

Comparative Analysis of Mast Cell Count in Oral Lichen Planus Andnormal Oral Mucosa

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Abstract: Oral lichen planus (OLP) is a common mucocutaneous disease of unknown etiology. It was first described by Wilson in 1869 and is thought to affect 0.5–1% of the world's population. It appears clinically as a persistent red, white or a mixed lesion. Though the precise pathogenesis is unidentified, evidences available at present strongly suggest that cell mediated immunity plays a major role in the initiation and evolution of this disease. The mast cell, the major immune effector cell of the connective tissue is thought to be mediating this synchronized cellular orchestra, the symphony of which results in the various clinical manifestations of oral lichen planus.

Aim: To evaluate mast cell density in oral lichen planus and to compare it with normal mucosa.

Materials & Methods: 60 cases of oral lichen planus and 20 normal oral mucosa were studied for mast cells using toluidine blue stain. Two sample T test was used to compare the mean values of the density of cells in the lesional area to that of control. The mean of the type and distribution of mast cells were compared using F test- One way ANOVA.

Result: An increase in mast cell density was noted in lichen planus when compared to normal buccal mucosa. There is a marked distribution of mast cells was seen below the inflammatory band and mast cell degranulation was prominent.

Conclusion: Marked increased density of mast cells and their distribution suggest there is a definite role in different phases of evolution of Oral Lichen Planus.

KeyWords: Mast cells, Oral lichen planus, Pathogenesis.

Photomicrogram:

Photo-1: mast cell with large eosinophilic cytoplasm and centrally placed nucleus seen in close association with a blood vessel. (H & E, 20x)

Photo-2: Spreading mast cells. Note the granules dispersed in the adjacent connective tissue (Toluidine Blue, 40x)

Photo-3: Intact mast cells packed with granules. (Toluidine Blue, 10x)

Photo-4: Degranulated mast cells with pale pink cytoplasm and well defined blue nucleus. (Toluidine Blue, 20x).

Photo-5: Spreading mast cells. Granular cells with indistinct cell border seen immediately below the inflammatory band. (Toluidine Blue, 10x)

I. Introduction

The data available suggests that oral lichen planus is a T-cell-mediated^{1,2} autoimmune disease in which cytotoxic CD8+ T-cells trigger the apoptosis of oral epithelial cells. with an unknown etiology³. and is thought to affect 0.5–1% of the world's population. Though various clinical subtypes have been described by WHO⁴, the subepithelial band of chronic inflammatory infiltrate is a consistent microscopic finding in all the cases. The inflammatory cellular components and its complementary essentials which make up the sub epithelial band in oral lichen planus acts against a self antigen, present in the epithelium as a self motivated synchronized orchestra. While there is sizeable literature on the T cell population^{1,2} in the oral lichen planus, other immunocompetent cells have attracted less attention. Hence a histochemical study using toluidine blue stain was conducted to assess the density, morphological characteristics and distribution of mast cells, the major immunoeffector cells of the connective tissue, in 60 cases of oral lichen planus

II. Material And Methods

The material used for this study consisted of Diseased group and control group. The diseased group comprised of 60 cases of oral lichen planus reported to the out patient department of Jodhpur Dental college, Jodhpur, and the control group consisted of 20 specimens taken from normal buccal mucosa for comparative analysis with the diseased tissue. Specimens from these cases were subjected for histopathological study under H&E, to read the histological changes and toluidine blue stain to analyze the mast cells. The working solution of toluidine blue was prepared as given below⁵.

Stock solution: Toluidine Blue O – 1.0 gm

70% Alcohol - 100ml

1% Sodium Chloride

Sodium chloride - 0.5gms

Distilled water - 50ml

Working solution:

Toluidine Blue, stock-50ml

1% Sodium Chloride- 45ml

III. Results

Toluidine blue stain revealed more number of mast cells and distinct features of mast cells could be well appreciated using toluidine blue stain. Our observations based on 60 cases studied, showed that there is definite increase in mast cell density in OLP when compared to the normal control group. In H & E sections mast cells were noticed in all cases of Lichen planus and they appeared as large eosinophilic cells with well-defined cell borders and acentrally placed nucleus (Fig.1).

Out of 60 cases, 19 cases showed severe increase, 19 cases showed moderate increase and 22 cases showed mild increase in mast cell density. When distribution of mast cells was studied, more concentration of mast cells was found immediately below the inflammatory infiltrate. 18 cases showed intense density of mast cells below the infiltrate whereas 34 cases showed moderate distribution and 8 cases showed mild distribution. Differences were noticed in the colour and morphology of mast cells distributed within the infiltrate and in the deeper connective tissue and three types of mast cells could be identified. The cells in the deeper connective tissue (except those seen in relation to the blood vessels) were found to be round/oval in shape and dark purple in colour. The cell borders were well defined and nucleus was not visible. These fully granulated cells with granules masking the nucleus were named as intact cells (Fig.2). In the superficial connective tissue, immediately below the infiltrate and near the blood vessels, the mast cells appeared flattened or irregular and cytoplasm appeared granular. Many cells showed spreading granules and in some cases granules were found dispersed in the connective tissue. The cell borders were not defined and the nucleus was only partially appreciable. They formed the spreading cells (Fig.3 & Fig.4). In addition to the types mentioned above, a third type named as degranulated cells (Fig 5) was found within the infiltrate. These cells appeared paler as the staining had changed from metachromatic violet to light pink; the nucleus was blue in colour and well defined. When the mean value of the cell types were statistically analysed it was found that spreading cells were significantly increased in number when compared to other cell types.

IV. Discussion

Our research reveals that only 20 out of the 60 cases examined, showed lining up of mast cells near the basement membrane. In these 20 cases only few cells (<10 cells) were seen in relation to the basement membrane. Though consistency regarding the increase in mast cell density was noticed no conformity seems to exist regarding their distribution. Abbey et al⁶ noted the distribution of mast cells through out the sub epithelial inflammatory infiltrate whereas Heyden G et al⁷ reported the distribution of mast cells in the non inflammatory part of lamina propria. Similar distribution of mast cells was noticed in our study also. Jontell M⁸ et al found an increased distribution of granulated mast cells below the infiltrate and noticed that those cells within the infiltrate lost their metachromatic property and appeared pale pink suggesting degranulation of the cells.

Increased density of mast cells found in our study was consistent with the previous studies reported. Walter B Hall⁹ et al found a significant increase in the number of mast cells in oral lichen planus and he noted that the mast cells lined up along the basement membrane. In our study we noted an increased accumulation of degranulating/spreading mast cells immediately below the inflammatory band, though the significance of infiltrate below the infiltrate is not known. The mast cell accumulation in the connective tissue along the borders of inflammatory infiltrate especially at the inflammatory infiltrate - connective tissue interface was noticed in recurrent aphthous ulcer by Natah S S et al¹⁰ and they suggested that mast cell degranulation at these interfaces may contribute to localized extra cellular degradation by mast cell proteinases, which is an essential step

for inflammatory cell movement and migration through extra cellular tissue. Kabashima et al¹¹ found close association of substance P immunoreactive nerve and mast cells in periapical granuloma and suggested that the synthesis of TNF α from mast cells is stimulated by substance P released by noxious stimulants. Similar non immunogenic mast cell degranulation could also occur in OLP. Tryptase can facilitate recruitment of T lymphocytes whereas Chymase, can cause the degradation of basement membrane either directly or indirectly via the activation of T-cell secreted MMP-9 thereby paving way for the CD8+ lymphocytes to enter the epithelium. Zhao et al¹² found increased number of mast cell in close apposition to the nerve fibers especially in the superficial layer and they suggested that mast cell nervous system axis may contribute to the pathogenesis of OLP. Mast cell degranulation releases a range of pro-inflammatory mediators such as Histamines, TNF α , Chymase and Tryptase, and each of these mediators have specific function in OLP. Histamine causes vasodilatation and increases the vascular permeability whereas TNF may up regulate endothelial cell adhesion molecule expression that is required for lymphocyte adhesion to the luminal surfaces of blood vessels and subsequent extravasation. The distribution of mast cells at different levels may suggest the role of mast cells at different phases of oral lichen planus. The initial phase may be involving the blood vessel to dilate and extravasate the lymphocytes. Subsequently these lymphocytes are attracted towards the subepithelial zone. The mast cells may also release some cytokines that causes the destruction of extracellular matrix and attract the targeting lymphocytes towards the basement membrane.

V. Conclusion

Our study reveals that the mast cell has definite role in the pathogenesis of oral lichen planus. Hence due importance should be given for further study to understand the disease process as well as to evolve a successful treatment.

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