	6.4	17.8
	Severe	3	10.0	6.0	8.0	22.3
Moderately differentiated	Mild	2	8.0	5.0	7.0	22.0
	Moderate	4	8.5	5.5	8.5	23.5
	Severe	9	11.1	7.2	9.0	26.1

DISCUSSION

Mast cells have for quite a while been considered to accept a specific part in the pathophysiology of various diseases. The release of mast cell mediators has been assumed to augment to tissue injury and inflammation. Recent information has shown that inflammation is a basic segment of tumor progression. Mast cells are the neighbourhood residents of the connective tissue. They are supposed to be proinflammatory and insusceptible enhancing in real life and they produce mitogenic cytokines.

Mast cells can deliver various proinflammatory, immunoregulatory and angiogenic molecules through various provocation pathways. The actuation of mast cells has been demonstrated to have numerous biological results, for example, mitogenesis, extracellular matrix degradation, angiogenesis, an increase of microvascular hyperpermeability and enlistment of provocative cells including macrophages. Expanded angiogenesis has been connected with neoplastic movement, metastasis as found in a few examinations and furthermore is linked with increase in the number of malignancies. The thickness of mast cells in a tissue can be premeditated histochemically by utilizing stains like toluidine blue and alcian blue; and immunohistochemically by utilizing mast cell tryptase, heparin, chymase, and carboxypeptidase A.

Oral squamous cell carcinoma stays a major issue of oral wellbeing around the world. Universally, head and neck squamous cell carcinoma is the 6th most common malignancy and records for roughly 5% of malignant tumors in developed nations. While, in the developing nations it is the most well-known malignancy representing up to half of malignant tumors. Oral squamous cell carcinoma has a complex biological conduct and notwithstanding progresses in the treatment modalities the 5-year survival rate actually contacts just 5%. This has prompted a premium in foreseeing its conceivable future behavior so that alternative therapeutic strategies can.

In the natural history of tumor development and growth, a population of cells within a host is exposed to an initiation factor and a new wave of gene expression occurs, removing normal growth constraints. Ultimately, the cells comprising a successful solid tumor develop two distinct new capabilities that are not possessed by the normal cells from which they arise. Firstly, they proliferate without internal constraints. Secondly, they create an environment in vivo where their growth potential can be realized. Numerous areas of tumor biology have been researched in-depth and an increasing number of molecular markers associated with malignant transformation have been successfully correlated with prognosis and clinical course of the disease.

Various studies have been done to assess the role of mast cells in precancerous and cancerous conditions. The present study was done for the quantitative and qualitative measurement of mast cells in OSCC and an attempt was made to correlate their number and type with the density of inflammatory cells. MCs assume a significant part in tumor advancement. MCs gather around the tumor edges and release pro-angiogenic and angiogenic factors that favor tumor progression.

MCs in tissues can be distinguished utilizing histochemical stains like toluidine blue, alcian blue, and immunohistochemistry utilizing tryptase, heparin, chymase, and, carboxypeptidase. In the current investigation, 1% toluidine blue stain was utilized for the identification and count of MCs. Anuradhaet al.,[9]showed altogether increased MCs counts in OSCCs when contrasted with the normal oral mucosa. The impact of the contribution of MCs to tumor invasion has additionally been shown in oral cancers. In this investigation, we have noticed a critical increase in mast cell when contrasted with normal, which affirms the significance of mast cells in the pathogenesis of oral squamous cell carcinoma. This outcome of an increased number of mast cells is predictable with past examinations. In OSCC, a more prominent number of mast cells were seen in more profound connective tissue (zone II) when compared with subepithelial connective tissue.

Jose *et al*.[10] noticed a bigger number of degranulating mast cells in the subepithelial zone. This was clarified by the way that mast cells that moved from blood vessels in the more profound connective tissue to the extravascular compartment later advanced toward the subepithelial zone, where they apply their biologic impact on blood vessels and help in enrollment of inflammatory cells to the lesional region. But, in our investigation utilizing toluidine blue, we couldn't conclusively differentiate between intact and degranulated mast cells.

In our investigation the mean number of mast cells is altogether more in moderately differentiated SCC which is 24.8±2.111, than in well differentiated SCC i.e 19.13±2.325 (p value<0.001)and in control group it is 3.86±1.187. This outcome is in harmony with the result of the examination done by Telagi *et al*. [11] They detected that there is an increase in mast cell density in squamous cell carcinoma of lip compared to normal oral mucosa which proposes their implication in the development of these lesions.

In the current examination the mean number of various types of mast cells were determined in both MDSCC and WDSCC. Mean number of Typical mast cell (TMC) was 10.133±1.4075 and 8.410±1.1212; atypical mast cell (AMC) was 6.41±1.2421 and 4.33±1.1127 granular mast cell (GMC) was 8.4±1.12and

6.420±1.110among both the groups respectively, which is as per the investigations done by Telavi *et al* [12] who noted abundant mast cells in grade I and II of OSF. They credited vesicle establishment and manifestations of itching sensation to histamine discharge from mast cells. Prostaglandins and leukotrienes emitted by mast cells are intense secretagogues for serous and mucous cells which would attribute to increased salivation Fig 1 to 6.

In our study comparative analysis of different types of mast cells in various degrees of inflammatory infiltrate was calculated between MDSCC and WDSCC. The result of the study is as follows. In mild inflammatory infiltrate the number of typical, atypical, and granular mast cells are 7.4, 3.4, 5.4 respectively. In moderate inflammatory infiltrate the number of typical, atypical, and granular mast cell are 8.6,4.6, 6.4 respectively. In severe inflammatory infiltrate the number of typical, atypical, and granular mast cell are 10.0, 6.0, 8.0 respectively.

It has been suggested by Walsh et al., [13] that cytokines delivered by tissue mast cells might be the triggering factor for the acceptance of vascular adhesion molecules to permit passage of mast cells to the extra vascular compartment. In the current study, an increased number of mast cells were found corresponding to an increase in inflammatory cell response. Hence, the concentration of inflammatory cells in tissue is the reflection of vascularity.

A significant higher count of mast cells was found in MDSCC and WDSCC. This firmly recommends that mast cell count/density might be utilized as indicators for disease advancement in oral carcinogenesis. This finding has a clinical importance by aiding in depicting a risk populace, which may profit by adjuvant therapeutic techniques for example antiangiogenesis therapy and vascular targeting of anticancer-gene therapy.

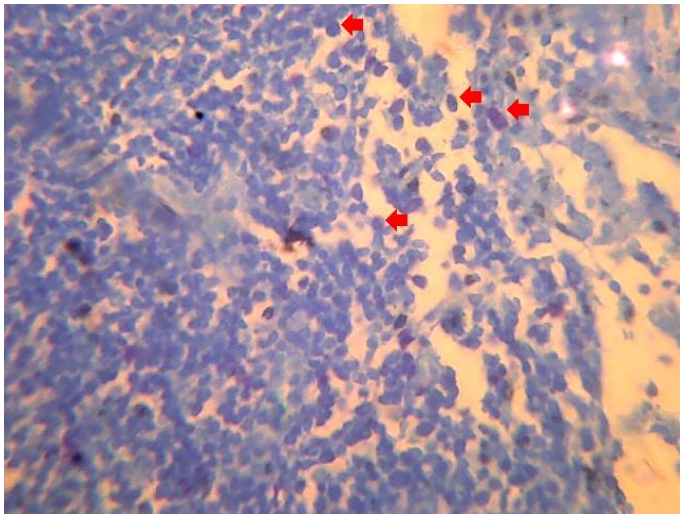

Fig 1: Photo Micrograph of 1% Toluidine Blue stain MDSCC- 40X

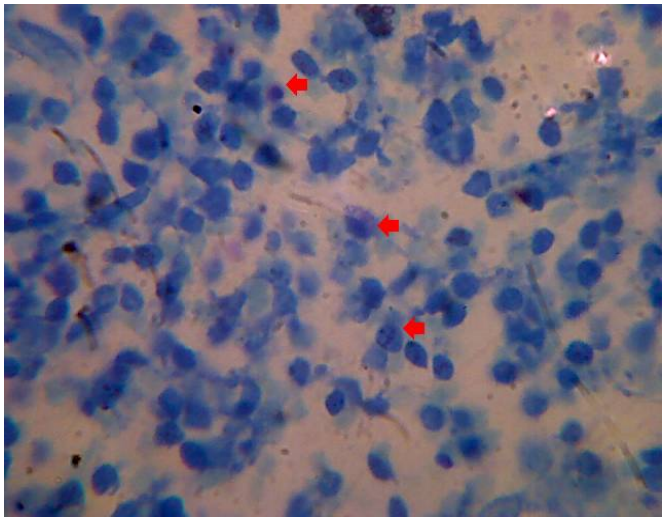

Fig 2: Photo Micrograph of 1% Toluidine Blue stain MDSCC- 100X

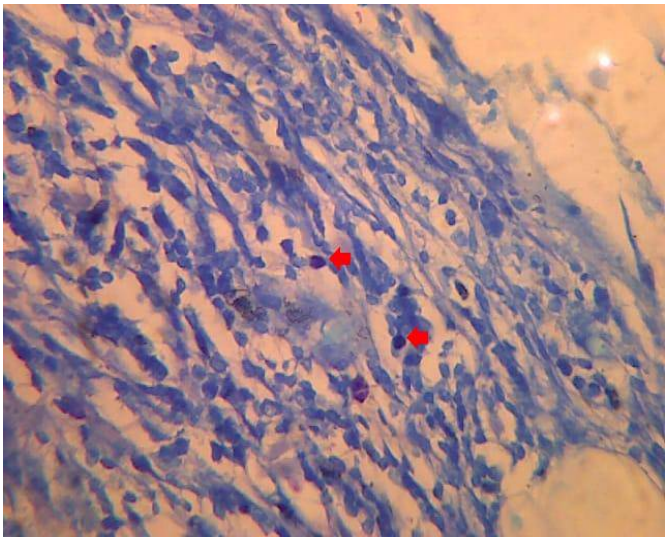

Fig 3: Photo Micrograph of 1% Toluidine Blue stain WDSCC- 40X

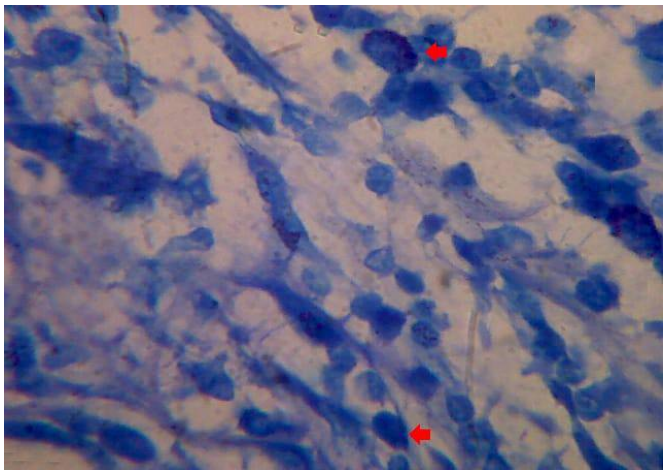

Fig 4: Photo Micrograph of 1% Toluidine Blue stain WDSCC- 100X

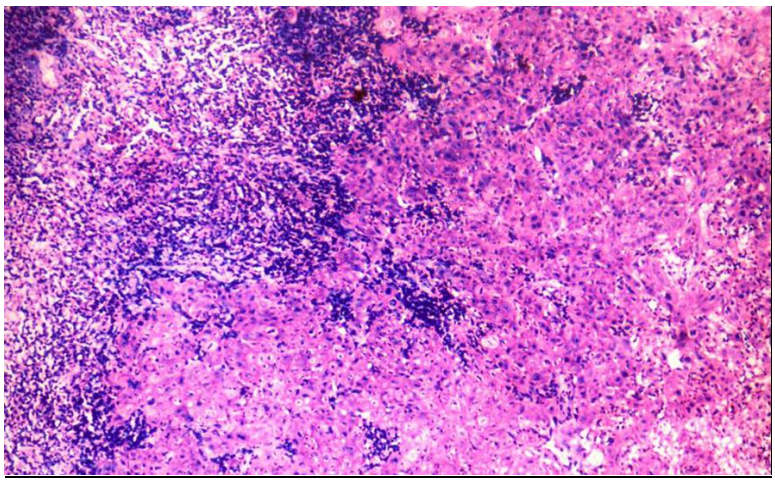

Fig 5: H&E-Stained Section of Moderately differentiated SCC

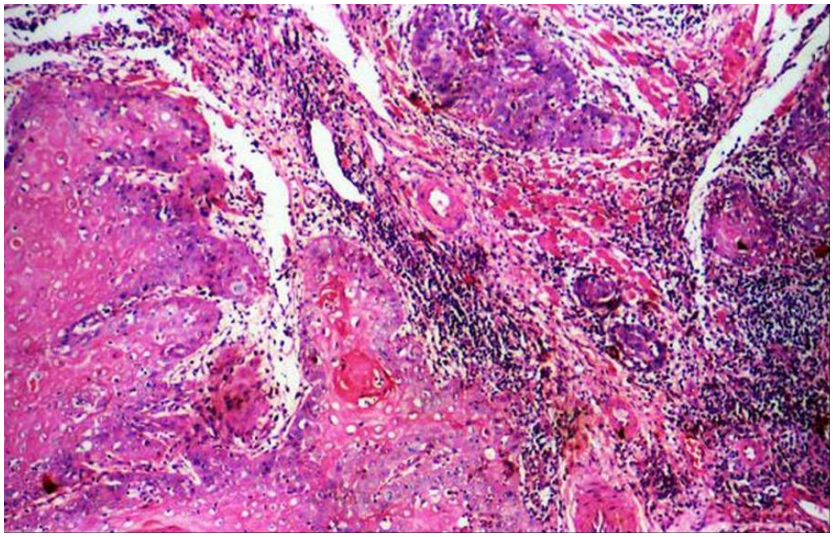

Fig 6: H&E-Stained Section of well differentiated SCC

CONCLUSIONS

The numbers of mast cells were considerably more in moderately differentiated squamous cell carcinoma as compared to well-differentiated squamous cell carcinoma and control group. The sum of all the three types of mast cellsi.e typical, atypical, and granular is more in case of moderately differentiated squamous cell carcinoma than well-differentiated squamous cell carcinoma. An increased number of TMCs, AMCs, and GMCs in more inflamed areas add to the effective involvement of mast cells in several stages of the inflammatory activity manifested by their degranulation. This explicitly suggests that mast cell count/density might be utilized as indicators for disease advancement in oral squamous cell carcinoma.This assessment reveals that there is a decisive increase in mast cells count when contrasted with normal mucosa confirming their contributing role in tumor progression.

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