

Quantitative Assessment of Mast Cells in Oral Reactive Lesions

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Abstract:

Objective: To identify and Quantify mast cells and Evaluate its localization in different oral reactive lesions.

Material And Method: 10 cases of each diagnosed reactive lesions were retrieved from the Departmental archives, which included pyogenic granuloma, irritational fibroma peripheral ossifying fibroma and peripheral giant cell granuloma. Confirmed cases were stained with 1% toluidine blue for mast cells. The mast cells were then counted under 40X magnification and was compared between the study groups.

Results: The Mean \pm SD value for mast cells in pyogenic granuloma were found to be 28.6 ± 15.68 , irritational fibroma 23.5 ± 3.02 , peripheral ossifying fibroma 65.7 ± 12.06 , peripheral giant cell granuloma 5.2 ± 2.04 . ONE WAY ANOVA test revealed that mast cells are highly significant in peripheral ossifying fibroma and was randomly distributed throughout the lesion. Mast cells were observed in all reactive lesions. The presence of mast cells was highest in Peripheral Ossifying Fibroma.

Conclusion: Mast cells seem to have a more potent role in peripheral ossifying fibroma than in other oral reactive lesions. However a study on larger sample size is required for a definitive association.

Keywords: mast cells, toluidine blue, oral reactive lesions

I. Introduction

Reactive lesions are painless production of hyperplastic tissue resulting from a repair response.¹ Reactive hyperplastic lesions represent the most frequently encountered oral mucosal lesions representing a reaction to some kind of irritation or to low grade injury.²

Kfir et al (1980) have classified oral reactive lesions into pyogenic granuloma (PG), peripheral ossifying fibroma (POF), peripheral giant cell granuloma (PGCG) and fibrous hyperplasia.²

Mast cell (MCs) are thought to influence the formation and course of reactive lesions by maintaining the leukocyte migration.⁵ Mature mast cells are usually found at the basement membrane, connective tissue and mucous membranes as well as at the central and peripheral nervous systems.³ Several studies have been done on mast cells in normal mucosa. It has been shown to vary to some extent from region to region.^{14,15}

Mast cell produce, store and release an array of relevant mediators³. Mast cells have both pro-inflammatory and anti-inflammatory effects.⁵

As an anti-inflammatory action mast cell proteases cleaves and inactivates various interleukins IL-5, IL-6, IL-13, TNF, Endothelin 1, Anaphylatoxin C3a thus restraining the excess inflammation.⁶

As a pro-inflammatory action mast cells promotes neutrophil aggregation, eosinophil aggregation, T lymphocyte stimulation and also influence the endothelial cells along with the mediators that are released immediately upon activation thus contributing during process of repair by neo-angiogenesis, fibrinogenesis, re-epithelization.³ Till now very few work has been done to evaluate the role of mast cells in oral reactive lesions. Hence this study is conducted to assess the mast cells in oral reactive lesions.

II. Materials And Method

40 reactive lesions were retrieved from the Departmental archives, which included 10 cases of each pyogenic granuloma, irritational fibroma, peripheral ossifying fibroma and peripheral giant cell granuloma

Staining For mast cells: The samples were retrieved from archives which were fixed in formalin embedded in paraffin, serially sectioned at 5um thick and stained with haematoxylin and eosin to confirm the diagnosis. Cases which had overlapping features were not considered in the study. Later Confirmed cases were stained with 1% toluidine blue to visualize the mast cells. Afterwards the sections were dehydrated, cleared and

mounted with DPX mountant. The mast cells were then calculated under 40X objective in 5 different clear fields without repetition in a zig zag pattern for their localization and quantification and was then compared between the study groups. This Semi Quantitative assessment for the presence of MC count was divided into grades as (Table I). The comparison between different groups was performed with one way ANOVA test. A p value <0.05 or less was designated as statistically significant.

III. Results

Statistical Analysis:

Among 40 cases the following lesions were recorded and their mean standard deviation (\pm SD) for mast cells was calculated. (Table II)

The reticular connective tissue was considered as superficial whereas connective tissue with adipose tissue, salivary gland, muscle was considered as deeper connective tissue for localisation of mast cell.

In our study compared to all other reactive lesions, the quantitative assessment with mean SD value for mast cells was found to be highest in peripheral ossifying fibroma and least in PGCG. (Graph I & Table III)

In our study localization of mast cells (MCs) were found more in deeper connective tissue than in superficial connective tissue (Pie chart I). According to the lesion per se in PG, irritational fibroma and POF, MCs were less in superficial CT than in deeper CT and were randomly scattered and in deeper CT they were mostly close to blood vessels and in the areas of inflammation. Whereas in POF mast cells are also seen around the areas of calcifications. (Pie chart II)

In PGCG the MCs were far and few and did not show any particular localization.

Hence, in our study MCs were found in all the reactive lesions. But the numbers were significant in POF, PG and irritational fibroma while they were insignificant in PGCG.

IV. Discussion

It was found that there is an increase in the average mast cell count in the inflammatory lesions which may lead to inflammatory changes by release of their chemical mediators. The mast cells on stimulation by various etiological factors may increase in number and subsequently undergo degranulation and cause inflammatory and vascular changes leading to formation of reactive lesions. It was found that mast cells number was more in inflamed mucosa as compared to normal mucosa.¹⁵

Mast cell contains potent mediators, including histamine, heparin, proteinases, leukotrienes, and multifunctional cytokines that contributes to processes of inflammation, angiogenesis and matrix degradation.¹⁴

In PG it is said that local etiological factors like gingival inflammation, calculus and trauma activate MC resulting in release of mediators that leads to subsequent inflammatory and vascular changes.⁶

In our study, the no. of MC's in Pyogenic granuloma were higher with mean (SD of 28.6 ± 15.68) than rest of the reactive lesions except POF which have highest number of mast cells and another observation made was that there was no sequential increase in the infiltration of MCs from early to late stage of pyogenic granuloma. This indicates that the number remains same irrespective of the stage of the disease process. At the same time in irritational fibroma the mean SD (23.5 ± 3.02) and also within the moderate grade. Thus it can be assumed that MCs may have a similar role as that of PG.

It is thought that the MCs have an impact on the formation of new blood vessels by stimulating the migration and proliferation of endothelial cells for the formation of neovascular sprouts.⁷ An increase in the average number of mast cell count was found in vascular lesions and it was suggested that release of mediators from mast cells may lead to neoangiogenesis. (de Oliveira Rodini et al.)¹⁵

The mast cells also activate fibroblast via tryptase that synthesize collagens as well as hyaluronic acid and contributes to fibrous tissue formation. Mast cells along with fibroblasts attaches to fibronectin hence attributing in increased fibrosis.⁶ Furthermore it is not clear in the literature that how a long standing PG changes from a highly vascular lesion to a fibrosed one. Since in our study the number of mast cells did not alter in early or late pyogenic granuloma so it can be presumed that only the mediators synthesized by MC may change rather than variation in the number.

In peripheral ossifying fibroma (POF), the mean number of MC's was 65.7 ± 12.06 and was highest than in other oral reactive lesions. However in spite of extensive search of literature there were no studies done so far that explains the pathogenesis of mast cells in POF. Several theories have been put forward to explain the pathogenesis of POF. Some believe that POF develops initially as pyogenic granuloma that undergoes fibrous maturation and subsequent calcification.⁸ But histologically late phase of pyogenic granuloma resembles fibroma rather than POF. While others believe that chronic irritation of periosteal and periodontal membranes causes metaplasia of connective tissue and resultant initiation of formation of bone or dystrophic calcification. It has been suggested that the lesion may be caused by fibrosis of granulation tissue.⁸

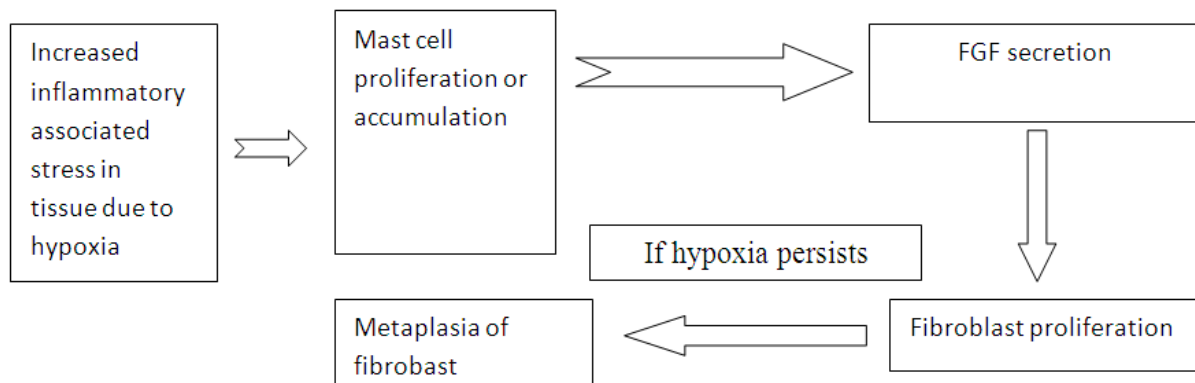
In our study the number of MCs in PG were found consistent irrespective of its stages, while in POF it was found that the MCs were almost three times higher than in pyogenic granuloma. So we believe the second

theory of metaplastic change of connective tissue could be the reason for the development of POF. This is further confirmed by Shafer's also that there is metaplastic change in POF. However the exact reason for this metaplastic change is still unknown, may be the foci of tissue injury may result in metaplasia of connective tissue.



The other supporting theories regarding calcification in POF are given by Theresa A Freeman suggested that due to tissue injury there will be mast cell proliferation and activation which results in increased mast cell number. This in turn will lead to the fibrosis. The presence of chymase, a proteolytic enzyme and cleavage of TGF- β have been associated with development of fibrotic diseases. This fibrotic disease undergoes metaplastic changes that progress to tissue calcification.

Other theory by Theresa A Freeman suggested that increased inflammatory associated oxidative stress, leading to an accumulation of mast cells (secreting FGF), driving fibroblast proliferation and creating avascular regions of hypoxia. This hypoxia and associated stress if persists induces the metaplastic conversion of fibrotic tissue to fibrocartilage and subsequent bone formation.



Thus we presume that MCs have a role in pathogenesis of POF.

Lastly the number of mast cells in Peripheral Giant cell Granuloma (PGCG) is very less in our study with mean SD of 5.2 ± 2.04 , which was within mild grade. We suppose that their presence is only an incidental finding. In PGCG multinucleated giant cells are actually the osteoclasts left from the physiological resorption of teeth or reaction to injury and MCs have no role to play in PGCG.¹³

V. Conclusion

This study demonstrated that MCs are present in all oral reactive lesions, thus having a possible role in pathogenesis of oral reactive lesions except PGCG. Mast cells have gained a lot of importance in recent years owing to the vast number of chemical mediators they release with wide range of actions in many of the disease processes. Once confirmed, it makes easier for us to direct the therapeutic modalities against mast cells and its granules to alter the course of disease/lesion.

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Figures:

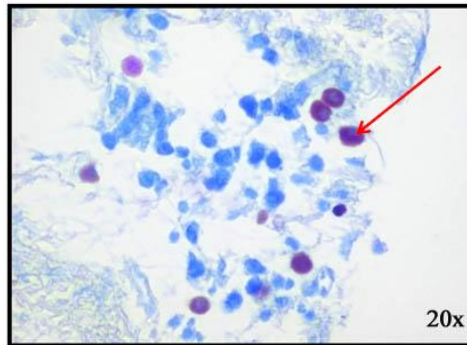


Figure 1 Peripheral Ossifying Fibroma, 20X mast cells are stained metachromatically violet with toluidine blue and are found around the areas of calcifications.

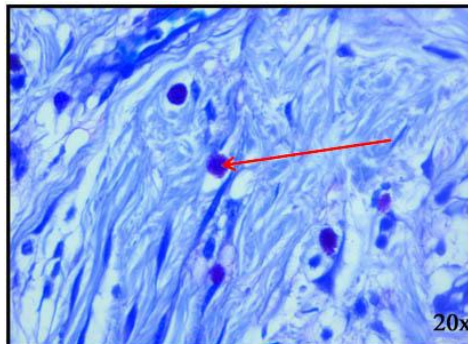


Figure 2 Irritational Fibroma, mast cells are stained metachromatically violet with toluidine blue.

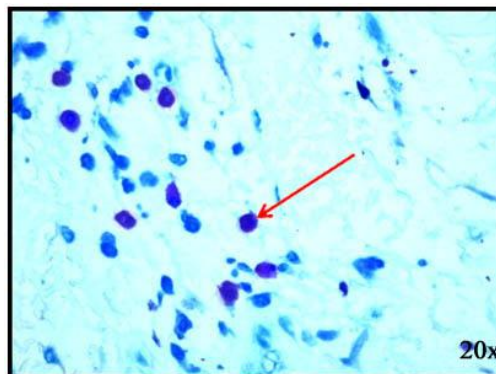
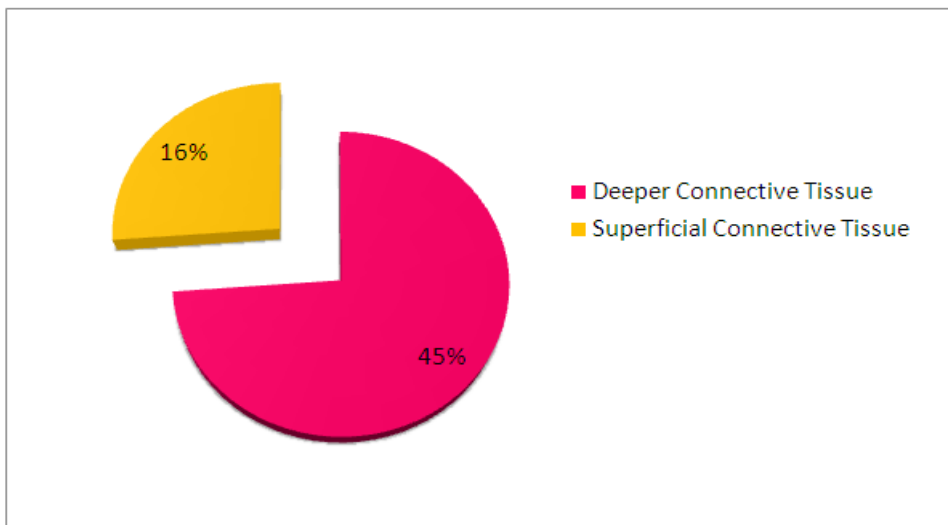
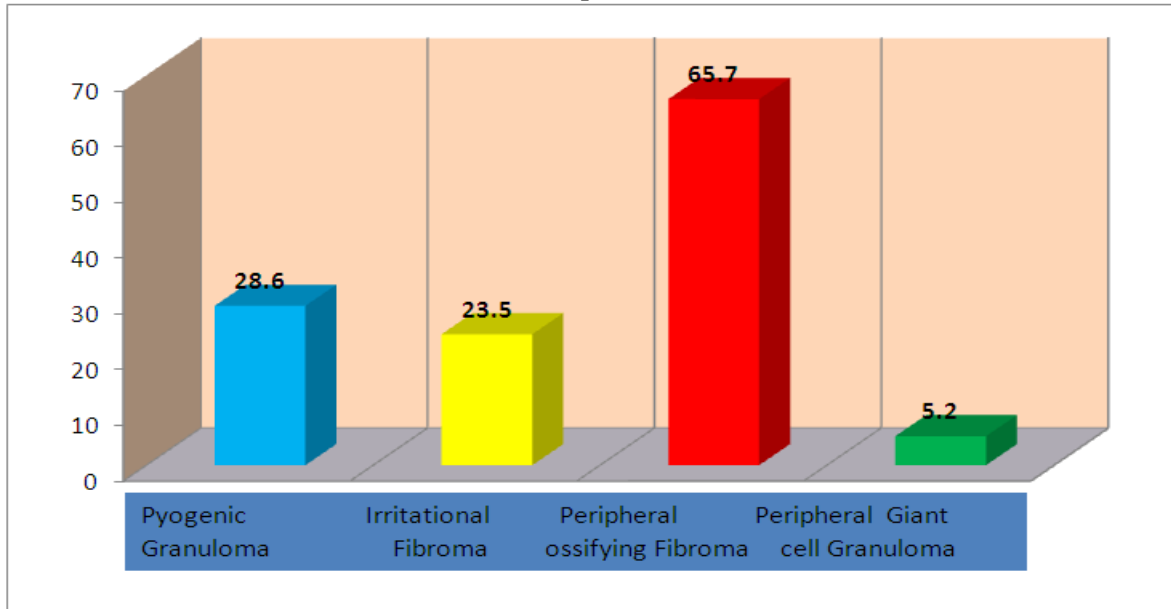


Figure 3 Pyogenic Granuloma, 20X mast cells are stained metachromatically violet with toluidine blue.

Graph And Tables:
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Pie chart I showing mast cell localization in deeper and superficial connective tissue

Pie chart II showing mast cell localization in deeper connective tissue .

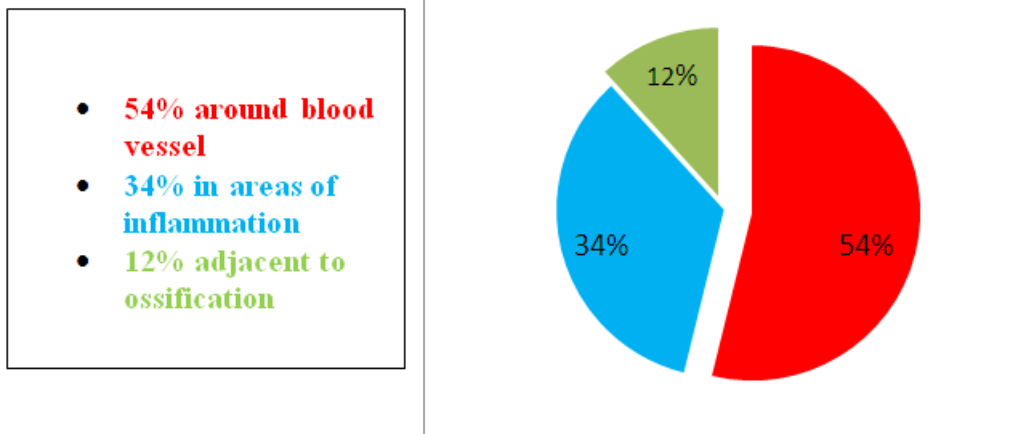


Table I:

Severe	More than 50
Moderate	10-50
Mild	Less than 10

Table II:

Lesions	Mean ± SD
Pyogenic granuloma	28.6 ± 15.68
Irritational Fibroma	23.5 ± 3.02
Peripheral ossifying fibroma	65.7 ± 12.06
Peripheral giant cell granuloma	5.2 ± 2.04

ANOVA analysis:

F value: 63.59 p value: 0.0000

Interpretation: Highly significant