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Role of Mast cell in Oral lesions

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Abstract
Background: *The present study was carried out for quantitative analysis of the mean MCC/optical field and MCD/sq. mm in the benign, premalignant and malignant oral lesions.*
Objective: *To analyse the role of mast cell in tumorogenesis.*
Study Design: *This study was carried out in the Department of Pathology, RNT Medical College, Udaipur. Punch biopsy of various oral lesions were selected. The sections were obtained and stained with Hematoxylin and Eosin and Toluidine blue. The number of mast cells/optical field and mast cell density/sq. mm were assessed. The following conclusions were drawn from our study.*
Result: *An increase in both mean MCC/optical field and MCD/sq. mm was found as the disease progress from benign to premalignant and to malignant oral lesions. This increase in the values was statistically significant between benign v/s malignant and premalignant v/s malignant. However value was found to be non-significant between benign v/s premalignant.*
Keywords: *Mast cells, Mast cell count, Mast cell density, Oral squamous cell carcinoma.*

INTRODUCTION

Oral cancer is a global health problem. It is the sixth most common cancer in the world and accounts for 2 to 4% of malignancies in the West, but in the Indian subcontinent it accounts for 40% of all cancers [1]. Oral cancers include cancers of the lip and the oral mucosa (buccal mucosa, gums, soft and hard palate, tongue and the floor of the mouth). A significant proportion of oral squamous cell carcinoma develops from the premalignant lesions such as leukoplakia and oral submucous fibrosis. The primary aim in the management of premalignant is the early diagnosis and prevention of malignant transformation.

The role of angiogenesis in neoplasia has been receiving increasing attention in recent times, since it can be used as independent prognostic indicator for tumour progression and metastasis. The induction of angiogenesis is mediated by several stimulatory and inhibitory molecules which are released by both the tumour and the host cells and it depends on a net balance between the stimulatory angiogenic and the inhibitory anti-angiogenic factors [2].

Among the various host immune cells, the mast cells have been implicated in tumour progression because they promote angiogenesis. There is considerable controversy with regard to the number and distribution of the mast cells in

clinically normal oral tissues as compared to the inflamed and diseased tissue.

Mast cells arise from a multipotent CD 34+ precursor in the bone marrow and circulate in the peripheral blood as agranular, monocytic appearing cells. They are small cells, round to ovoid in shape with a diameter of 12 – 15 microns and are packed with numerous cytoplasmic granules of 0.2-0.5 microns in size.

As far as oral diseases are concerned, mast cells have been implicated in variety of diseases like periapical lesions, oral submucous fibrosis, odontogenic cysts, gingivitis, and pyogenic granulomas [3].

Mast cells exert their influence locally and systemically by releasing a variety of potent mediators through degranulation [4].

Hence a histochemical study using toluidine blue stain was conducted to quantify the number of mast cells in various oral lesions and to signify their role in tumour growth and progression.

MATERIAL AND METHOD

The present study was conducted on two hundred patients presenting with oral lesions in the ENT Outpatient Department and Histopathology section of Pathology Department, RNT Medical College, Udaipur, in the year 2012- 2013.

Patients with suspicious oral inflammatory lesions, potentially malignant and malignant lesions, such as leukoplakia, erythroplakia, palatal erythema, actinic cheilosis, erosive lichen planus and oral carcinoma were selected irrespective of their age and gender.

Punch biopsies of the oral lesions taken after thorough oral and general physical examination in the ENT department were sent in 10% formalin fixative solution to the histopathology section. The sections were standardized by maintaining the thickness at 3-4 microns. One set of sections were stained by Harris hematoxylin and eosin for histopathological diagnosis. The other section was stained with 1% toluidine blue for mast cells.

The stained sections were examined under binocular research light microscope "LABOMED".

Mast cells were counted in 10 random high power fields (40X) having larger number of mast cells, and average per high field was determined [5]. Further the mast cell count was expressed per sq mm using the following formula:

Radius of one high power field (40X) - 0.235 mm (measured with the occulo-micrometer).

Area - $22/7r^2$

Area of the field - $22/7x\ (0.235)^2$ approximately 0.2 sq mm.

Suppose number of mast cells counted per one high power field i.e. in 0.2 sq mm is A

Mast cell density i.e. number of mast cells per sq mm = 5 x A

The results were then subjected to statistical analysis for obtaining significance value using't' and 'z' test.

RESULTS

Out of 200 patients included in this study, 67 patients (33.5%) had benign lesion, 16 patients (8%) had premalignant lesion and 117 patients (58.5%) had malignant oral lesions. Amongst the malignant group, 55 patients (27.5%) had well differentiated squamous cell carcinoma while 62 patients (31%) had moderately keratinizing squamous cell carcinoma.

Majority (76.5 per cent) of the patients were adults (31-70 years), however, the age ranged from 10 – 90 Years. Males (77.0 per cent) were predominantly affected with male to female ratio of 3.3:1. The most commonly affected site was tongue (35.5 per cent) followed by buccal mucosa. Tobacco chewing and smoking were found to be equally responsible for both benign and malignant lesions. The patient with benign lesions were more commonly found in the age group of 21-50 years. Those with premalignant lesions showed an equal predilection in the 5^{th}, 6^{th} and 7^{th} decade of life (25%) whereas most of the oral lesions (58.5 per cent) in our study were malignant and were common in the age group of 41-50 years.

Staining of mast cells was carried out using Toluidine blue and enumeration of mast cells was done. Mast cells visible in each optical field (40X) were individually counted and the total number of mast cells per optical field was determined. Further the mast cell count was expressed per sq. mm. The mean mast cell count (MCC) per optical field and mast cell density (MCD) per sq. mm was calculated for benign, premalignant and malignant oral lesions. All the values were expressed in terms of mean ± Sd. The results obtained were tabulated and subjected to statistical analysis.

Mean mast cell count/optical field was found to be 4.039 in benign lesions, 4.050 in pre malignant lesions and 7.215 in malignant lesions. Mast cell density also showed a step wise increase as the lesion progressed form benign (20.194) to premalignant (20.250) to malignant lesion (36.077).

The statistical analysis in the present study revealed p value to be non-significant between inflammatory and hyperplastic V/s dysplasia (p = 0.972). However p value was found to be highly significant between inflammatory and hyperplastic V/s malignant (p=0.000); dysplasia V/s malignant (p=0.000); and between well differentiated squamous cell carcinoma V/s moderately differentiated squamous cell carcinoma (p=0.000).

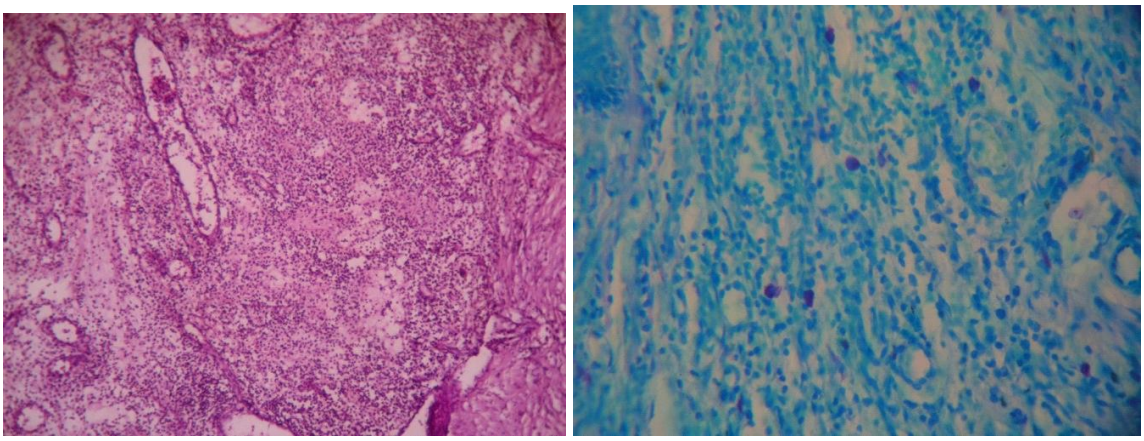

Figure1: (A) Microphotograph showing inflammatory oral lesion (H and E X 100).
(B) Mast cells admixed with dense lymphocytic infiltrate (Toluidine blue X400).

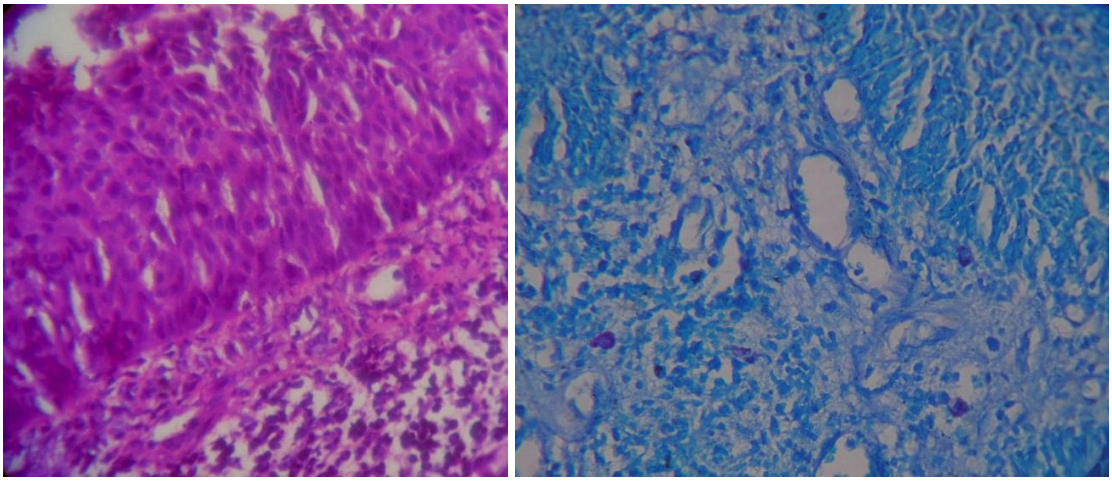

Figure 2 : (A) Dysplastic squamous epithelium (H and E X 100).
(B) Toluidine blue stained section showing mast cells and dysplastic squamous epithelium (Toluidine blue X 400).

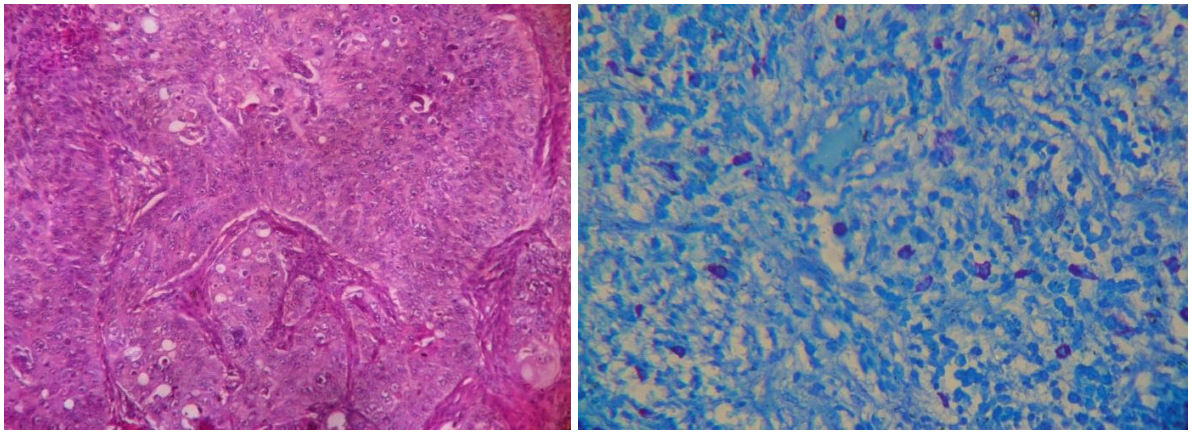

Figure 3: (A) Microphotograph showing squamous cell carcinoma (H and E X 100).
(B) Hot spot showing numerous mast cells in squamous cell carcinoma (Toluidine blue X 400).

DISCUSSION

Mast cells were first described in 1878 by Paul Ehrlich as metachromatically-staining basophilic cells found in connective tissues [6] as fixed elements implicated in "tissue tropism". Mast cells are being currently regarded as key effector cells in tissue vascularization, remodeling and homeostasis.

Mast cells are ubiquitous cells that are dispersed throughout in almost all the major organs of the body. They are large spherical or elliptical mononuclear cells, mobile bone marrow-derived elements having a diameter of about of 12 microns. They have a life span of weeks to months [7]. These cells are found particularly in association with connective tissue structures such as blood vessels, lymphatic vessels, nerves and in proximity to surfaces that interface the external environment such as those of the respiratory, genitourinary, gastrointestinal system and the skin. Mast cells typically contain 80-300 granules that originate from hematopoietic stem cells [8].

Mast cells release low molecular weight substances, such as histamine, as well as cytokines and chemokines that exert profound effects on inflammation and cancer [9]. Mast cells also produce a variety of lipid & protein alpha mediators with pro-inflammatory activities including chemotactins, cell activating & cell growth factor.

Histamine released from mast cells can induce tumour proliferation through H1 receptors and suppress the immune system through H2 receptors. Both may be involved in human carcinogenesis [10].

Angiogenesis is a critical process in tumour progression because the vascular network produced by the host is essential to allow neoplastic cell populations to form a clinically observable tumour [11].

Mast cells are an abundant source of angiogenic factors [12]. Under physiological conditions, mast cells are particularly prominent in the close vicinity of capillaries and lymphatic channels. In many inflammatory disorders characterized by a profound vascular remodeling, the infiltrate exhibits numerous mast cells that show the structural features of degranulating elements. In various tumour models, mast cells appear at the edges of invasive tumours, where they facilitate angiogenesis by releasing preformed mediators or by triggering proteolytic release of extracellular matrix-bound angiogenic compounds.

The role of mast cells in bringing about neo-angiogenesis has been studied in oral squamous cell carcinomas and a positive relation has been shown [13].There is also evidence that these cells, by regulating angiogenesis, can influence growth and progression in human cancer [4].

The present study showed mean mast cell count / optical field in benign oral lesions was found to be 4.039, in pre malignant lesions 4.050 and in malignant lesions 7.215. The findings was consistent with the study of Kinra et al [3] who

also showed an increase in both mean MCC/optical field and MCD/sq. mm in oral submucous fibrosis, oral leukoplakia, oral lichen planus and oral squamous cell carcinoma.

The mast cell density in the present study also showed increasing trend from benign (20.194) to premalignant (20.250) to malignant lesions (36.077).

Similar results were observed in the study carried out by Iamaroon et al [13]. The density of mast cells appeared to increase with disease progression. The mast cell counts were significantly higher in oral squamous cell carcinoma than in hyperkeratosis and normal oral mucosa.

In our study the mean mast cell count of 5.658 and 8.597 was observed in well differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma. These findings were in consonance with the study carried out by Sharma et al [14] and Anuradha et al [15]. The latter study revealed 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 tumour cells are not dependent on them anymore for the neovascularization effect thereby resulting in the mast cells increase in moderately differentiated when compared to well-differentiated oral squamous cell carcinoma.

The findings were however, inconsistent with the study carried out by Kalra et al [16] on oral squamous cell carcinoma which showed decreased mast cell from well to moderately to poorly differentiated carcinoma and stated that this may be due to the massive degranulation of mast cell making their identification difficult.

In the present study we observed a definitive increase in mast cells count with the disease progression which strengthens the possibility of role of mast cells in the pathogenesis of disease.

The procedure of the assay of mast cell was cheap and easy to perform and hence can be targeted to enhance the prognosis of the disease.

CONCLUSION

As Oral Squamous cell carcinoma 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. After analysing the results of the present study, it was concluded that there was definitive increase in mast cells count as the disease progresses thus substantiating their contributing role in tumour progression. Therapeutic intervention to influence the mast cell secretions should be considered at early precancerous stage.

In our set up due to unavailability of sophisticated diagnostic techniques and the prevalence of tobacco and gutka chewing, mast cells may serve as a novel therapeutic target for cancer treatment and inhibiting mast cell functions may inhibit tumour growth.

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