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INCREASED MAST CELLS NUMBER IN LEUKOPLAKIA, ORAL LICHEN PLANUS AND ORAL SQUAMOUS CELL CARCINOMA SUGGESTIVE OF THEIR PRO-INFLAMMATORY ACTION

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ABSTRACT

Introduction: Oral leukoplakia, oral lichen planus (OLP) and oral squamous cell carcinoma (OSCC) are the commonly occurring oral diseases. These diseases at some stage are associated with chronic inflammation in adjacent connective tissue. Mast cells are the local residents of the connective tissue, and are said to be pro-inflammatory and immunoamplifying in action. These functions of mast cells may play a significant role in the pathogenesis of other oral diseases. **Aims:** This study was done to histologically evaluate the number of mast cells in tissue sections of oral leukoplakia, lichen planus and squamous cell carcinoma. **Materials and Methods:** ten cases each of normal oral mucosa, oral leukoplakia, lichen planus and squamous cell carcinoma were studied for mast cell number using 1% Toluidine blue. **Results:** Increase in mast cell number was seen in all the above mentioned oral diseases, with the highest mast cell count obtained in oral lichen planus. The mast cell number/sq.mm in oral leukoplakia, lichen planus, squamous cell carcinoma were; 59.50, 59.75 and 56.75 respectively. **Conclusion:** As compared to normal oral mucosa, increase in the mast cell number was noted in all the four conditions. Mast cell hyperplasia in oral leukoplakia, OLP, OSCC suggests their probable role in the pathogenesis of these diseases.

KEYWORDS: Mast cells, oral leukoplakia, oral lichen planus, oral squamous cell carcinoma

INTRODUCTION

Mast cells and basophils represent distinct haematopoietic lineages that can express complementary or overlapping functions in the context of acute and chronic immunoglobulin E (IgE)-associated allergic responses.[1,2] They both contribute to leukocyte recruitment, stromal and tissue cell activation, modulation of immune reactions, tissue remodelling and angiogenesis. Both cell types play a critical role in innate immunity to parasite and bacterial infection[3] and can be activated by bacterial and viral proteins. being intimately involved in wound healing and defense against pathogens. The development of staining techniques for histologic sections led to the initial definitive description of mast cells by a medical student named Paul Ehrlich over a 100 years ago.[4]

The commonly occurring oral diseases like oral leukoplakia, submucous fibrosis, lichen planus, squamous cell carcinoma have chronic inflammation in common. In addition, autoimmunity is strongly associated with OLP and angiogenesis is associated with the proliferation of carcinoma. Therefore the role of mast cells was evaluated in these four diseases. The present study was carried out to estimate and compare mast cell number in oral leukoplakia, OLP and OSCC.

MATERIALS AND METHODS

Ten cases each of oral leukoplakia, oral lichen planus and oral squamous cell carcinoma were retrieved from the archives of the Department of Oral Pathology and Microbiology, Patna dental college and hospital, Patna. Biopsies of normal oral mucosa were obtained from adult patients undergoing extraction for orthodontic treatment. Two sections each of 5 microns thickness were cut; one section was stained with Hematoxylin and Eosin; the other was stained with 1% toluidine Blue at about pH 4 for mast cells. Toluidine blue stains the mast cell granules metachromatically due to its reaction with sulphated mucopolysaccharides.[5]

Mast cells were counted using an oculometer grid in 30 grid fields under a magnification of x400 under Motic microscope with a magnification. Mast cell count was expressed as the number of mast cells per grid field and the number of mast cells per square millimeter.

Criteria to identify the mast cells

Mast cells are spindle to oval-shaped and have the same staining characteristics as the fibroblasts with hematoxylin and eosin stain. Therefore, they are difficult to differentiate from fibroblasts. Selective stain of 1% toluidine blue is used for mast cells. Mast cell granules are purplish red and the nuclei of mast cells appear sky blue in color.

RESULTS

The results of the study showed a maximum mast cell count in oral lichen planus of 59.75/sq.mm, in leukoplakia of 59.50/ sq.mm and in OSCC the mast cell count was 56.75/sq.mm respectively as compared to 25.50/ sq.mm of mast cell count in normal oral mucosa [Table 1].

Table 1: Mast cell count in normal oral mucosa, leukoplakia, OPL and OSCC.

Oral disease	No. of cases	Number of Average number of mast cells/grid	Average field mast cells/sq.mm
Normal oral mucosa	10	1.02	25.50
Oral leukoplakia	10	2.38	59.50
Oral lichen planus	10	2.39	59.75
Oral squamous cell carcinoma	10	2.27	56.75

DISCUSSION

Mast cells are the local residents of the connective tissue. The role played by the mast cell mediators and their interaction with other inflammatory cells has been intriguing. Mast cells have been studied in normal gingiva, chronic inflammatory gingivitis, desquamative gingivitis, lichen planus, OSMF and oral cancer.[6,7] Mast cells exhibit phenotypic plasticity.[8] There is variation in the mast cell mediators with the change in the microenvironment, which makes the study of this cell in various diseases interesting. Therefore, the present study was done to evaluate the mast cell number in 10 cases each of normal oral mucosa, oral leukoplakia and submucous fibrosis. 1% toluidine blue was used as a selective stain for mast cells. Mast cell count was done using an oculometer grid in 30 grid fields.

The results obtained showed an increased mast cell number in oral leukoplakia. The observations by Biviji *et al.*[6] showed a mean increase in the number of mast cells /unit microscopic field in oral leukoplakia compared to normal oral mucosa. It can be concluded that the biologically and pharmacologically active agents in the mast cells might contribute to inflammatory reaction seen in leukoplakia. These stimulated mast cells may release interleukin-1, which causes increased epithelial proliferation[9] that is seen in leukoplakia. Histamine may cause increased mucosal permeability, which could facilitate increased access for the antigen to the connective tissue [Table 2].

Table 2: Illustrating the probable effect of mast cell mediators in oral leukoplakia leading to the following clinical and histopathological changes.

Mast cell mediators	Histopathological features	Clinical features
Interleukin-1 and TNF Increased epithelial cell proliferation	Increased thickness of the epithelium	White patch or a plaque.
Histamine Enhances permeability across the epithelial surface.	Increased mucosal permeability despite hyperkeratosis	Chronicity of the lesion.
Heparin Causes endothelial cell proliferation and migration	Increased vascularity of the stroma and ulceration	Erosive leukoplakia.

Maximum numbers of mast cells were seen in oral lichen planus (59.75/sq.mm) as compared to 25.50/sq.mm seen in normal oral mucosa. These results are similar to the studies carried out by Xijing *et al.*[7] who observed a mast cell count of 151.5/sq.mm in lichen planus. They considered mast cells as the offenders in basement membrane destruction. TNFalpha released from the mast cells causes increased synthesis of matrix metalloproteinases like collagenase, which cause the basement membrane destruction. TNF-alpha also causes increased expression of adhesion molecules like E-selectin, ICAM. This could probably cause increased leukocytic migration. Histamine causes vasopermeability leading to submucosal edema and antigen induced T-cell proliferation. This could attribute for the characteristic trafficking of lymphocytes. The cytotoxic lymphocytes thus recruited by the mast cells cause the basal cell degeneration, keratinocyte apoptosis and thus the characteristic Civette bodies seen in oral lichen planus[10] [Table 3].

Table 3: Illustrating the probable effects of mast cell mediators in oral lichen planus leading to the following clinical and histopathological changes.

Mast cell mediators	Histopathological features	Clinical features
Histamine Induces vasopermeability. Antigen induced T-cell proliferation.	Submucosal edema. Trafficking of T-lymphocytes.	Vesicles, bullae and erosive lesions. Chronic persistence of the lesion.
TNF-alpha Increased production of matrix metalloproteinases like stromyelsin, collagenase. Destruction of basement membrane.	Necrosis and liquifactive degeneration of basal cell layer.	Vesicles, bullae and erosive lesions.

The mean mast cell count in oral squamous cell carcinoma in the present study was 56.75/sq.mm. Rooney *et al.*[11] suggested that heparin from the mast cells cause vasoproliferation and increases the half-life of basic fibroblastic growth factor (FGF), which is a potent angiogenic substance, thereby promoting tumour angiogenesis and facilitating local tumour invasion. Interleukin- 1 leads to epithelial proliferation[11] [Table 4].

Table 4: Illustrating the probable effects of mast cell mediators in oral squamous cell carcinoma leading to the following clinical and histopathological changes.

Mast cell mediators	Histopathological features	Clinical features
IL-1 AND TNF-alpha Causes increased epithelial cell proliferation.	Increased thickness of the epithelium.	Exophytic growth or a plaque.
TNF-alpha Causes destruction of the basement membrane.	Invasion of epithelial cells into the connective tissue.	
IL-1 Causes increased T and B cell proliferation.	Increased lymphocytic infiltration and increased plasma cell infiltration.	
Heparin Causes angiogenesis and type-VIII collagen synthesis.	Increased vascularity of the stroma.	Tumour angiogenesis.

In this study, mast cell hyperplasia was observed in all the oral diseases considered. The mediators in mast cells are known to vary with the variation in microenvironment in various diseases. Thus it is probable that mast cells play a key role in mediating the cross talks between the external antigenic agent and the local immunologic factors.

CONCLUSION

Mast cells serve a critical role in the development of inflammation in the oral mucosa and the dental pulp, both in the early, vaso-inductive events and in the transition from acute to chronic inflammation. Because of the unique properties of mast cells, these cells are ideally poised to serve as "gatekeepers" of the microvasculature in the oral cavity. An appreciation of the multiple interactions among mast cells, endothelial cells, nerves, and other immune system provides a basis for therapies for targeting mast cell responses. Therefore more studies are needs to be carried out in greater number of cases. The tissue level and the type of mediators should be analyzed in the various diseases considered.

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