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Research Article

Comparison of Distribution of Mast Cells in Leukoplakia and Oral Squamous Cell Carcinoma - A Retrospective Study

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Abstract

Oral leukoplakia and oral squamous cell carcinoma (OSCC) are the commonly occurring oral diseases, with characteristic clinical and histological features. These diseases at some stages are associated with chronic inflammation in adjacent connective tissue. Mast cells are granule-containing secretory cells which are local residents of the connective, scattered along the capillaries of oral mucosa. They are proinflammatory and expressed the serine proteases, tryptase and chymase along with cytokines and may play a significant role in the pathogenesis of oral diseases. The aim of the study was to histologically evaluate and compare the presence of mast cells in Normal mucosa, Leukoplakia and Well differentiated oral squamous cell carcinoma. Thirty cases each of normal oral mucosa, oral leukoplakia, and oral squamous cell carcinoma (OSCC) were studied for mast cell number using 1% Toluidine blue. There was a linear increase in mast cell numbers were seen in leukoplakia and OSCC as compared to normal mucosa. Mast cell hyperplasia in oral leukoplakia and OSCC suggests their probable role in the pathogenesis of these diseases.

Keywords: Mast Cells; Oral Submucous Fibrosis; Oral Leukoplakia; Oral Squamous Cell Carcinoma

Abbreviations

LT: Leukotriene; TNF: Tumor Necrosis Factor; IL: Interleukin; MMP: Matrix Metalloproteinases

Introduction

Mast cell was discovered by Paul Ehrlich (1877), who termed it Mast Zellen i.e. fattened or well-fed cells [1]. Mast cells are key effector cell in allergic diseases, but it has become apparent that they also contribute to other pathologies, including autoimmune diseases and cancer. Mast cells secrete a wide range of proangiogenic, proinflammatory, immune-modulatory and mitogenic cytokines. Mast cells are large connective tissue cells (diameter-10 - 15 microns), with a life span of weeks to months, scattered along the capillaries, containing numerous basophilic granules in their cytoplasm. Mast cells release preformed secretory mediators like histamine, heparin, tryptase; lipid derived mediators like leukotrienes B4 (LTB4), LTC4, LTD4 and LTE4; pro-inflammatory cytokines like TNF-alpha, IL-1; mitogenic cytokines: IL-3, IL-5 and immunomodulatory cytokines like IL-4, IL-10 [2,3]. The commonly occurring oral diseases like oral leukoplakia, submucous fibrosis, lichen planus, squamous cell carcinoma have chronic inflammation in common it is probable that mast cells play key role in mediating the cross talks

between the external antigenic agent and local immunogenic factors [4-6]. The present study was carried out to evaluate the mast cell number in oral leukoplakia, OSCC and compare it with normal oral mucosa.

Materials and Methods

30 cases each of oral leukoplakia and OSCC were retrieved from the archives of the Department of Oral Pathology and Microbiology, from the Department of Oral Pathology, K M Shah Dental College and Hospital. Biopsies of normal oral mucosa were obtained from 30 adult patients undergoing extraction for orthodontic treatment. Two sections of five microns thickness each were cut; one section was stained with Hematoxylin and Eosin; the other was stained with 1% toluidine blue for mast cells. Toluidine blue stains the mast cell granules metachromatically due to its reaction with sulphated mucopolysaccharides.

Evaluation of mast cells was performed using light microscope under 400X magnification and counting was done in zigzag manner by evaluating maximum up to 10 microscopic fields.

Collected data was tabulated and followed by statistical analysis using Mann Whitney test with SPSS software.

It is difficult to differentiate mast cell from fibroblasts in hematoxylin and eosin staining, So Selective stain of 1% toluidine blue is used for mast cells. Mast cells stain purple with blue background in toluidine blue stain.

Results

The present study compared 90 cases which comprised 30 cases of normal mucosa, 30 cases of oral leukoplakia (22 cases oral leukoplakia with hyperkeratosis and 8 cases leukoplakia with dysplasia) and 30 cases of Oral well differentiated SCC. Out of 30 cases of leukoplakia mean number of mast cell/microscopic field in leukoplakia with hyperkeratosis (22) and leukoplakia with dysplasia (8) were 4.01 ± 1.757 and 4.98 ± 1.674 respectively which was statistically significant when compare to normal group (p = 0.001).

The results of the study showed a maximum mast cell count in OSCC of 5.14 ± 2.531, in leukoplakia mast cell count was 4.27 ± 1.761 as compared to 2.55 ± 1.908 of mast cell count in normal oral mucosa. Mast cell count show linear progression from normal oral mucosa; leukoplakia with hyperkeratosis, leukoplakia with dysplasia to oral squamous cell carcinoma (Table 1 and 2 and graph 1 and figure 1-4).

Discussion

Mast cells are local residents of the connective tissue. The role played by mast cell mediators and interaction with other inflammatory cells has been intriguing. Mast cells have been studied in normal gingiva, chronic inflammatory gingivitis, desquamative gin-

Group	Total No. of Patients	Mean No. of Mast Cell/Microscopic Field ± Stdev	Mann Whitney Test- P Value
Control	30	2.55 ± 1.908	Mast Cell Count/Microscopic Field; Control and Leukoplakia 0.000 (HS) Mast Cell Count/Microscopic Field; Control And OSCC 0.000 (HS) Mast Cell Count/Microscopic Field; Leukoplakia and OSCC 0.145 (NS)
Leukoplakia	30	4.27 ± 1.761	
OSCC	30	5.14 ± 2.531	
Ns- Nonsignificant, Hs- Highly Significant			

Table 1: Comparison of mean number of mast cell in control, leukoplakia and OSCC.

Study Groups	Number of Cases	Mean Number of Mast Cell/Microscopic Field	Std Deviation
Normal Mucosa (Control)	30	2.55	1.908
Leukoplakia with Hyperkeratosis	22	4.01	1.757
Leukoplakia with Dysplasia	8	4.98	1.674
OSCC	30	5.14	2.531

Table 2: Comparison of mean number of mast cell in control, leukoplakia with hyperkeratosis and leukoplakia with dysplasia and OSCC.

Graph 1: Linear increase in mast cell count/microscopic field in normal mucosa, leukoplakia with hyperplasia, leukoplakia with dysplasia and OSCC.

Figure 1: Photomicrograph showing mast cell in normal mucosa. (40X) Toluidine Blue stain.

Figure 2: Photomicrograph showing mast cell in hyperkeratotic leukoplakia. (40X) Toluidine Blue stain.

Figure 3: Photomicrograph showing mast cells in dysplastic leukoplakia (40X) Toluidine blue stain.

Figure 4: Photomicrograph showing mast cells in OSCC (40 X) toluidine blue stain.

givitis, lichen planus, oral submucous fibrosis and oral cancer. Mast cells exhibit phenotypic plasticity. There is variation in mast cell mediators with the change in the microenvironment, which makes the study of this cell in various diseases interesting [1-5].

It has been well established that oral SCC progresses multistep fashion: initially from normal epithelium, hyperkeratosis, premalignant dysplasia, and carcinoma in situ to invasion.

In this study, significant (p = 0.000) increase in mean mast cell number/microscopic field was found in oral leukoplakia as compared to controls. Results of the present study were in line with the previous studies of premalignant lesions of oral cavity.

The observations by Biviji., *et al.* showed similar results i.e. mean increase in number of mast cells/microscopic field in oral leukoplakia than normal mucosa [7]. Studies has showed the same results as in our study that increase mast cell hyperplasia in oral leukoplakia and oral epithelial dysplasias as compared to normal oral mucosa [4,5]. Imaaroon., *et al.* (2003) also concluded similar results of increase density of mast cell with disease progression [6]. The results of I G Rojas., *et al.* (2004), in Actinic Chelitis, a premalignant condition, showed that mast cells were significantly increased as compared to normal, are also consistent with findings of our study [8]. The biologically and pharmacologically active agents in mast cells might contribute to inflammatory reaction seen in leukoplakia. These stimulated mast cells may release IL-1, which causes increased epithelial proliferation that is seen in leukoplakia as hyperkeratosis. Histamine may cause increase mucosal permeability, which could facilitate increased access for antigen to the connective tissue. Heparin further causes endothelial cell proliferation and migration which results in increased vascularity of the stroma and in epithelial ulceration [2-5]. Lisa M Coussens., *et al.* (1999) demonstrated the significance of mast cell as a key accessory during premalignant stages of squamous cell carcinoma [9].

In our study mast cell count increased with progression of disease, with a significant increase in mast cell count in hyperkeratosis (0.001) and dysplastic leukoplakia (0.001) as compared to control.

In the present study, we found mast cell count was significantly higher in oral SCC as compared to normal oral mucosa. Many studies support the results of our study that mast cell hyperplasia was observed in oral squamous cell carcinoma compared to normal oral mucosa [4-6,10]. Threefold increase in the average number of mast cells/slide was noted in OSCC when compared to controls. Angiogenic regulation is biphasic in carcinogenesis. In the premalignant stage as early phase of hyperplasia and dysplasia, infiltration of mast cells leads to degranulation and activation of 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 and regulation of angiogenesis [11].

These results are similar to Rojas., *et al.* (2005) who found out significantly increase in mast cell count in lip SCC as compared to normal lip [8]. Mast cells shown to accumulate near sites of new capillary formation have long been implicated in angiogenesis, perhaps via Mast Cell tryptase [2,6]. Direct association of mast cell with vascular tube formation was clearly shown *in vitro* (Blair RJ., *et al.* 1997). It is believed that mast cell play a significant role in promoting tumor angiogenesis, probably by secreting several potent angiogenic factors including histamine, VEGF, bFGF and tryptase. A study by Blair., *et al.* (1997) revealed that tryptase could directly induce cell proliferation of human dental microvascular endothelial cell in a dose dependent fashion and suggested that mast cell act as sites of new vessel formation by secreting tryptase [12].

However, Oleiveira-Neto HH., *et al.* found Mast cell density is lesser in OSCC and premalignant lesions compared normal controls. They attributed it to migration failure of mast cells, which may reflect a modification in the microenvironment during tumor initiation and progression [13].

The solid tumors needs the formation of new vasculature, i.e. angiogenesis for growth. This results from an imbalance between positive and negative angiogenic factors produced by tumor and host cells. Mast cells are an very important source of several proangiogenic and angiogenic factors and so around this area of angiogenesis mast cell density is seen to be high [6,10,14].

Enhancing the cytotoxic functions of mast cells and suppressing their angiogenic functions, could lead to a new anti-cancer treatment strategy. Furthermore, the mast cell heparin inhibitors, platelet factor 4 and protamine have been reported to inhibit angiogenesis [15].

Our study observed that mast cell counts show: a linear progression with the pathological progression of oral lesions starting from normal oral mucosa; leukoplakia with hyperkeratosis, leukoplakia with dysplasia to well differentiated oral squamous cell carcinoma.

All of the above discussion implies that mast cell in different oral lesions still demands studies of increasing complexity beyond those merely assessing mast cell numbers. Because role of mast cell in oral pathological lesions is still far beyond the reams of our present knowledge.

Conclusion

Viewing the role and importance of mast cell in oral lesions, there is still dire need for further clinical studies and researches on mast cell for validation using the large sample size including recurrent cases and follow up studies. Various studies have been done on mast cells in pathogenesis of many oral lesions. But still more specific and newer staining techniques, like immunohistochemical staining, for the demonstration of mast cells may provide a better insight into the role of these immunocompetent cells in the pathogenesis of potentially malignant and malignant oral lesions. What we know about mast cell is still only the tip of iceberg, lot to be revealed what remained far beyond from our knowledge.

Conflict of Interest

The authors declare that there was no conflict of interests.

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