Original Article

Role of Mast Cells in Progression of Oral Epithelial Dysplasia

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DOI:10.37821/ruhsjhs.6.2.2021.373

ABSTRACT

Introduction: Mast cells (MC) are the local residents of the connective tissue and are associated with the release of certain pro-inflammatory and mitogenic cytokines. These substances, when released from the mast cells, may play a significant role in the pathogenesis of diseases. MC infiltration and degranulation occur during oral carcinogenesis and the activation of these cells highly correlates with the distinct phases of dysplasia, carcinoma in situ, and invasive carcinoma. The objective of this study was to compare mast cell density (MCD) in different grades of oral epithelial dysplasia (OED) and determine its correlation with clinical and histopathologic parameters.

Methodology: Forty samples, of which 10 each of control (normal oral mucosa), mild, moderate and severe epithelial dysplasia were analyzed using toluidine blue. Mast cells were counted and compared in different groups.

Results: Mast cell density was significantly increased from mild to severe epithelial dysplasia. With no significant difference was detected between mild and moderate epithelial dysplasia samples.

Conclusion: Our findings indicate that MCs are present in the tumor microenvironment and mast cell mediators play an important role in progression of dysplastic lesions.

Keywords: Mast cell, Mast cell density, Mast cell mediators, Oral epithelial dysplasia.

INTRODUCTION

Mast cells are major immunoeffector cells of connective tissue. They are well engineered, multifunctional cells which play a central role in acquired and innate immunity and have a phagocytic function that might thereby contribute to host defense. Mast cells (MCs) are ubiquitously distributed, resident connective tissue cells. They are particularly frequent in close proximity to epithelial surfaces in the skin, the respiratory system, and the gastrointestinal mucosa where they are strategically located for optimal interaction with the environment and for their putative function in host defence. MCs secrete wide range of pro-inflammatory, immune-modulatory, and mitogenic cytokines.[1]

MCs possess many properties that enable them to participate in a diverse range of biological activities. They phagocytose, process antigens, produce cytokines, and release a variety of preformed mediators (e.g., histamine, proteoglycans and proteases) and newly formed physiological mediators (e.g., leukotrienes (LTs) and prostaglandins). MCs carry an array of adhesion molecules, immune response receptors, and other surface molecules, which permit them to react to multiple specific and nonspecific stimuli.[2] These wide-ranging biological characteristics, their ubiquitous distribution and strategic location near blood vessels, nerves, inflamed tissues, and neoplastic foci enable them to play a central role in a multitude of physiologic, immunologic, and pathologic processes.[3]

Recent data suggest that the accumulation of mast cells around the tumor margins and their release of potent pro-angiogenic and angiogenic factors may represent a tumor-host interaction which probably favors tumor progression.[4,5] The accumulation of mast cells is usually estimated by counting the mast cell density, which is the number of mast cells per optical field in tissue sections.[6]

The purpose of the current study was to examine the

relationship between mast cells and the histological progression from normal oral tissues through leukoplakia lesions with varying degrees of dysplasia.

METHODS

The study was undertaken after approval from the Institutional Ethics Committee. A retrospective study was done on archival tissue received from Department of Oral Pathology. The study sample comprised of 40 patients, which were divided into four groups, consisting of 10 cases of control group comprising of normal buccal mucosa and 10 cases each of mild, moderate, and severe epithelial dysplasia, respectively.

Two tissue sections of 5µm thickness of each case were obtained. Sections were stained with hematoxylin and eosin (H&E) and categorized to the particular groups. Specific staining for mast cells was done using 1% toluidine blue (TB) solution.[7]

Counting procedure

Haematoxylin and eosin stained tissue sections were reviewed to confirm the histological diagnosis and representative tissue blocks were selected. Mast cells (MCs) were counted in TB stained sections, under a magnification of 40X (Figure 1). The mast cells were counted in 10 representative and consecutive fields (40X magnification). The slide was scanned in a step ladder fashion to avoid overlapping of fields. The mean of ten values was calculated and expressed as mean mast cell density per field.

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

Out of the 40 patients, 28 were men and 12 were women. Figure 2 shows the distribution of cases according to gender. The age range of cases was 19-65 years. Primary tumor sites were: the vermilion border, the buccal mucosa, the tongue, the floor of mouth, and other sites within the oral cavity (Table 1).

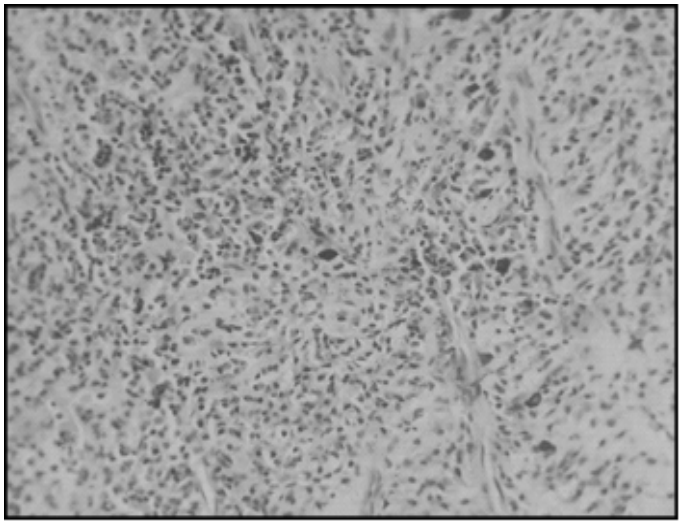

Figure 1: Mast cells seen in oral mucosa stained by toluidine blue at 40X magnification.

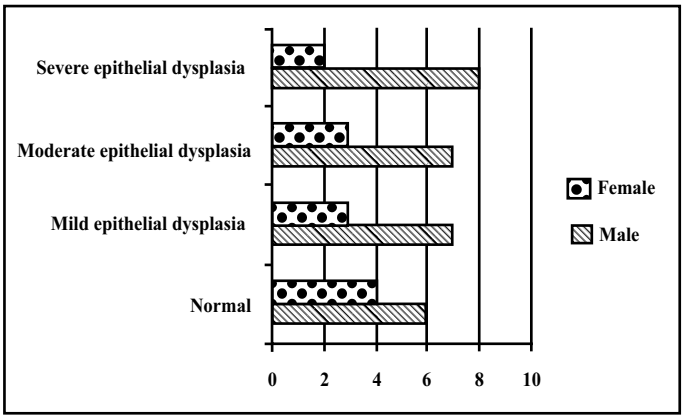

Figure 2: Distribution of cases according to gender.

Table 1: Site of lesion in different histologic type

Histological Diagnosis	Lesion Site				
	Vermilion border	Buccal mucosa	Tongue	Floor of mouth	Other sites
Normal oral mucosa	3	5	1	1	0
Mild epithelial dysplasia	4	3	1	2	0
Moderate epithelial dysplasia	2	6	1	1	0
Severe epithelial dysplasia	2	4	2	2	0

Numerical mast cell density

Toluidine blue staining revealed mast cells as large, purple, oval, and highly granulated cells (Figure 1). Mast cells were observed at the lamina propria highly populating areas around the tumor margins. In normal oral mucosa, mast cells were very few and looked small without sign of activation around blood vessels. In case of epithelial dysplasla, mast cells looked larger and intact: the histological location was in the underlying connective tissue and predominantly around the blood vessels. Increased numbers of mast cells were also found in areas of high vascularization (namely the 'hot spots'). MCD was counted in every tissue. In normal oral tissues, the mean MCD was 2.2/grid field, in mild dysplasia was 4.4/grid field, in moderate dysplasia the mean mast cell density was 5.1/grid field and in severe dysplasia the mean mast cell density was 7.6/grid field (Figure 2). The number of mast cells were found to be increased significantly between normal oral mucosa and mild dysplasia and moderate and severe dysplasia. The number of mast cells were also found to be increased significantly between mild dysplasia and severe dysplasia (p=0.05) (Table 2). No correlation was found between mast cell density and the age of patients or the site of primary tumor.

DISCUSSION

From its derivation 'mast' refers to feeding and was applied by Ehrlich in 1877 to certain cells of the connective tissue, he thought, looked so stuffed with granules. He imagined that they might have been over eating.[8] Mast cells are relatively small granule-containing secretory cells, which are round or oval in shape, having a diameter of about 12-15 μm. Their numerous cytoplasmic granules frequently obscure the small, round nucleus. In some sections, these cells seem to have degranulated, so that many of the granules are located outside the cell. Mast cells are present in mucosal and connective tissue environment. In oral mucosa and skin, they are distributed preferentially about the microvascular bed, being in close proximity to the basement membranes of blood vascular endothelial cells and nerves.[9]

Mast cells are known as unicellular endocrine glands, since on discharge of mast cell granules, a number of mediators are released which include heparin, histamine, and serotonin, which have major physiological and pharmacological significance.[10]

Mast cells are a common feature of lymphocytic and macrophagic infiltrations which make up the local cellular immune response. They are also observed in substantial numbers around tumors lacking a pronounced inflammatory response. Their presence in stromal connective tissues at the tumor periphery is especially evident. Whether mast cells are innocent bystanders with regard to tumor development or whether they contribute a functional role in tumor growth, invasion, metastasis, and angiogenesis, are questions that are currently receiving attention by many investigators.[11]

The fact that squamous cell carcinoma progresses in a multistep fashion have been well established: initially from normal epithelium, hyperkeratosis, premalignant dysplasia, and carcinoma *in situ* to invasive squamous cell carcinoma.[5] A study by Coussens et al[12] demonstrated the significance of mast cells as a key accessory during the premalignant stages of squamous carcinogenesis. In the premalignant early phase of hyperplasia and dysplasia, infiltrating mast cells degranulate and turn on and progressively intensify angiogenesis by releasing sequestered angiogenic activators.

In accordance to our study, the studies by Iamaroon et al[5] and Michailidou et al[13] demonstrated an increase in the number of mast cells as well as increase in the number of

Table 2: Mean mast cell density in different groups

Histological diagnosis	No. of cases	Mean mast cell density (per grid field)
Normal oral mucosa	10	2.2
Mild epithelial dysplasia	10	4.4
Moderate epithelial dysplasia	10	5.1
Severe epithelial dysplasia	10	7.6

vascularity during transition from normal tissue through different degrees of dysplasia to early and late carcinoma, suggesting the role of mast cells in tumor angiogenesis.

Study by Jandinski et al[14] showed an increase in the number of mast cells from normal to benign hyperkeratotic and dyskeratotic tissues, but this increase was not significant while the mast cell number increased significantly from normal tissue to low grade carcinoma. He suggested that this increase in number is because of antigenic stimulation. Further, he reported that the number of mast cells decreased in medium-high grade carcinomas, and attributed this to unfavourable cellular environment.

A similar increase in the number of mast cells from normal mucosa to oral leukoplakia was also observed by Biviji.[15] They concluded that the biologically and pharmacologically active agents in the mast cells might contribute to the inflammatory reaction seen in leukoplakia.

An increase in the number of mast cells in tissue sections of oral leukoplakia, submucous fibrosis, lichen planus, and oral squamous cell carcinoma as compared to normal oral mucosa was also observed by Ankle et al.[16] They showed a mean increase in the number of mast cells/unit and concluded that the biologically and pharmacologically active agents in the mast cells might contribute to inflammatory reaction seen in leukoplakia. Interleukin 1 contribute to increased epithelial proliferation seen in leukoplakia and the release of proangiogenic and angiogenic factors, such as histamine and heparin, chymase, beta fibroblast growth factors (βFGF), and vascular endothelial growth factors (VEGF) by mast cells may lead to increase in density of microvessels significantly between normal oral mucosa and oral leukoplakia without dysplasia, oral leukoplakia with mild, moderate or severe dysplasia and OSCC.

Researchers showed a direct correlation between mast cells activity and different phases of hyperkeratosis, dysplasia-carcinoma in situ and oral carcinoma. Sathyakumar et al[17] reported the increase of MCD in leukoplakia in comparison with normal mucosa.

Khare et al[18] in their study observed an increase in mast cell count from non dysplastic (NDL) to dysplastic lesion (DL) to squamous cell carcinoma (OSCC). They observed that the densities of mast cells and mean vessels increased from NDL to DL to OSCC with the exception that the densities of MVs was slightly higher in NOM group than NDL, but this difference was not statistically significant.

Study by Flynn et al[19] in experimental carcinogenesis demonstrated sequential mast cell migration and degranulation towards progressive mucosal dysplasia and subsequent development of squamous cell carcinoma.

In our study, we observed that similar to the above mentioned studies the number of mast cells increased from normal oral mucosa to epithelial dysplasia. Upon comparison of mast cells with age or sex, the results were non-significant in either of the groups. This was in accordance to the study conducted by Raniere et al[20] and Oliviera-Neto et al[21], who also did not find any correlation of mast cell density with age or sex.

Many tumor associated mast cells have been found to undergo degranulation and release of granular components such as heparin and histamine, which have been shown to potentiate endothelial cell migration and proliferation and to induce adhesion molecule expression on epithelial cells, potentially leading to increased tumor angiogenesis and metastasis.[22]

CONCLUSION

Based on the concept that mast cells play an important role in chronicity of inflammation, it may be possible to use drugs therapeutically in order to influence mast cell secretion and thereby thwart inflammation.

Deeper understanding of mast cell activation mechanisms, immune-modulatory capacity, and proangiogenic potential will open new perspectives on the development of future therapeutic strategies targeted at such multifunctional cells.

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