Immunoregulatory Role of Mast Cells in Apical Granulomas and Periapical Cysts-An Immunohistochemical Study

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Abstract

Objective: The aim of the study was to examine the immunohistochemical expression and localization of mast cell tryptase in apical granulomas and periapical cysts in order to enhance the understanding of inflammatory and immunologic phenomenon associated with the evolution of periapical lesions.

Materials and methods: Biopsy specimens of 30 periapical lesions were stained with hematoxylin–eosin, and immunohistochemical Mast Cell Tryptase from Bio SB (IHC detection system kit) antibody. The tryptase positive mast cells and mononuclear inflammatory cells were counted in 10 consecutive high power fields (100X) using the binocular microscope from Motic attached to a computer with Motic Advanced Images 3.2 software.

Results: Mast cells were present in active inflammatory areas as well as peripheral regions of both periapical lesions. In the periapical cyst group, mast cells were located just beneath the cystic epithelium, in the connective tissue and in the intraepithelial areas. The presence of mast cells was greater in cysts than in granulomas (the percentage mean and standard deviation of mast cells in the apical granuloma and periapical cyst group, being 6.05 ± 5.27% and 10.91 ± 7.92%). Degranulated mast cells were more in cyst than periapical granulomas (p=0.002).

Conclusions: The findings of present study could suggest a potential role of mast cells in regulation of cellular immune mechanisms in periapical lesions.

Key Words: *Periapical lesions, Mast Cells, Degranulated mast cells*

Introduction

The chronic inflammatory periapical lesions appear small in size, they should not be underestimated as even the best endondontic treatment modalities, at times, fail to conquer these tiny looking lesions. This may be due to the failure to understand the pathogenesis. The periapical inflammatory lesion is the response of the periapical tissues to chronic irritation caused by microbial, chemical, and mechanical stimuli coming from the pulp through the root canal systems [1]. The presence of different inflammatory cells such as polymorphonuclear leucocytes, macrophages, lymphocytes, plasma cells, mast cells, basophils and eosinophils, indicates the existence of local immune reactions in these lesions. The role of mast cells as the key effector of allergic inflammation, anaphylactic inflammatory reactions and in the pathogenesis of chronic inflammation, is well known [2]. Torbinejad and Bakland proposed that IgE-mediated reactions could play an important role in the initiation and perpetuation of the periapical lesion if mast cells and plasma cells containing IgE are present. Perrini and Fonzi suggested that IgE has an active role in the pathogenesis of the periapical granuloma, and that the anaphylactic and hypersensitivity reactions represent an active immunologic phenomenon in the pathogenesis of these lesions [3].

The expression of mast-cell-specific serine proteases (tryptase and chymase) is the current gold standard for determining mast cell phenotypes in various tissue compartments [4]. Staining mast cells with toluidine blue seems to be dependent on an intact number of mast cell granules, whereas the most sensitive immunohistochemical techniques are able to detect partially degranulated mast cells, which still contain enough tryptase [2].

The aim of this study was to examine the immunohistochemical expression and localization of mast cell tryptase in apical granulomas and periapical cysts in order to enhance the understanding of inflammatory and immunologic phenomenon associated with the evolution of periapical lesions.

Materials and Methods

Biopsy specimens of 30 periapical lesions, a total of 15 cases each of apical granuloma and periapical cyst were included in this study. Cysts generally showed well defined cavities lined by stratified squamous epithelium of varying thickness, with a moderate to intense infiltration of inflammatory cells and well encapsulated. Periapical granulomas generally showed intense infiltration of inflammatory cells, and foamy macrophages with no evidence of epithelial lining. Samples were selected based on microscopic examination of slides stained with Hematoxylin and Eosin For microtomy, paraffin blocks of the selected subjects were taken and sectioning was done using semiautomatic Rotary Microtome for making 5µ thick sections (Leica RM 2245) and were mounted on super frost slides (size 25×75×1.0mm). Immuno histochemical study was carried out using polymer labeling technique (Dako Envision). Sections were dewaxed, washed in alcohol and antigen retrieval was carried out in a Decloaking Chamber (Biocare) with 10 mM Citra solution at 125ºC for 30 seconds followed

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by 90ºC for 10 seconds. Slides were cooled naturally and brought to room temperature. Then they were placed inside the Dako Autostainer Universal Staining System (Automated Immunohistochemistry Staining System). Endogenous peroxidase was blocked by using 0.3% hydrogen peroxide in methanol at room temperature for 10 min. Sections were washed PBS (Phosphate Buffered Saline) briefly and incubated with primary antibody (Mast Cell Tryptase from Bio SB) for 60 min. Diaminobenzidine (DAB) was used as the chromogen in hydrogen peroxide for 10 minutes. Sections were then counterstained with haemotoxylin and mounted. The mast cells usually take up brown stain against blue staining stroma, fibroblasts and inflammatory cells. Mast cells could be of varying shapes, some of them with intact cell membrane whereas others showing degranulation. In some of the cells, the nucleus could be masked by the mast cell granules. So, these criteria were followed for evaluation of slides.

Mast cells were counted in a representative section of each slide. These cells were counted in 10 consecutive high power fields (100X) using the binocular microscope from Motic attached to a computer with Motic Advanced Images 3.2 software. The grid consisted of 100 squares (*Figure 1*) each measuring 1µmx1µm in size. The fields containing artefactual changes such as chatter, tears etc were omitted from the study.

Granules lying irregularly in the stroma were not taken into account. Data analysis and database management were done using SPSS (statistical package for social science) version 16.00. Level of significance was set up at $p < 0.05$. T-test for equality of means was applied.

Results

General and qualitative microscopic analysis indicated mast cells to be present in active inflammatory areas as well as peripheral regions of both periapical regions (*Figure 2*). In the periapical cyst group, mast cells were also located just beneath the cystic epithelium, in the connective tissue and in the intraepithelial areas (*Figure 3*). Negative controls used the study did not show any staining, confirming the specificity of the procedure. The readings of average number of mast cells, inflammatory mononuclear cells and degranulated mast cells

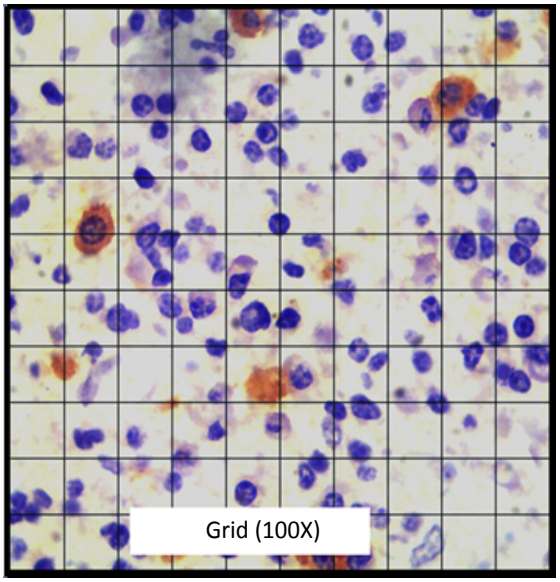

Figure 1. *Grid (100x).*

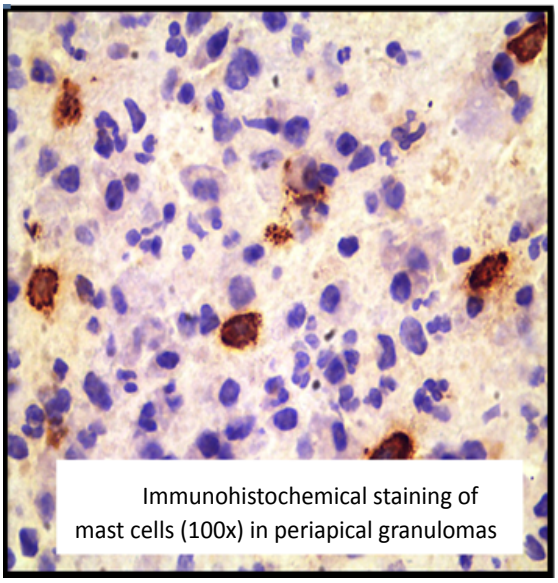

Figure 2*. Immunohistochemical staining of mast cells (100x) in periapical granulomas.*

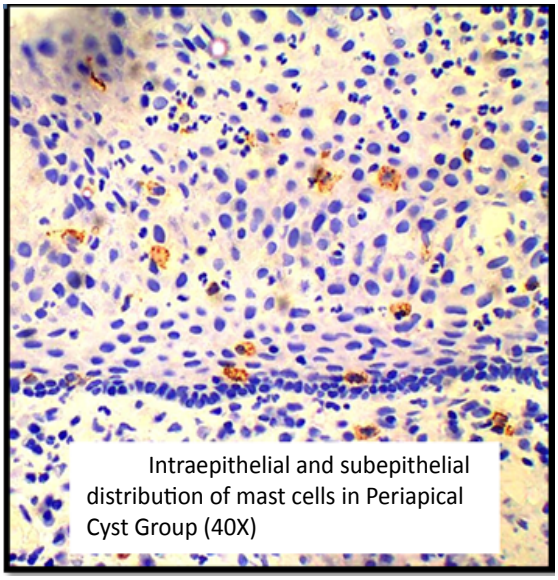

Figure 3*. Intraepithelial and subepithelial distribution of mast cells in Periapical Cyst Group (40 x).*

are shown in recorded are shown in *Tables 1,2* in each of the two groups. *Figure 5* shows comparison of mast cells between two groups and *Figure 6* shows comparison of degranulated mast cells between two groups

Table 3 shows the percentage mean and standard deviation of mast cells in the apical granuloma and periapical cyst group, being 6.05 ± 5.27% and 10.91 ± 7.92%. Comparison of percentage mean of number of mast cells between the two groups using T test for equality of means showed a significant difference between the two groups, being higher in the periapical cyst group. (p=0.000). *Table 4* shows comparison of mean and standard deviation of the number of degranulated mast cells between the two groups. Degranulated mast cell (*Figure 4*) mean is more in the cyst group as compared to the granuloma (p=0.02).

Discussion

The periradicular lesions formed are the result of the local defense reactions against the bacterial challenge. Periradicular granulomas represent a subsequent reparative

Table 1. *Mean number of mast cells in Apical Granuloma group.*

Mean MC	Mean of total number of mast cells
Mean DMC	Mean of total number of degranulated mast cells

Table 2. *Mean number of mast cells in Periapical Cyst group.*

Sr.no	Mean MC	Mean DMC
1	3.2	0.5
2	4	0.8
3	3.8	0.6
4	4.1	0.8
5	3.2	0.3
6	3.6	1.2
7	2.9	0.3
8	2.9	0.1
9	2.7	0.3
10	2.2	0.1
11	4	0.6
12	2.9	0.4
13	3.1	0.7
14	1.9	0.3
15	3.2	0

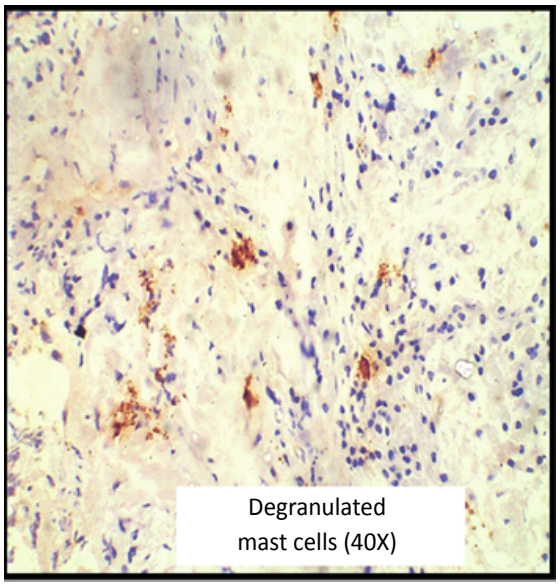

Figure 4. *Degranulated mast cells (40x).*

process of chronic local inflammation whilst periradicular cysts are thought to be derived from epithelial rests within or adjacent to granulomatous tissue. The tissues of both cysts and granulomas are infiltrated by specific and nonspecific cells involved in the local immunological responses. The local antigen presentation taking place in periradicular tissues leads to activation of immune cells [5].

Among the immunological studies, both humoral and cell-mediated reactions have been implicated during the course of periapical lesions. Infiltrates of T lymphocytes, indicating the cellular immune reactions and the presence of B lymphocytes, plasma cells and immunoglobulins, indicating a humoral immune response suggest the contribution of immunological phenomenon associated with the periapical lesions. Mast cells may also contribute to the immune mechanism thereby regulating the immune reactions [2].

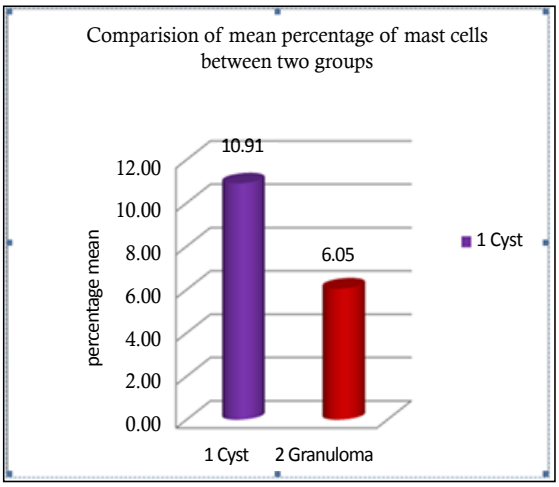

Figure 5. *Comparison of mast cells between two groups.*

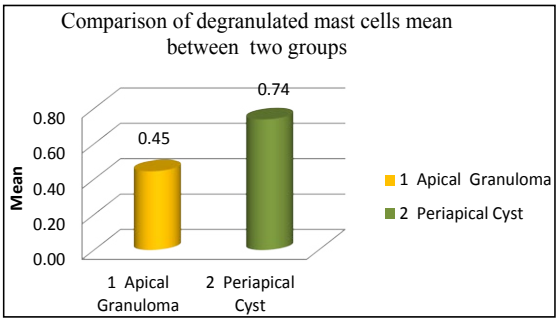

Figure 6. *Comparison of degranulated mast cells between two groups.*

Table 2. *Mean number of mast cells in Periapical Cyst group.*

Sr.no	Mean MC	Mean DMC
1	4.2	0.1
2	3	0.3
3	5	0
4	6	0
5	3.8	0.5
6	6.6	2.6
7	4.2	0.6
8	3.6	0.9
9	4.9	0.8
10	5.5	1.9
11	3.5	1.2
12	3.8	0.2
13	5.2	0.3
14	4	0.8
15	3.2	0.7

The present study was adopted to localize the mast cells and quantify their number and compare them between the apical granulomas and periapical cysts so as to propose a role of mast cells in the pathogenesis of these lesions. One of the first studies by the Matheisen demonstrated the presence of mast cells in granulomas and cysts, but without description of its localization. Another study quantified number of mast cells restricting only to periapical granulomas, based on staining with toluidine blue [2]. Till date, only six reports quantified the number of mast cells in periapical lesions.

Mast cells cannot be viewed using H & E stain. Various

special stains and immunohistochemical methods have been implied to study mast cells. Toluidine blue, Azure A, Alcian blue, Bismarch brown and Thionin are some of the special stains used to identify mast cells. All these use the property of metachromasia of mast cell granules [2,3,6].

In a study conducted by AC Batista et al. on mast cells in periodontal disease, the number of tryptase-positive mast cells was significantly higher than the number of toluidine blue-stained cells within any groups examined [7]. Therefore, immunohistochemical method using mast cell tryptase was used in the present study which is more specific and stains both immature and degranulated mast cells.

Based on immunohistochemical analysis, we observed periapical cysts to exhibit greater percentage of mast cells than that found in apical granulomas. It is possible that mast cells are more frequently found in cysts as these longstanding lesions are composed of fibrous capsule which contain more mast cells than granulomas.

These results are in accordance with the study carried out by Rodini et al. however, another study conducted by Dražić et al. found no difference in semiquantitative intensity of the presence of mast cells between granulomas and cysts [2,3]. Mast cells could be associated with bone tissue destruction and growth of human periapical granulomas [3]. More number of mast cells in the cyst group may indicate more destruction of bone and hence contribute to the expansion of the cyst. The presence of degranulated mast cells in periapical lesions has been studied by many authors but none of them have quantified their number, nor have they made any comparison between the cyst and granuloma group. According to the present study, significantly more number of degranulated mast cells has been found in the cyst group than the granulomas (*Table 4*, *Figure 6*). More degranulation indicates more active mast cells and hence more release of mediators. The presence of degranulated mast cells in the periapical cyst, as recorded in the present study may be responsible for the release of various mediators such as heparin, histamine, proteolytic and hydrolytic enzymes. One of the mediators is TNF-α which has been implicated as an important factor in promoting the chronicity of the lesion [8]. So, based on this concept it may be possible to use drugs therapeutically in order to influence mast cell secretion and therefore thwart inflammation. The secretory products such as proteases (chymase and tryptase) contribute to connective tissue breakdown which may justify the abscess theory in the pathogenesis of periapical cysts [9].

Another effect of the presence of degranulated mast cells in the periphery of the lesion may suggest their role in the resorption of bone thus promoting cyst growth. Mast cell tryptase, heparin and prostaglandins have been implicated in the process. In addition, many of the cytokines produced by activated mast cells, such as IL-1, IL-6 and TNF-α in particular have been shown to increase local osteolytic activity. Furthermore, in systemic mastocytosis, which is characterized by an abnormal proliferation of mast cells, approximately 60-70% of the patients develop radiographically detectable lesions. Inhibition of mast cell mediator release with ketotifen was shown in one case to lead to reversal of bone changes [10]. So, more number of degranulated mast cells in the cyst group suggests more resorption of the bone and hence expansion of the lesion.

The presence of mast cells in the epithelium, as seen in the present study, indicates the movement of intact mast cells through the epithelium and would account for the presence of glycosaminoglycans in cyst fluid. Mast cells are recognized as a mobile cell population, capable of migration within the tissues [9]. This implies a chemotactic stimulus to mast cells in periapical cysts attracting them to the epithelial lining or luminal fluid contents. The nature of such stimuli is unclear, but it is interesting that the secretary matrix proteins of normal odontogenic epithelium have been reported to be chemotactic to mast cells [11]. As the hydrostatic pressure of the luminal fluid is important in cyst enlargement, mast cell activity may contribute to this by increasing the osmotic pressure of the fluid. Therefore mast cells may be implicated in the pathogenesis of periapical cysts.

Another observation was the presence of mast cells lying close to the plasma cells among the inflammatory infiltrate. Perrini and Fonzi suggested that hypersensitivity is an active phenomenon in the periapical granulomas. Their opinion was based on the fact that these lesions contain IgE and that possibly mast cells degranulate. The mast cells have specific immunological receptors for IgE antibody [12]. Torbinejad and Bakland mentioned that IgE- mediated reaction could play a role in the initiation and perpetuation of the periapical lesions if mast cells and plasma cells containing IgE are present in periapical granulomas [4]. Therefore, hypersensitivity reaction could be implicated in the pathogenesis of periapical lesions. This finding suggests possible contributions of mast cells and their mediators in chronic inflammation, as well as the functional relationship of mast cells and immunocompetent cell populations in periapical lesions.

Taking together our and previously discussed results, it could be presumed that mast cells are implicated in regulation of cellular immune mechanisms in periapical inflamed tissue. The interaction of mast cells and other immune cells (T cells and plasma cells) indicates an immunoregulatory role of mast cells in the pathogenesis of periapical lesions. The localization of these cells in different zones of the periapical lesions suggests their participation in mechanisms of cyst expansion by various mechanisms and their mobile nature.

Table 3. *Percentage of mast cells to total cells - comparison between the two groups. Group Statistics (mean and standard deviation of percentage of mast cells).*

Group		N	Mean	Std. Deviation	Std. Error Mean
Percentage	**1 Cyst**	150	10.9107	7.91839	0.64653
	2 Granuloma	150	6.0546	5.27238	0.43049

Table 4. *Comparison of the Number of Degranulated Mast Cells between the Two Groups. Table Group Statistics (mean and standard deviation of number of degranulated mast cells).*

	Group	N	Mean	Std. Deviation	Std. Error Mean
DMC	**Apical Granuloma**	150	0.45	0.848	0.069
	Periapical Cyst	150	0.74	1.266	0.103

The various mediators released on degranulation of mast cells, each in their own way, contribute to the pathogenesis of periapical lesions. Since only a few studies have been carried out to investigate the nature of mast cells in these lesions, further research in this field is required to define/unfold the exact participation of mast cells and their products in the initiation and perpetuation of these lesions.

Conclusion

Mast cells may play a potential role in regulation of cellular immune mechanisms in periapical lesions but further study would be required for further confirmation.

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