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Oncology

Comparative analysis of mast cell density in various grades of Oral Squamous Cell Carcinoma – A trial study

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ABSTRACT The commonly occurring oral neoplasm – Oral Squamous Cell Carcinoma (OSCC) which is being associated with the chronic inflammation in the adjacent connective tissue. Mast cells are the local residents of the connective tissue and they are considered as complex and multifunctional cells, playing a major role in immunopathology and a substantial role in tumor angiogenesis. The purpose was to compare the Mast Cell Density (MCD) in Normal oral mucosa and among the different grades of OSCC and to validate the role of mast cells in tumour progression of OSCC. Assessment of was done using the 1% Toluidine blue stain in a study sample of 24 cases of different grades of OSCC and 6 cases normal oral mucosa as controls. The MCD was statistically analysed and the results revealed a statistically significant increase in mast cell count in Well Differentiated OSCC than other grades of OSCC and controls.

KEYWORDS : mast cells, immunopathology, tumor angiogenesis, toluidine blue

INTRODUCTION:

The sixth most common cancer in the world is Oral Squamous Cell Carcinoma (OSCC). It is one of the leading causes of death in India. The molecular biological markers of OSCC have been extensively studied to aid in the prevention and the prognosis of OSCC. However, no marker has been universally accepted so far.[3]

By the time of detection of oral cancers, approximately 60% of them are very well advanced. Despite innovation being made in various treatment modalities, the long term survival rate for OSCC remains lesser than 50 percent.[3]

By the time oral cancer is diagnosed, most individuals have localized or regional disease (37% localized; 43% regional; 10% distant and 10% unstaged). Five year survival rates for all oral cancers cases are 79% for those with localized disease, 42% for regional disease and 19% for disease with distant metastases. Development of oral cancer proceeds through discrete molecular genetic changes that are acquired from loss of genomic integrity following continued exposure to environmental risk factors.[11]

These genetic changes generate concomitant phenotypic changes in the tumor cells allowing them to continually survive, spread out and having an ability to invade surrounding tissues and metastasize.[4,31]

Angiogenesis or neovascularization is the growth of new blood vessels from pre-existing ones. This is an important component in many biological processes, both in physiological conditions (proliferating endometrium and embryogenesis) as well as pathological conditions (rheumatoid arthritis and neoplastic diseases). This is a complex phenomenon that is absolutely required for the continued growth and survival of neoplasms.[19,34]

The progression and the metastasis of malignant tumours of the lung,[38] breast,[40] oesophagus[10,15] and the oral cavity.[5,13,16,20,28,36,41] are known to be aided by angiogenesis. As described in melanomas, breast carcinomas and other malignant neoplasms, the fact is that tumors are angiogenesis dependant and metastatic cells are only shed after tumor establishes its microcirculation.[12]

It has been proposed that Angiogenesis is a central process in many human malignancies and being regulated by a balance between angiogenic stimulators and inhibitors.[18]

The "angiogenic switch" depends on net balance of positive and negative angiogenic factors in the tumor. Thus, the angiogenic phenotype may result from the production of growth factors, such as FGF-2 and VGEF, by the tumor cells and/or the down-regulation of negative modulators in tissues with quiescent vasculature like TSP-1.[24]

Among the various host immune cells, the mast cells have been implicated in tumour progression because they promote angiogenesis.[5,10,13,16,20,28,36,37,41]

Mast cells are normally present in small numbers in the connective tissue of all organs and more particularly in the dermal layer of skin (around blood vessels and nerves), of size ranging from 5 to 15 μm in diameter and in histologic sections often appear ovoid, tadpole, or spindle shaped cells with cytoplasmic granules of 0.2 to 0.5 cm in size.

Mast cells exert their influence both locally and systemically by releasing a variety of potent mediators such as histamine, leukotrienes, and cytokines through degranulation. They cause neovascularization by releasing angiogenic mediators such as fibroblast growth factor (FGF), transforming growth factor-β (TGF), tumor necrosis factor- β (TNF), and vascular endothelial growth factor (VEGF). These factors are also occurring in various pathological states and also in some benign and malignant tumors.[23,32,35]

To examine the relationship between the mast cell density and the histological grades of OSCC, we analyzed the mast cell density (MCD) in different grades of OSCC and compared it with that of the normal mucosa by using the 1% Toluidine blue stain as the mast cells stain metachromatically with Toluidine blue.[33]

Materials and methods:

Formalin-fixed, paraffin-embedded tissue specimens of 25 cases of OSCC (10 well differentiated OSCC, 10 moderately differentiated OSCC, and 4 poorly differentiated OSCC) were retrieved from the archives.

Six cases of normal gingival tissues were included in the study as controls. The relevant information regarding the clinical parameters was obtained from the records of the patients.

The cases which were diagnosed clinically and confirmed by histopathological means as squamous cell carcinoma alone were

included in the study. These cases were graded histologically into well differentiated, moderately differentiated and poorly differentiated squamous cell carcinomas.

In brief, 5 µm sections of formalin-fixed, paraffin blocks were deparaffinized with xylene and they were rehydrated with graded alcohols. The sections were stained with 1% Toluidine blue, mounted with DPX and observed under a microscope. The stained sections were studied for metachromasia, which was taken as a positive identification of the mast cells. Toluidine blue stains the mast cell granules metachromatically due to its reaction with sulphated mucopolysaccharides.[32] Sections of neurofibromas were used as the positive controls for the mast cells.

The slides were studied under a light microscope. The mast cell granules stained brilliant red/purple and the background stained in different shades of blue.

For the determination of the Mast Cell Density (MSD), the stained sections were screened at low power (10X) to identify the areas of the hot spots. A mast cell count was performed at the high power (40X) magnification in three randomly chosen fields in the hot spot areas. The mast cell count was expressed as the number of mast cells per high power field. The average figures which were obtained in the counted hot spot fields were considered as MCD for a given case. All the counts were performed by a single investigator who had the knowledge of the clinical or the histopathological variables, to eliminate an interobserver variation.

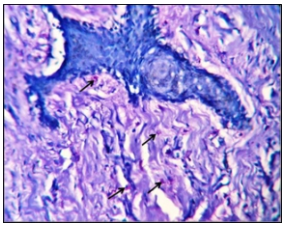

Figure.1
Toluidine blue stained normal gingival tissue section under 40X magnification showing mast cells

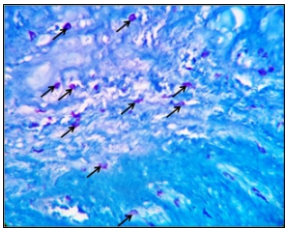

Figure.2
Toluidine blue stained section of WDSCC under 40X magnification showing more number of mast cells

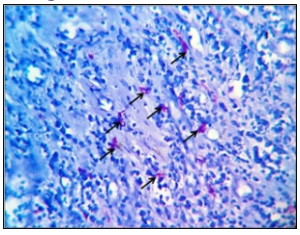

Figure.3
Toluidine blue stained section of MDSCC under 40X magnification showing mast cells

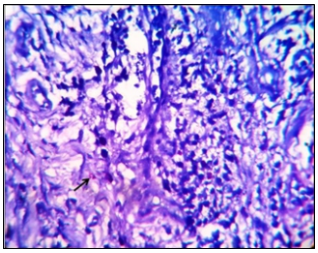

Figure.4
Toluidine blue stained section of PDSCC under 40X magnification showing less number of mast cells

Results:
Mean value of Mast Cell Density (MCD) were studied (Table 2). MCD among various grades of OSCC with and without controls studied (Table 3 & 4) using ANOVA. Comparison of MCD between any Two Grades of Oral Squamous Cell Carcinoma including Normal Tissue studied using Student's-t test (Table 5).

Histopathological Diagnosis	No. of Cases
Normal	6
Well differentiated squamous cell carcinoma	10
Moderately differentiated squamous cell carcinoma	10
Poorly differentiated squamous cell carcinoma	4
Total	30

Table. 1
Distribution of Cases by Histopathological Grading of OSCC and Normal Tissue.

Histopathological Diagnosis	Mast Cell Density (Mean±SD)		
Normal	10.83	±	4.63
Well differentiated SCC	19.19	±	10.89
Moderately differentiated SCC	4.45	±	3.16
Poorly differentiated SCC	2.81	±	1.74

Table.2
MCD in Different Histopathological Grades of OSCC and controls.

WDSCC (N = 10)			MDSCC (N = 10)			PDSCC (N = 4)			F value	p Value
Mean	±	SD	Mean	±	SD	Mea	±	SD		
19.19	±	10.89	4.45	±	3.16	2.81	±	1.74	12.21	< 0.01

Table.3
Comparison of between Different Grades of Oral Squamous Cell Carcinoma (not including controls)

Normal (N = 6)			WDSCC (N = 10)			MDSCC (N = 10)			PDSCC (N = 4)			F value	p Value
Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD		
10.83	±	4.63	19.19	±	10.89	4.45	±	3.16	2.81	±	1.74	9.24	< 0.01

Table .4
Comparison of MCD between Different Grades of Oral Squamous Cell Carcinoma (including controls)

Normal (N = 6)			WDSCC (N = 10)			MDSCC (N = 10)			PDSCC (N = 4)			F value	p Value
Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD		
10.83	±	4.63	19.19	±	10.89							-1.77	Ns
10.83	±	4.63				4.45	±	3.16				3.29	< 0.01
10.83	±	4.63							2.81	±	1.74	3.26	< 0.05
			19.19	±	10.89	4.45	±	3.16				4.11	< 0.01
			19.19	±	10.89				2.81	±	1.74	2.92	< 0.05
						4.45	±	3.16	2.81	±	1.74	0.96	Ns

Table.5
Comparison of MCD between any Two Grades of Oral Squamous Cell Carcinoma (including controls)

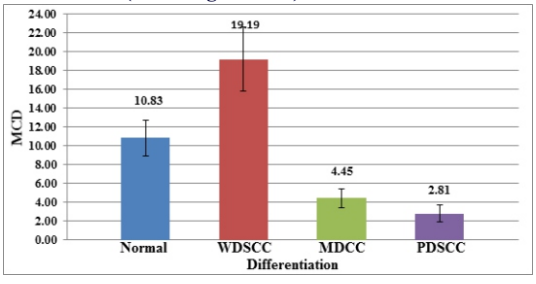

Figure.9
Bar diagram representing Mast Cell Density in normal and different grades of OSCC

Discussion:
Mast cell was discovered by Paul Ehrlich in 1877 as a granular cell of

loose connective tissue and he named it as "Mastzellen"— a well fed cell. Studies on these cells have shown them to be complex, multifunctional and well-engineered cells playing an essential role in acquired and innate immunity. They take origin from multipotent CD 34+ precursor in the bone marrow, later circulate in the peripheral blood as agranular monocytic cell, and then migrate into tissues, assuming their typical granular morphology from their immature state. They are being normally distributed throughout the connective tissue, adjacent to blood or lymphatic vessels, and also near or within peripheral nerves. They are more in number especially beneath the epithelial surfaces of the skin, in the respiratory system, gastrointestinal and genitourinary tracts. Many of the mediators are stored within cytoplasmic granules of mast cells. They include preformed mediators like histamine, heparin, and tryptase; lipid derived mediators like leukotriene's B4 (LTB4), LTC4, LTD4, and LTE4; proinflammatory cytokines like TNF- , IL-1; mitogenic cytokines like IL-3, IL-5; and immunomodulatory cytokines like IL-4, IL-10, and serotonin and other mediators are produced at the time of mast cell stimulation such as IL-1.[1,17]

Mast cells have been considered the tissue equivalent of the circulating basophils though they arise from a common precursor cell in the bone marrow. But there is no evidence that mature basophils are able to differentiate into mast cells. The two cell types are readily distinguished by their morphology on light microscopy and the presence of chloroacetate esterase activity in mast cells. They have been studied in normal gingiva, chronic inflammatory gingivitis, desquamative gingivitis, lichen planus, oral submucous fibrosis (OSMF), and OSCC. They exhibit phenotypic plasticity and variation in the mast cell mediators with the change in the microenvironment. Studies have shown that in some malignancies, large numbers of mast cells were detected before the occurrence of neovascularisation.[21,35]

A sustained tumour growth requires a positive balance between the tumour cell proliferation and cell death or apoptosis. Experimental animal model have shown that the initiation of angiogenesis appeared following a decrease in the tumour cell apoptosis, while the levels of the tumour cell proliferation remained constant, thus leading to the net tumour growth.[20] The preinvasive malignant cells are known to remain dormant until they become angiogenic, and this is followed by a rapid phase of tumour growth.[22,30]

A solid tumour growth is dependent upon an adequate blood supply which is achieved by the generation of stroma where the formation of capillaries is a central event and it is also gives an entry site for the immune inflammatory cells. In other words, in a particular tumour, the number of the micro vessels and the mast cells could be related to the amount of the stromal component. Due to this reason, the variations in the amount of stroma and the tumour cells may influence the average number of the mast cells.[38]

The mast cell density in a tissue was studied by using histochemical stains like toluidine blue[10] and alcian blue[38] and immuno histochemically[14] by using mast cell tryptase, heparin, chymase, and carboxypeptidase A. Sudhakar R et al.[33] found an inverse relationship between the mast cells and their vascularity and inflammation, in oral inflammatory lesions.

In the present study, the correlation between MCD and the progression of oral squamous cell carcinoma from well differentiated to poorly differentiated revealed a linear decrease in the MCD, thus suggesting a negative correlation between them. However, if the presence of the mast cells was the key factor in the angiogenesis, there would have been an exponential increase rather than a decrease, thus indirectly suggesting the role of other factors that could have modulated the angiogenesis.

In the present study, Mast cell Density (MCD) among various grades of OSCC with and without controls was studied using ANOVA which revealed highly significant difference ($P < 0.01$) in MCD. Student's 't' test revealed highly statistically significant difference ($P < 0.01$) in MCD between controls and MDOSCC and between WDOSCC and MDOSCC. A significant ($P < 0.05$) difference in MCD between controls and PDOSCC and between WDOSCC and PDOSCC. No statistical differences ($P > 0.05$) were found in MCD between controls and WDOSCC and between MDOSCC and PDOSCC.

In carcinogenesis, angiogenic regulation is biphasic. In the early premalignant phase of hyperplasia and dysplasia, infiltrating mast cells degranulate and activate dermal fibroblasts which intensify angiogenesis. They also activate progelatinase B (a member of the matrix metalloproteinase (MMP) family) which is involved in both extracellular remodeling[7] and regulation of angiogenesis.[39]

By releasing sequestered angiogenic activators the mast cells activate and progressively intensify angiogenesis. As neoplastic progression proceeds, angiogenic growth factor gene expression is upregulated in the cancer cells, giving progression to the second cancer phase, wherein the tumor cells control their angiogenic phenotype directly instead of depending on the inflammatory cells to indirectly affect neovascularisation.[9,8,25,29,27]

This implies that mast cells have significant role in the early stages of cancer progression and increase in mast cells is observed in the initial stages whereas, in second cancer phase, the mast cells decrease because the tumor cells are not dependent on them anymore for the neovascularization effect and this could be the reason for the decrease in the mast cells in moderate when compared to well-differentiated OSCC in our study and also might be due to interobserver mystification in classifying the well and moderate differentiated forms of OSCC when compared to poorly differentiated.

The progressive depletion of the mast cells in the present study from well and moderately differentiated OSCC to poorly differentiated OSCC, could be probably because the mast cells may have been degranulated as the disease progressed. The lack of mast cell granules in the advanced disease states may have resulted in the negative staining with Toluidine blue. The ultra structural observation of the mast cells in different stages of degranulation and their progressive reduction and disappearance in the advanced states were substantiated by Rajendran R et al.[26] in OSMF and by Clamon N H et al., in GVHD.[6]

The outcome of present study is in accordance with Anuradha A et al.[2] who found that a remarkable increase in mast cell count in well differentiated OSCC than normal tissue and a decrease in mast cell count in both moderately and poorly differentiated OSCC.

Our results were in concordance with those of Coussens LM et al.[8] who studied on role of the mast cells during squamous epithelial carcinogenesis in a mouse model. They observed that the stroma in poorly-differentiated carcinoma was devoid of mast cells. They have also suggested that the angiogenic regulation in squamous carcinogenesis was biphasic tumour growth pattern..

Conclusion:

This study reveals that there is a definitive increase in mast cells density in well differentiated squamous cell carcinoma when compared to normal mucosa substantiating their contributing role in tumor progression. Decrease in mast cells in poorly differentiated OSCC reflects important modification in the microenvironment.

Even though exact functional relevance is unclear, Evidences substantiate that mast cells may induce the tumour progression by providing mitogenic stimulation or angiogenesis. MCD may be used as an indicator of the progression by helping in delineating a risk population, which might benefit from an attractive adjuvant therapeutic strategy for OSCC.

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