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ORIGINAL RESEARCH

A Quantitative Analysis of Mast Cells in Inflammatory Periapical and Gingival Lesions

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ABSTRACT

Aim: The aim of the study was to quantify the presence of mast cells in various inflammatory lesions like periapical granuloma, periapical cyst, inflammatory gingival hyperplasia and pyogenic granuloma. Mast cell degranulation and association with lymphocytes were also recorded in an attempt to understand the role of mast cells in the pathogenesis of these inflammatory lesions.

Materials and methods: The quantification of mast cells was done on toluidine blue stained sections of all the four groups of lesions, using the image analyzer software, Image-Pro-Express (Media Cybernetics, USA).

Results: An increased number of mast cells in various inflammatory lesions with a significant difference between the four groups were noted. Mast cell number tended to be greater in the lesions present in the anterior region of the mouth than in the posterior region of the oral cavity. The mean mast cell number decreased with the increasing age which was directly correlated with the age of the patients. Mast cell site, distribution, degranulation and its association with fibroblasts, lymphocytes and blood vessels were noted.

Conclusion: The location of mast cells in different areas, their association with lymphocytes, fibroblasts, endothelial cells, and the phenomenon of degranulation helps to appreciate the release of various mediators and multiple interactions among these cells, leading to increased vascular permeability, angiogenic response, collagen synthesis, regulation of inflammation, bone resorption, and extracellular matrix destruction, thus contributing to the pathogenesis of these inflammatory lesions.

Keywords: Mast cells, Periapical lesions, Gingival lesions.

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INTRODUCTION

Over the last century, mast cells and their granules have captured the interest of investigators from a variety of scientific disciplines.[1] Mast cells are mobile, bone marrow-derived, granule-containing immune cells that are found in all connective tissues and mucosal environments and in the peripheral and central nervous systems.[2]

Following activation by immunologic or non-immunologic stimuli, mast cells release via their granules a range of preformed mediators like serine proteases tryptase, chymase, cathepsin-G, histamine, heparin, serotonin, acid hydrolases and cytokines. Mast cells may subsequently synthesize and secrete additional mediators as well; those are not preformed in their granules. They include interleukins IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13 and IL-16, granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet activating factor (PAF), RANTES, macrophage inflammatory protein, and the arachidonic acid metabolites; prostaglandin 2 and leukotriene C4.[3] The induction of inflammation by mast cells is consequent upon the release of these preformed potent biological mediators as well as secondary mediators.[2]

Most of the periapical lesions and gingival lesions are inflammatory in origin and these lesions are the response of the periapical tissues to the microbial and chemical stimuli coming from the pulp through the root canal systems.[4] Among the cells found in periapical lesions, mast cells have been detected in the inflammatory infiltrates of granulomas and cysts, suggesting a role for mast cells in the inflammatory mechanisms of these lesions.[5]

Chronic generalized inflammatory gingival hyperplasia and pyogenic granuloma are common inflammatory gingival lesions. They represent inflammation and repair attempts that are stymied due to ongoing etiologic stimulation.[6] Among the cells found in periodontal tissues, mast cells have been detected in both healthy and inflamed gingiva, in different densities at different sites.[7] It is suggested that mast cells are present and active in chronic inflammatory periapical reactions[8] and inflammatory gingival lesions.[9,10]

AIMS AND OBJECTIVES

The present study aims to identify and quantify the presence of mast cells in various inflammatory lesions like periapical

granuloma, periapical cyst, inflammatory gingival hyperplasia and pyogenic granuloma. Mast cell degranulation and association with lymphocytes are also recorded in order to attempt to clarify the role of mast cells in the pathogenesis of these inflammatory lesions.

MATERIALS AND METHODS

The present study was undertaken by retrieving archived records and paraffin embedded tissue blocks of previously diagnosed cases of periapical granuloma, periapical cyst, inflammatory gingival hyperplasia and pyogenic granuloma from the archives of the Department of Oral Pathology. The age of the patient and the location of the lesion of each case were also noted. As control for staining procedure, sections from a case of neurofibroma was used. The study group comprised of 50 cases, of which 11 cases were of periapical granuloma, 14 cases were of periapical cyst, 12 cases were of inflammatory gingival hyperplasia and 13 cases of were pyogenic granuloma.

Sectioning and Toluidine Blue Staining Procedure for Mast Cells

From each paraffin embedded block, sections of 6 µm thickness were cut by using rotary microtome.

Churukian and Schenk's acidified Toluidine blue stain[11] was used as it gives rapid, crisp staining of mast cells. Mast cells were stained purple and the nuclei, blue.

Quantifying Mast Cells

In a representative section of each specimen, 10 consecutive microscopic fields were photographed under ×40 high power magnification using a 3 chip digital camera attached to a trinocular microscope with a ×40 objective lens.

Both intact (Fig. 1) and degranulated (Fig. 2) mast cells were identified by the structure of purple-colored granules. The quantification of mast cells was done using a single parameter, namely, manual tag for the number of positively stained cells irrespective of the staining intensity by using the image analyzer software Image-Pro-Express (Media Cybernetics, USA) (Fig. 3). The cells counted in each case and their association with lymphocytes and degranulation was also recorded.

Depending on the age of the patients, cases were grouped into 4 groups: group A—9 to 19 years, group B—20 to 39 years, group C—40 to 59 years and group D—60 years and above. On the basis of location they were grouped into lesions present in anterior region, molar region and canine-premolar region. These data were submitted for statistical analysis.

RESULTS

Mast cells were found in different areas of periapical and gingival lesions, distributed in several patterns: subepithelially, in isolated groups in the connective tissue, and as isolated cells. Some cells were seen in association with fibroblasts, some in association with blood vessels and several in association with lymphocytes. Many of the mast cells were found to be degranulated.

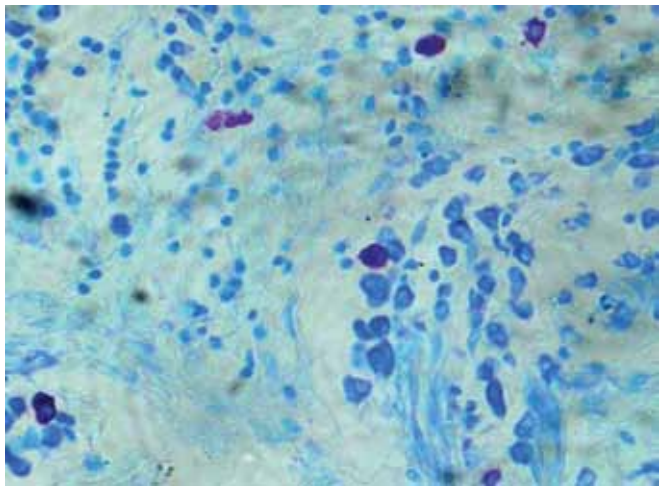


Fig. 1: Intact mast cell (H&E, ×40 magnification)

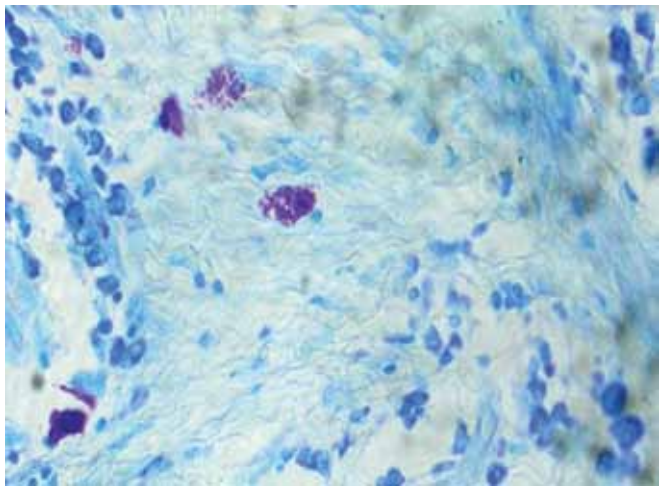


Fig. 2: Degranulated mast cell (H&E, ×40 magnification)

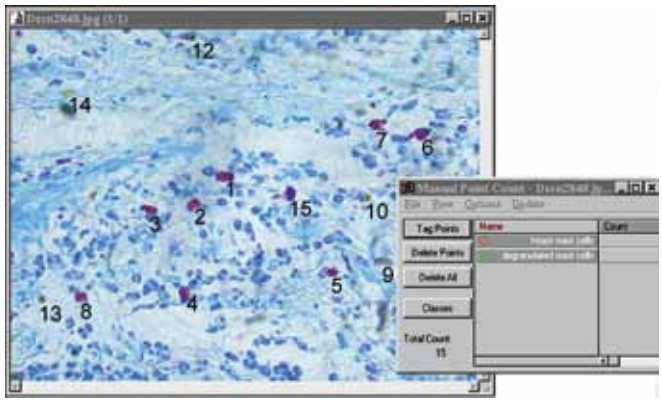


Fig. 3: Counting of intact and degranulated mast cells using the image analyzer

The location and the number of mast cells counted in the 50 cases are represented (Table 1). Mast cell number tended to be greater in the anterior region and higher numbers of mast cells were found in the anterior and molar regions compared to the canine-premolar region.

The age range of the patients was 9 to 72 years. Age distribution and total number of mast cells counted along with mean and standard deviations (Table 2). The mean mast cell number was directly correlated with the age of the patients.

STATISTICAL ANALYSIS

Null hypothesis: There is no significant difference in the mean total mast cells of the 4 groups, i.e. $\mu1 = \mu2 = \mu3 = \mu4$

Alternate hypothesis: There is a significant difference in the mean total mast cells of the 4 groups, i.e. $\mu1 \neq \mu2 \neq \mu3 \neq \mu4$

Level of significance: $\alpha = 0.05$

Statistical test: In order to compare the means of the 4 groups ANOVA was used.

Decision criterion: Comparison of p-value with the level of significance was done. If $p < 0.05$, the alternate hypothesis was accepted and concluded as significant difference exist in the mean total mast cells of the four groups. Otherwise, the null hypothesis was accepted.

Computations: Table 3 gives us the various computations and the p-value.

Table 1: Quantification of mast cells and its location

Location	*No. of cases (%)*	*No. of mast cells*	*Mean*	*SD*
Anterior region	18 (36)	1104	61.3	64.9
Molar region	18 (36)	819	45.06	36.4
Canine-premolar region	14 (28)	427	30.5	36.44

Table 2: Age distribution and mast cell correlation in four groups

Age groups	*No. of cases*	*Total no. of mast cells*	*Mean*	*SD*
A—9 to 19 years	7	444	63.4	32.1
B—20 to 39 years	23	1126	48.96	42
C—40 to 59 years	15	666	44.4	68.8
D—60 years and above	5	129	25.8	25.4

There was a significant difference between the four groups with respect to the total mast cells as $p = 0.010$ ($p < 0.05$) (*see* Table 3). The mean of total number of mast cells in various lesions are represented in Graph 1 and the percentages of intact and degranulated mast cells in various lesions are represented in Graph 2.

Mast cells were more frequently observed in areas with chronic inflammatory infiltrate. Their percentage to the total number of mast cells counted in all lesions is 52% (51.941489). Their percentage to the total number of mast cells in each lesion is represented in the Graph 3. Mast cells were also seen in fibrotic zones but with less frequency when compared to inflammatory areas. Cellular degranulation was regularly seen in zones with inflammatory infiltrates.

DISCUSSION

Several studies have reported the presence of mast cells in periapical inflammatory lesions and inflammatory gingival lesions. Quantification of mast cells in periapical lesions was done by Kontiainen S et al (1986),[12] Smith G et al (1989),[13] Montes LC et al (2004),[8] Sudhakar R et al (2005);[14] and in inflamed gingiva by Hagiwara K et al (1999),[15] Batista AC et al (2005),[16] and Prakash S et al (2006).[7]

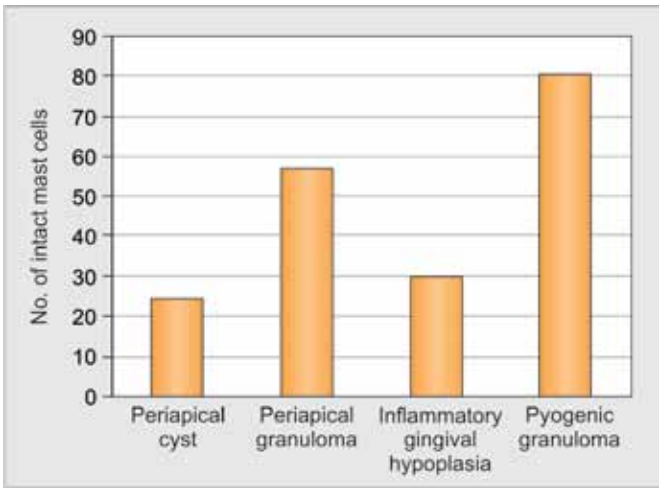
Our results showed that differences in the mast cell number exist in different locations, age groups and types of inflammatory periapical and gingival lesions. Mast cell number tended to be greater in the anterior region of the mouth in the present study whereas their number tended to be greater in the posterior region in the study conducted by Montes LC et al (2004)[8] on periapical lesions. In the present study the mast cell number was directly correlated with the age of the patients but it was not so in the study conducted by Montes LC et al (2004) on periapical lesions.[8]

Rodini DOC et al (2004) stated that microscopic analysis of periapical lesions revealed mast cells to be present in greater numbers in periapical cysts (22.30 ± 8.23) than in apical granulomas (17.44 ± 4.34),[17] which was not so in the present study, where we found greater number of mast cells in apical granulomas (57.553 ± 8.08) than in periapical cysts (24.36 ± 24.08).

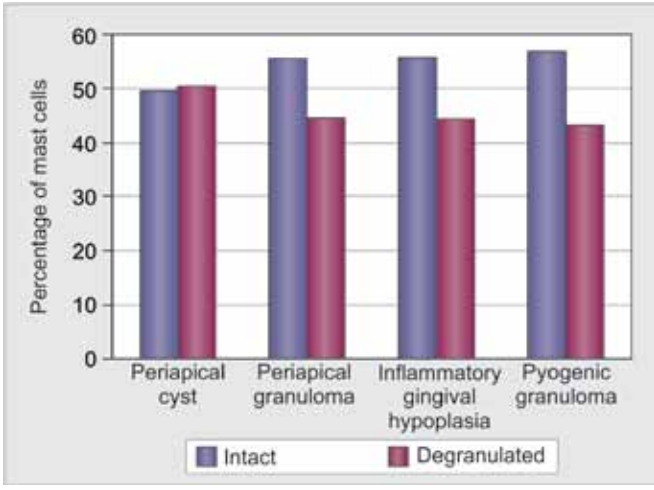
Hagiwara K, et al (1999) stated that the average density of mast cells in pyogenic granuloma was 103.5 ± 25.2[15]

Table 3: Compilation of values with p-value in lesions

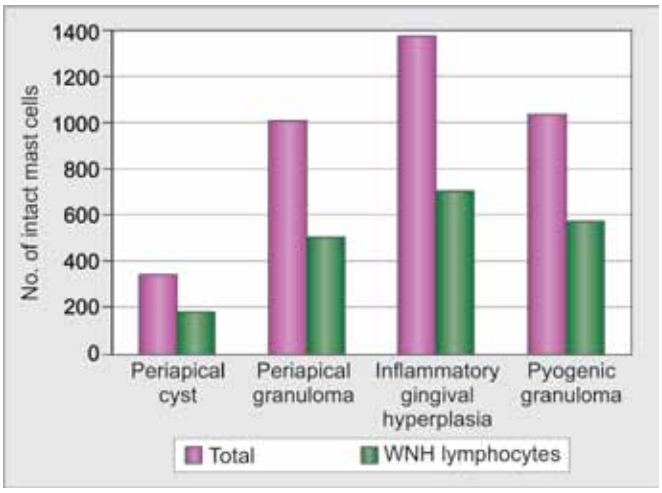
Groups	*N*	*Mean*	*SD*	*Min*	*Max*	*F*	*p-value*
Periapical cyst	14	24.36	24.08	0	66	4.268	0.01
Periapical granuloma	11	57.55	38.08	2	106		
Total periapical lesions	25	38.96	34.65	0	106		
Inflammatory gingival hyperplasia	12	29.75	31.26	0	95		
Pyogenic granuloma	13	79.54	70.51	3	260		
Total gingival lesions	25	55.64	59.82	0	260		



Graph 1: Mean total of mast cells in the groups



Graph 2: Percentages of intact and degranulated mast cells in the groups



Graph 3: Total number of mast cell and mast cells associated with lymphocytes

per hpf, which was much higher in comparison to the mean value in the present study, which is 79.54 ± 70.51 mast cells per hpf.

Evaluation of Association of Mast Cells with Lymphocytes and Comparison with Other Studies

We found mast cells to be more numerous in regions of active inflammation as seen in other studies done by Rodini DOC et al (2004),[17] Montes LC et al (2004),[8] Smith et al (1989),[13] Steinsvoll S et al (2004),[10] Günhan M et al (1991)[18] and Günhan M (1989).[19]

But, this finding is in contrast to the study done by Sudhakar R et al (2005)[14] and Zachrisson BU (2006),[9] where an inverse relationship between mast cells and inflammation was found.

In our study, most of the mast cells (52%) were in close proximity to lymphocytes. This finding is in concordance with the studies of Oliveira D et al (2004),[17] Ledesma et al (2004),[8] and Batista et al (2005).[16] Degranulation of mast cells was a frequent finding in the inflamed areas. This was in concordance with the studies conducted by Montes LC et al (2004)[8] and Walsh LJ et al (1995)[20] and in contrast to the study conducted by Babál P et al (1989)[21] where they found mast cells in periapical granulomas showed no signs of marked degranulation.

Role of Mast Cells in the Pathogenesis of Inflammatory Periapical and Gingival Lesions

The induction of inflammation by mast cells is consequent upon the release of preformed potent biological mediators as well as secondary mediators.[2] In gingival lesions, products from the bacterial plaque at the gingival margin may directly or indirectly induce proliferation of mast cells in the adjacent connective tissue. Above a certain degree of stress, however, the mast cell may respond by degranulation and release its active substances, which contribute to the inflammatory reaction.[9]

The association of IgE with mast cells in periapical lesions allows us to consider the possibility of IgE hypersensitivity reaction occurring within these lesions. Proposed sources of allergen have included not only the bacteria and their products but also denatured host tissue. Activation of the mast cells leads to release of preformed elements and generation of potent biological substance leading to inflammation.[2]

In previous studies and in the present study, mast cells are found to be more numerous in regions of active inflammation in both inflammatory periapical and gingival lesions.[3,8,13,17]

A functional relationship between mast cells and T lymphocytes has been suggested by Olivera D et al (2004). T cell-mast cell interactions have been shown to be bidirectional, fulfilling mutually regulatory and/or modulatory roles, including influences on cellular processes, such as growth, proliferation, activation and antigen presentation. Also, T-cell-derived mediators, such as β-chemokines, directly

induce mast cell degranulation. A functional relationship between these two cell population may facilitate elicitation of an immune response contributory to the pathogenesis of periapical lesions[17] and the same may apply to inflammatory gingival lesions also.

It is believed that mast cells play a significant role in promoting angiogenesis, probably by secreting several potent angiogenic factors, including heparin, histamine, VEGF and tryptase.[22] These findings and the presence of mast cells in association with blood vessels in the present study indicate that mast cells may function as one of the factors for angiogenesis in inflammatory gingival and periapical lesions.

Mast cell derived interleukin-1 causes increased fibroblastic response and tryptase causes increased production of type I collagen and fibronectin thereby leading to increased fibrosis.[23,24] Histamine may also interact with and promote increased fibroblast proliferation.[1,25-27] These findings and the presence of mast cells in association with fibroblasts in our study suggest a possible role of mast cells in enhanced collagen synthesis and fibrosis seen in the later stages of inflammatory periapical and gingival lesions.

Studies have shown that stimulated mast cells may release interleukin-1, which causes increased epithelial proliferation in leukoplakia.[23] This property of mast cell derived interleukin-1 may act as one of the factors in the proliferation of epithelium (cell rests of Malassez) in periapical granuloma leading to cyst formation. Interleukin-1 may also play a role in epithelial thickening in inflammatory gingival lesions.

Role of Mast Cells in Degradation of Extracellular Matrix and Bone Resorption

Mast cells contain tryptase and chymase; these are proteolytic enzymes that take part in degradation of extracellular matrix.[8] Thus, they help in breakdown of proteoglycans of the connective tissue capsule of the periapical cyst leading to its expansion.

The mast cell derived mediators like histamine, heparin, tryptase, TNF, IL-1, IL-6, prostaglandins and other arachidonic acid metabolites are known to cause bone resorption.[8,13,20] Mast cell derived tryptase can activate matrix metalloproteinases which help in extracellular matrix degradation and bone resorption.[8,10] These findings suggest that one of the main activities of the mast cells in periapical lesions is related to bone destruction for enlargement of the lesions.

The hydrostatic pressure of the luminal fluid is important in cyst enlargement and mast cell activity might contribute to this by increasing the osmotic pressure in at least three ways:

- By direct release of heparin into the luminal fluid.
- By release of hydrolytic enzymes which could degrade capsular extracellular matrix components thereby facilitating their passage into the fluid.
- By the action of histamine on smooth muscle contraction and vascular permeability encouraging transudation of serum proteins.[13]

CONCLUSION

In the present study, increased number of mast cells in various inflammatory periapical and gingival lesions was found. Mast cells were found to be distributed in different location of histological sections of various inflammatory lesions and in several patterns: subepithelially, in isolated groups in the connective tissue and as isolated cells. Some cells were in association with fibroblasts, some with blood vessels and several with lymphocytes. Many of the mast cells were found to be degranulated. Taking together our results and previous studies, it can be stated that mast cells serve a critical role in initiation, development, and progressin of inflammatory periapical and gingival lesions by their association with increased vascular permeability,[20] angiogenic response,[15,23] collagen synthesis,[23,24] regulation of inflammation,[3,9,28] bone resorption[8,13,20] and extracellular matrix destruction.[8,10]

This information about the role of mast cells in the initiation, development and progression of these lesions goes a long way in improving our understanding of their pathogenesis and therapeutic implications.

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