Original Article

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Dentistry Section

Incidence of Mast Cells in Gingival and Periapical Inflammation- A Kaleidoscopic Study

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ABSTRACT

Introduction: Mast cells are large granular cells that have classically been related to neutrophil stimulation during early step of inflammation.

Aim: The objective of this work was to identify the incidence of mast cells in inflammatory lesions like periapical granuloma, pyogenic granuloma, gingival hyperplasia.

1. To assess the staining intensity of mast cells by using different metachromatic stains.

2. To correlate the above findings histopathologically.

Materials and Methods: In this study, we used 5 micron thick sections from paraffin-embedded tissue blocks of previously diagnosed periapical and gingival inflammatory lesions. The sections were stained with routine H & E and metachromatic stains like Toluidine blue, Alcian blue, Aldehyde fuchsin and Giemsa. The number of mast cells was quantified. Statistical analysis was done and mast cell numbers were compared.

Results: In both gingival and periapical inflammatory lesions, toludine blue showed more number of mast cells followed by giemsa. Giemsa stain showed statistical significance in differentiating both periapical and gingival lesions ($p<0.05$) in terms of mast cell count. Moderate inflammation (46.4%) was seen in a higher propotion of gingival inflammations whereas periapical inflammatory lesions revealed severe inflammation (53.3%).

In both types of inflammatory lesions, higher staining intensity was shown by toludine blue followed by giemsa which was statistically significant.

Conclusion: Mast cell number is inversely proportional to inflammatory response in gingival inflammatory lesions and directly proportional to inflammatory response in periapical inflammatory lesions. Although, toludine blue is found to be a better stain, giemsa has equivalent properties as that of toludine blue.

Keywords: Alcian blue, Aldehyde fuchsin, Giemsa, Metachromasia, Metachromatic stains

INTRODUCTION

Mast cells were first described by Paul Ehrlich in 1879 [1]. Mast cells are unicellular endocrine glands [2], which originate from CD 34+ precursor cells in the bone marrow and contain metachromatically staining cytoplasmic granules [3]. Mast cells play an important role in host reactions, IgE associated disorders, hypersensitivity reaction, mitogenesis, ECM degradation, angiogenesis, augmentation of microvascular hyperpermeability and recruitment of macrophages [4,5]. They are the initial cells which induce type recruitment of neutrophils which is the early step of inflammatory responses [5]. Stimulation of mast cells release secretory granules into the surrounding tissue by a process called Piecemeal Degranulation [6]. The degranulation causes of release immunoregulatory molecules, proinflammatory molecules, mediators like tryptase, TNF—α, IL-4 and angiogenic molecules which in turn leads to an increase in fibroblast proliferation.

Heparin, FGF, and VEGF induce endothelial cell migration and neoangiogenesis which play an important role in oral inflammatory lesions and granuloma formation. TNF-α causes leukocyte infiltration during inflammation [7-11].

Mast cell inhibitors can be used in severe inflammation, due to which it acts as a prognostic indicator, thus, keeping in mind the importance of mast cells, we made an attempt to check the incidence of mast cells in different inflammatory conditions using metachromatic stains to evaluate which stain is best to differentiate these cells in various inflammatory lesions (Kaleidoscopic study). These metachromatic stains were used to stain mast cell granules which exhibit the property of metachromasia.

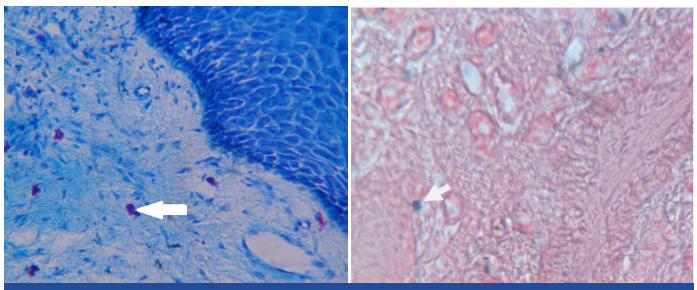
MATERIALS AND METHODS

Sample selection -The present retrospective study used paraffin-embedded tissue blocks retrieved from archives of Department of Oral Pathology and Microbiology, BBDCODS. Study sample included Periapical Granuloma, Gingival Inflammatory lesions i.e., Pyogenic Granuloma and Inflammatory Gingival Hyperplasias (15 each) and 3 normal tissues were taken as control.

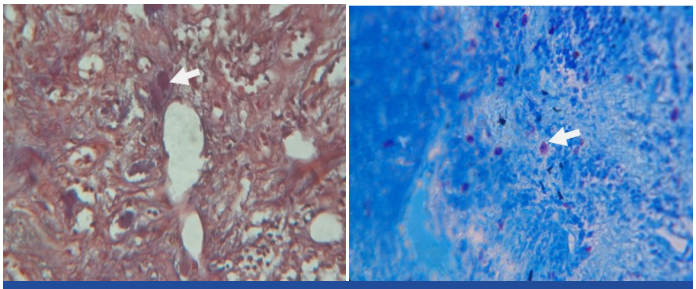
Sectioning of lesions and stains used- 5 micron thick sections were cut from paraffin embedded tissue blocks of previously diagnosed cases and processed for routine Hematoxylin & Eosin and metachromatic stains like Toluidine Blue, Alcian Blue, Aldehyde Fuchsin and Giemsa respectively.

Method of Counting- Under 40X magnification, we randomly selected 10 microscopic fields and evaluated them for staining intensity by three expert observers. The results were statistically analysed using Student t- test and one-way ANOVA.

Criteria to identify mast cells- Mast cells are oval to spindle shaped with similar staining characteristic as fibroblasts and so are



[Table/Fig-1]: Depicts mast cells stained with Toludine blue.
[Table/Fig-2]: Depicts mast cells stained with Alcian blue.



[Table/Fig-3]: Depicts mast cells stained with Aldehyde Fuchsin.
[Table/Fig-4]: Depicts mast cells stained with Giemsa.

difficult to differentiate with Haematoxylin and Eosin. Under different metachromatic staining, mast cell granules will appear purplish blue with nuclei- sky blue in toluidine blue stain [Table/Fig-1], blue in alcian blue [Table/Fig-2], purple in aldehyde fuchsin stain [Table/Fig-3] and blue in Giemsa stain [Table/Fig-4].

RESULTS

The data was collected in a systematic manner and formulated as tables and graphs derived from statistical analysis, for the interpretation of results. In both gingival and periapical inflammatory lesions, toludine blue showed more number of mast cells followed by Giemsa, (Toluidine blue>Giemsa >Alcian blue=Aldehyde fuchsin) [Table/Fig-5].

Giemsa stain showed statistical significance in differentiating both periapical and gingival lesions ($p<0.05$) in terms of mast cell count [Table/Fig-6].

Depending upon inflammatory cell density per microscopic field, on a scale of 0-3, we graded inflammation as no inflammation-0, mild-1, moderate-2 and severe inflammation-3. Among cases of gingival inflammation, higher proportion of cases were moderate (46.4%). In Periapical lesions, higher proportion of cases showed severe inflammation. (53.3%) [Table/Fig-7].

In both gingival and periapical lesions, higher staining intensity was shown by Toludine Blue Gingival Inflammation=2.60, Periapical Inflammation=2.53) which was more than giemsa (gingival Inflammation=2.40, Periapical Inflammation=2.40) and was statistically significant [Table/Fig-8]. Most of Periapical lesions showed severe inflammation (53.3%) [Table/Fig-9] whereas gingival

	Gingival				Periapical			
	Toluidine Blue	Alcian Blue	Aldehyde Fuchsin	Giemsa	Toluidine Blue	Alcian Blue	Aldehyde Fuchsin	Giemsa
Case 1	1	1	1	1	6	1	1	5
Case 2	1	0	0	1	1	0	0	2
Case 3	9	0	0	5	2	0	0	1
Case 4	2	0	0	1	1	0	0	0
Case 5	0	0	0	0	4	0	0	1
Case 6	4	0	0	4	0	0	0	0
Case 7	6	1	1	7	4	1	1	4
Case 8	0	0	0	0	2	0	0	1
Case 9	3	0	0	7	3	0	0	1
Case 10	0	0	0	0	2	0	0	2
Case 11	2	0	1	4	1	0	0	1
Case 12	8	1	0	3	0	0	0	0
Case 13	0	0	0	0	1	0	0	0
Case 14	4	1	0	2	0	0	0	0
Case 15	3	0	0	6	0	0	0	0
Mean	2.87	0.27	0.20	2.73	1.80	0.13	0.13	1.20
SD	2.90	0.458	0.414	2.604	1.78	0.352	0.352	1.521

[Table/Fig-5]: Depicts the mean number of mast cells according to different stains in gingival oral inflammatory lesions and periapical granuloma.

Stains	t-test	p-value
Gingival Toluidine Blue vs Periapical Toluidine Blue	1.214	0.235
Gingival Alcian Blue vs Periapical Alcian Blue	0.894	0.379
Gingival Aldehyde Fuchsin vs Periapical Aldehyde Fuchsin	0.475	0.638
Gingival Giemsa vs Periapical Giemsa	1.969	0.05*

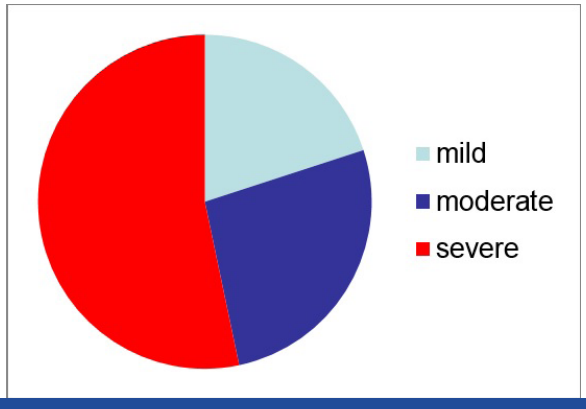
[Table/Fig-6]: Depicts the relationship between different stains among oral inflammatory lesions.

Severity of inflammation	Gingival n (%)	Periapical Granuloma n (%)
Mild	4(26.7)	3(20)
Moderate	7(46.6)	4(26.7)
Severe	4(26.7)	8(53.3)
Total (n=30)	15(100)	15(100)

[Table/Fig-7]: Depicts severity of inflammation among study population.

	Gingival				Periapical			
	Toluidine Blue	Alcian Blue	Aldehyde Fuchsin	Giemsa	Toluidine Blue	Alcian Blue	Aldehyde Fuchsin	Giemsa
Case 1	3	2	2	3	2	2	2	2
Case 2	3	2	0	3	3	2	0	3
Case 3	3	0	1	3	2	0	1	3
Case 4	3	3	2	2	2	2	2	2
Case 5	3	0	0	2	2	0	0	3
Case 6	2	2	2	2	3	2	1	3
Case 7	3	2	2	3	3	2	2	2
Case 8	3	2	0	3	3	2	0	2
Case 9	2	2	0	2	2	2	0	2
Case 10	3	0	0	3	2	0	0	0
Case 11	3	2	2	3	3	2	2	2
Case 12	3	2	1	3	3	2	1	3
Case 13	0	0	0	0	3	0	0	3
Case 14	2	1	0	2	2	0	0	3
Case 15	3	0	0	2	3	0	0	3
Mean	2.60	1.33	0.80	2.40	2.53	1.20	0.73	2.40
SD	0.828	1.047	0.941	0.828	0.516	1.01	0.88	0.828

[Table/Fig-8]: Depicts the staining intensity according to different stains in gingival oral inflammatory lesions and periapical granuloma.

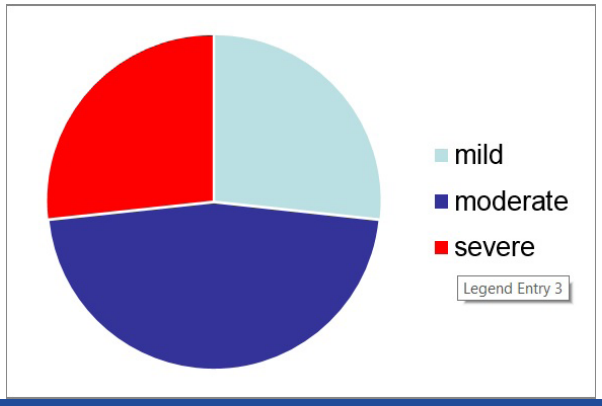


[Table/Fig-9]: Depicts periapical lesion showing severe inflammation.

lesions showed mild to moderate inflammation (46.4%) [Table/Fig-10].

DISCUSSION

Mast cells form first step in neo- vascularization and inflammatory reactions. Their degranulation activates endothelium through TNF-dependent mechanism- important in initiative phase of inflammation. In the present study the number of mast cells was more in lesions with mild inflammation [Table/Fig-5].



[Table/Fig-10]: Depicts gingival lesions showing mild to moderate inflammation.

R Sudhakar et al., stated that the predominance of mast cells under degranulation would take place in a stage much before the active stimulation of angiogenesis and subsequent inflammatory reaction [2]. This early stage could be defined as a pre-inflammatory stage which was seen before active vascularity. The increase in inflammatory cells caused a clear reduction in the number of mast cells in most of the well established inflammatory lesions [2]. Hence there is reduction in mast cell number in severe inflammatory lesions in our study. Walsh LJ et al., stated mast cells are responsive to neuropeptides; in the mucosal tissues [12], they form neural elements with langerhan cells. [2]. This causes mast cell degranulation releasing cytokines, enzymes and vasoactive amines leading to inflammation and vascular changes in pyogenic granuloma. Mast cells are not identified in Haematoxylin and Eosin stains because their metachromatic granules (heparin) are refractive and don't take up stains.

In our study toluidine blue showed more number of mast cell count in both gingival and periapical inflammations compared to other stains; Toluidine blue (gingival inflammation=2.87, periapical inflammation=1.80) >Giemsa (gingival inflammation=2.73, periapical inflammation=1.2) >Alcian blue (gingival inflammation=0.27, periapical inflammation=0.13) =Aldehyde fuchsin (gingival inflammation= 0.20, periapical inflammation=0.13); in that order.

According to Priyanka Debta et al., Toluidine blue gives good staining intensity, because mast cells granules contain heparin which are highly sulfated. Sulfated substances are highly metachromatic [13]. It stains mast cells as it is a metachromatic stain.

Toluidine blue has been the most widely used Metachromatic stain till date. It is inexpensive, reproducible, gives rapid results. In the present study giemsa showed satisfactory results in differentiating both periapical and gingival lesions ($p<0.05$) in terms of mast cell count which is in comparison with the findings of Leclere M et al., [14].

Staining intensity of toluidine blue was satisfactorily significant and it was more compared to ($p<0.05$) giemsa than alcian blue and aldehyde fuchsin, as the background contrast is better in Toluidine blue than other stains. Alcian blue and Aldehyde fuchsin were not as crisp as others staining and assessing the mast cell count. Alcian blue stains both sulfated and carboxylated acid mucopolysacchrides and sialomucins. It forms salt linkages with acid group of mucopolysacchrides. G I Horsefield stated that these mucopolysacchrides are removed when tissues are formalin fixed and paraffin embedded suggesting the failure of Alcian blue in demonstrating the mast cells as the case in our study [15].

S. Strobel et al., reported that Carnoys solution fixed specimens showed more number of mast cells [16]. Aldehyde fuchsin stains sulfated mucopolysacchrides which are removed in formalin fixed, paraffin embedded sections suggesting the failure in demonstrating the mast cells in our study.

CONCLUSION

Mast cells were more in moderately inflamed gingival lesions. (vice versa for periapical inflammation). Thus, mast cell count is inversely proportional to the inflammatory response in gingival lesions (vice versa for periapical inflammation).

Special stains allow us to see structures which we cannot appreciate using routine Haematoxylin & Eosin stain.

Toluidine staining method to demonstrate mast cells is better than Alcian blue and Aldehyde Fuchsin. We found Giemsa stain also has equal properties of toluidine blue in assessing mast cells.

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