

A Study on Assessment of Mast Cells in Oral Squamous Cell Carcinoma

Zaidi MA, Mallick AK¹

Department of Oral and Maxillofacial Pathology and Microbiology, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, ¹Department of Biochemistry, Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India

Address for correspondence:

Dr. Meesam Abbas Zaidi,
Department of Oral and Maxillofacial Pathology and Microbiology,
Uttaranchal Dental and Medical Research Institute,
Mazri Grant Near Doliwala,
Haridwar Road, Dehradun - 248 001,
Uttarakhand, India.
E-mail: meesam.az@gmail.com

Abstract

Background: Apart from the role of mast cells in maintenance of homeostasis and inflammation, their association with tumors has been described recently. In several malignancies, mast cell density has been found to correlate with angiogenesis, increased risk of metastasis and poor prognosis. **Aim:** The aim of the following study is to compare the number, topography and distribution of mast cells between normal oral mucosa and oral oral squamous cell carcinoma (SCC) and study the significance of mast cells in development of oral SCC. **Subjects and Methods:** A prospective case-control study including 100 patients was conducted after obtaining informed consent and ethical committee clearance. Forty cases were normal controls and 60 cases had oral SCC. Biopsy was performed and both qualitative and quantitative study of mast cells was done. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) 17.0 version (SPSS Inc., Chicago, IL, USA). **Results:** Buccal/labial mucosa was the most common site of lesion in SCC. Total mast cells count was a higher in SCC when compared with controls, which was a statistically significant ($P < 0.001$). SCC had significantly ($P < 0.001$) higher levels of degranulated mast cell. **Conclusion:** Role of mast cells in tumors may have direct clinical relevance and consequently, important clinical implications. Mast cells serve as a novel therapeutic target for cancer treatment and that inhibiting mast cell function may inhibit tumor growth.

Keywords: Mast cells, Oral squamous cell carcinoma, Toluidine blue stain

Introduction

Oral squamous cell carcinoma (SCC) is the most frequent malignancy in the mouth, corresponding to 95% of all oral malignant lesions.^[1] It progresses in a multistep fashion initially from normal epithelium, hyperkeratosis, pre-malignant dysplasia and carcinoma *in situ* to invasive carcinoma. In spite of vast advance in the field of cancer research, availability of sophisticated diagnostic techniques and improved therapeutic options prognosis of patients presenting with this type of tumor still remains very poor. This is probably due to unpredictable behavior of these

tumors, which show a variable aggressiveness independent of clinico-pathologic and histological grading.^[2]

Mast cells are mobile secretory cells containing granules which are distributed around the microvascular endothelium in the oral mucosa and dental pulp. They have diverse biological functions which include phagocytosis, antigen processing, production of cytokines and release of variety of preformed mediators (e.g., histamine, proteoglycans and proteases) and newly formed physiological mediators (e.g., leukotrienes and prostaglandins). Mast cells carry an array of adhesion molecules, immune response receptors and other surface molecules, which permit them to react to multiple specific and non-specific stimuli. This wide range of biological function, ubiquitous distribution and strategic location near blood vessels, nerves, inflamed tissues and neoplastic foci enable mast cells to play a central role in a multitude of physiologic, immunologic and pathologic processes.^[3] Recently, apart from their roles in the maintenance of homeostasis and in inflammation, the association of mast cells with various tumors has been described.^[4] In several malignancies, mast cell density

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DOI:
10.4103/2141-9248.133479

has been found to correlate with increased risk of metastasis and poor prognosis.^[5] Currently, the exact functional relevance of mast cells surrounding various tumors is being studied.^[6] Although, it has been suggested that mast cells are important in connective tissue disease and allergic inflammatory disease, the functional significance of mast cells in tumor locations is still not clearly understood.^[7]

As oral SCC is associated with chronic inflammation in adjacent connective tissue, immune reaction and angiogenesis with the progression of dysplastic changes, there is a need to evaluate the role of mast cells in it. Therefore, this was done to compare the number, topography and distribution of mast cells between normal oral mucosa and oral SCC and study the significance of mast cells in development of oral SCC.

Subjects and Methods

This prospective case control study was carried out on 100 patients, which were divided into two groups, consisting of 40 controls having a normal oral mucosa and 60 cases of SCC. Forty males and 20 females suffering from oral SCC were included in the study [Table 1]. The mean age of normal controls was 43 years ranging from 21 to 70 years and that of cases was 52 (9.2) years ranging from 30 to 75 years. Informed consent and Ethical Committee clearance was obtained for the study. All patients with systemic disorders, who were likely to have complications during the biopsy procedure, were excluded from the study. After selection of suitable patients, site for biopsy was selected and incisional biopsy was performed under aseptic conditions and local anesthesia. Biopsy specimens were preserved in 10% neutral buffered formalin solution.

The biopsied tissue of the study groups were processed and embedded in paraffin wax. The paraffin blocks were sectioned with a rotary manual soft-tissue microtome into two tissue sections of 5 µm thickness. Sections were stained with hematoxylin and eosin and categorized to the particular groups. Specific staining for mast cells was carried out using 1% toluidine blue in 1% sodium chloride solution. In brief, 5 µm thickness of paraffin blocks were deparaffinized with xylene and then rehydrated with graded alcohols. The slides were stained with 1% toluidine blue, mounted with dibutyl phthalate xylene and observed under a microscope. The stained sections were studied for metachromasia, which was taken as a positive identification of 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.

Mast cells were counted using an Olympus BX51 (Olympus Optico Co. LTD, Japan) trinocular light microscope with provision for photomicrograph with Olympus E331 SLR digital camera (Olympus Optico Co. LTD, Japan). Cell count was done in an oculometer grid under a magnification of $\times 40$. The toluidine blue stained sections were first screened at low power ($\times 10$). Mast cell counting was then performed under $\times 40$

magnification. The area of the microscopic field was calibrated with an ocular grid fitted inside the eyepiece with an area of 0.04 mm². The mast cells were counted throughout each of the tissue sections in 10 representative and consecutive grid fields ($\times 40$ magnification). The mean of 10 values was calculated and expressed as mean (standard deviation) per mm². The field was studied in a step ladder fashion and care was taken to prevent the overlapping of fields. The cells extending over the left and bottom sides of the grid were considered to be within the grid.

Based on the intensity of metachromasia, mast cells were categorized into two groups:

1. Intact mast cells exhibiting intense metachromasia and dense granules obscuring the nucleus
2. Degranulated mast cells with less intense metachromasia and a clear outline of the nucleus.

Aggregations of externalized mast cell granules not obviously associated with mast cells were excluded from the analysis.

Statistical analysis of the data was carried out using the Statistical Package Social Sciences (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was studied using the independent 't' test. $P < 0.05$ was considered as statistically significant.

Results

In this study, the total number of mast cells was found significantly increased in oral SCC (57.25 [10.38] per mm²) in comparison to normal controls (22.75 [12.65] per mm²). Cases with oral SCC showed significantly higher degranulated mast cells when compared with healthy controls [Table 2, Figure 1]. All these changes were highly significant ($P < 0.001$).

Discussion

Mast cells have long been considered to play a specific role in pathophysiology of many diseases. Release of mast cell mediators has been thought to contribute to tissue injury and

Table 1: Oral squamous cell carcinoma cases and normal cases

Study group	Total no. of cases	Males	Females
Squamous cell carcinoma	60	40	20
Control (normal oral mucosa)	40	28	12

Table 2: Intact, degranulated and total mast cells in cases and controls

Type of cells	Normal oral mucosa	Squamous cell carcinoma
Intact mast	13.9 (6.87)	24.5 (7.09)*
Degranulated mast	8.15 (5.46)	32.35 (10.29)*
Total mast	22.75 (12.65)	57.25 (10.38)*

* $P < 0.001$

inflammation. Recent data have shown that inflammation is a critical component for tumor progression.^[8]

Tumor genesis is a multi-step process in which interaction between genetically altered malignant cells and surrounding non-neoplastic cells appear to play a vital role. Various factors, like tumor derived peptides as well as regulated on activation normal T cell expressed and secreted or monocyte chemotactic protein-1, released during the development of the tumor may induce the chemotactic migration of mast cells.^[4] Some researchers suggest accumulation of mast cells is a part of a general immunological host defense mechanism since mast cells have been shown to be cytotoxic for some tumors.^[9,10] However, some studies have concluded that there is a possibility that mast cell mediators promotes tumor growth and metastasis.^[11-13] Mast cells associated with the tumor have been found to undergo degranulation and release of granular components such as heparin and histamine, which potentiates endothelial cell migration and proliferation and to induce adhesion molecule expression on epithelial cells, potentially leading to increased tumor angiogenesis and metastasis.^[14] Mast cells are also rich sources of proteases, specifically tryptase and chymase, which by their proteolytic activities, directly degrade extracellular matrix components or release matrix associated growth factor, thereby indirectly stimulating angiogenesis and facilitating invasion and metastasis by extracellular matrix remodeling. Matrix metalloproteinases are also produced by mast cells and may contribute to extracellular matrix degradation. Thus, mast cells may have an impact on both primary tumor development and subsequent tumor progression and metastasis.^[15,16]

The most common site of SCC in our study was the buccal mucosa [Figure 2]. Huang *et al.*, in their study, reported male with buccal mucosa as the commonly affected site because of frequent exposure to carcinogens.^[15] Although Oliveira-Neto *et al.* showed a decrease in the number of mast cells in oral SCC our study shows that the mean density of mast cells/mm² were significantly increased in cases with oral SCC ($P < 0.05$) when compared with normal controls [Table 2, Figures 1 and 3].^[17] This was in accordance with the study of Iamaroon *et al.* and Markopoulos and Antoniades. They suggested that mast cells release potent angiogenic factors like tryptase which plays a significant role in angiogenesis of oral SCC.^[11,18]

Although, not many researches have been conducted in humans to study the significance of the degranulation of the mast cells in cancer progression, the studies conducted on animal models suggests that degranulation is critical in the ability of mast cells to enhance tumor growth. Inhibition of mast-cell degranulation using disodium cromoglycate has been shown to impede tumor growth.^[19] In our study cases with oral squamous cell cancer showed significantly increased degranulation of mast cells ($P < 0.05$). Walsh *et al.* elicited that mast cell degranulation is a common feature of inflammatory lesion.^[16] The involvement of mast cells and their proteases in progression of cancer has implications for the pathogenic mechanism and potential

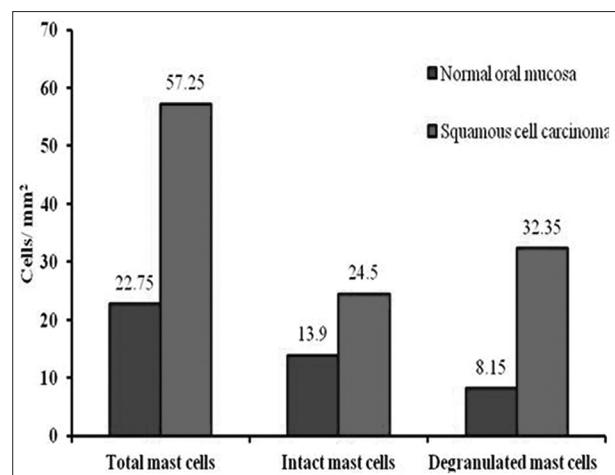


Figure 1: Bar chart showing the mean total, intact and degranulated mast cells in cases and controls

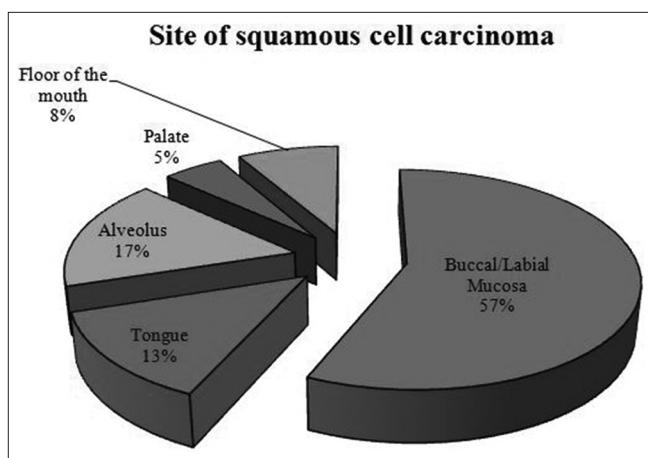


Figure 2: Diagram showing the site of oral squamous cell carcinoma

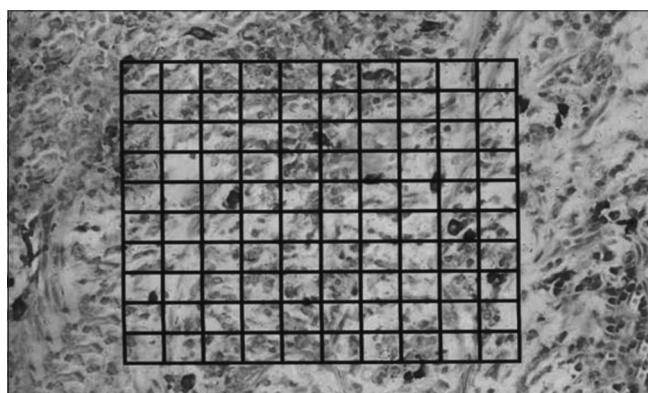


Figure 3: Photomicrograph showing mast cells infiltrated into the connective tissue with ocular grid fitted inside the eyepiece (x40)

therapeutic intervention in oral malignancy, in particular for anti-angiogenic strategies aimed at arresting neoplastic progression prior to the emergence of overtly malignant tumors. However, studies with larger samples and better methods for identification of mast cells like the ultra-structural study can increase the accuracy of the findings. Furthermore, there is a

need to study the role of mast cell stabilizers in preventing the progression of lesion. As mast cells play a vital role in chronicity of inflammation which eventually leads to oral SCC, therapeutic intervention to influence the mast cell secretion should be considered at early precancerous stage. Deeper understanding of mast cell activation mechanisms, immunomodulatory capacity and proangiogenic potential will open new perspectives on the development of future therapeutic strategies targeted at such multifunctional cells.

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How to cite this article: Zaidi MA, Mallick AK. A study on assessment of mast cells in oral squamous cell carcinoma. *Ann Med Health Sci Res* 2014;4:457-60.

Source of Support: Nil. **Conflict of Interest:** None declared.