

**MAST CELL DENSITY IN ODONTOGENIC CYSTS USING SPECIAL STAIN – A
CASE STUDY**

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Abstract

Background: The odontogenic cysts are the most commonly occurring destructive lesions of jaws .The origin of these cysts is attributed to dental apparatus or developmental or inflammatory. However, the cysts of developmental origin show secondary changes to infection The degranulation of mast cells plays an important role in the pathogenesis of these cysts.

Aims: The purpose of this study was to determine the mast cell density in different odontogenic cysts using special stain and its role in the pathogenesis of the cysts.

Material and Methods: The retrospective study was carried out in archival paraffin embedded tissues of 10 histopathologically diagnosed cases of radicular cyst, dentigerous cyst and odontogenic keratocyst which were stained by haemotoxylin and subsequently by toluidine blue. The toluidine blue sections were evaluated under microscope at 400x magnification in 4 zones and results were compared .

Results: The mast cell density was compared in all the four zones of these cysts and results were tabulated after statistical analysis.

Conclusion: Based upon observations of present study it can be concluded that mast cells play an important role in expansion of cysts. Further studies on activation of mast cell products will impart a new diagnostic approach to researchers and will unmask the fascinating aspects of this cell..

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INTRODUCTION

Odontogenic cysts are the most commonly encountered cysts in the jaws. They could be developmental or inflammatory in origin. Three most common jaw cysts – radicular cysts, dentigerous cysts and odontogenic keratocyst tumours can collectively represent upto 95% of all diagnoses. These pathologic conditions can destroy bone and undergo expansive growth in jaws as a consequence of breakdown of the extracellular matrix, built up of osmotic pressure in cystic fluid, and /or perilesional bone resorption^[1]. Mast cells are mobile, granule containing, bone marrow derived secretory cells, also known as mastocyte or labrocyte. The most striking feature of mast cells is that their cytoplasm is filled with dense metachromatic granules that stain red or violet when treated with basic aniline dyes^[2]. Mast cells or “mastzellen” (maestung—a root of the English word mastication) discovery is a result of genius and tenacity of Paul Ehrlich, who described this cell when he was medical student. Mast cells are known as ‘unicellular endocrine glands’; since on degranulation, they release a number of mediators. Mast cells are sensitized with IgE to particular foreign antigens that have already entered the body and thus serve as ‘sentinels on lookout’. Mast cells have a rather unique position among cells of the immune response^[3]. The mast cells are involved in a number of processes including bone remodeling by stimulation of tumour necrosis factor (TNF)^[4]. Mast cells contribute to increase in size by directly releasing heparin in to the lumen^[5]. Mast cells degranulation plays an important role in the inflammatory response and it is speculated that alteration in their number and distribution could contribute to the pathogenesis of odontogenic cysts^[6].

The present study aimed at evaluation of mast cell density in different types of odontogenic cysts and its possible role in their expansion

Objectives –To determine mast cell density in different odontogenic cysts.

SUBJECTS AND METHODS:

The present retrospective study was carried in the department of Oral Pathology and Microbiology, Sharad Pawar Dental College, Sawangi (M), Wardha. The Institutional Ethical Committee gave the approval for the study. Paraffin embedded tissue blocks 10 each of radicular cyst, dentigerous cyst, odontogenic keratocyst sections of size 5 microns were cut and stained with toluidine blue (1% toluidine chloride), mounted with DPX followed by counting under 400 x magnification .

Inclusion Criteria:

Histopathologically confirmed cases of Dentigerous cyst, Radicular cyst, Odontogenic Keratocyst was included in the study .

Exclusion Criteria:

1. The histopathologically stained tissues that are devoid of the epithelium will not be considered for the study.
2. Cases with Insufficient case records are excluded from the study.
3. Insufficient tissue samples are excluded from the study.

Criteria to identify mast cells

Mast cells are spindle to ovoid shaped and have same staining characteristics as fibroblasts in haematoxylin and eosin staining. Hence, they are difficult to differentiate from fibroblasts. Selective stain of 1 % toluidine blue is used for mast cells. Mast cells are purplish red and nuclei appear sky blue in colour.

Counting of mast cells:

Mast cells were counted in 10 areas and under 400x magnification using graticule scale which was divided into four zones: Intraepithelial zone, intermediate zone, subepithelial zone and deep zone. For intraepithelial zone the graticule scale was oriented along the basement membrane zone and along the epithelial - connective tissue

interface for counting in subepithelial zone. The graticule was then moved further down into two microscopic fields into the capsule and the procedure repeated for intermediate zone. It was then moved further down two microscopic fields into the capsule to a third level (deep zone) and the counting was performed in similar manner.

Statistical Analysis:

The statistical analysis was carried out using SPSS (Statistical and analysis software package) version 16.0 .The mean standard deviation was calculated for mast cell counting in each zone and Kruskal Wallis and Mann-Whitney test was applied and results were noted.

RESULTS

The presence of mast cells were counted in each cyst in all the four zones mentioned above. The distribution of mast cells in odontogenic

keratocysts is higher in subepithelial zone as compared to intraepithelial zone ($p < 0.05$). Also, a statistical significant difference was noted between intermediate and deep zone . [Table -1], [Fig-1].

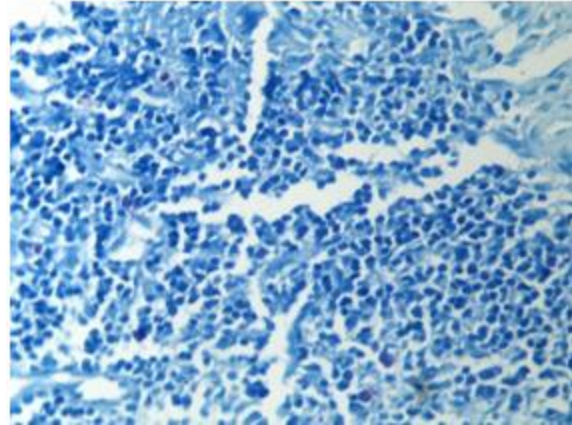


Fig -1 Mast cells in OKC as stained by toluidine blue under 400 x magnification.

Zones	Mean	Standard Deviation	Mean \pm S.D	Range
Intraepithelial	3.6000	5.12510	3.6000 \pm 5.12510	- 13.00
Subepithelial	18.2000	24.28	18.2000 \pm 24.28	1.00 - 85.00
Intermediate	13.1000	14.77	13.1000 \pm 14.77	- 45.00
Deep	5.7000	7.024	5.70 \pm 7.024	- 21.00

[Table -1]- The distribution of mast cells in various zones of OKC. The results of the study revealed that significant difference was observed between intraepithelial and subepithelial zone along with intraepithelial and deep zones and

intermediate vs deep zones ($p < 0.05$) Also, non-significant difference was seen between intermediate and intraepithelial zones along with intermediate and deep zones of OKC .($p > 0.05$)

The distribution of mast cells in various zones of dentigerous cysts [Table- 2], [Fig -2]

Zones	Mean	Standard Deviation	Mean \pm S.D	Range
Intraepithelial	2.6000	2.50	2.6000 \pm 2.50	- 8.00
Subepithelial	18.3000	14.283	18.3000 \pm 14.28	3.00- 42.00
Intermediate	9.9000	12.520	9.9000 \pm 12.520	- 30.00
Deep	5.9000	12.705	5.900 \pm 12.705	- 35.00

The results revealed that there was significant difference between the distribution of mast cells was statistically significant in subepithelial and intraepithelial zones along with subepithelial and deep zones ($p < 0.05$) whereas non –significant differences were observed in intraepithelial vs

intermediate zones , intraepithelial vs deep zone and intermediate vs deep zones ($p > 0.05$).

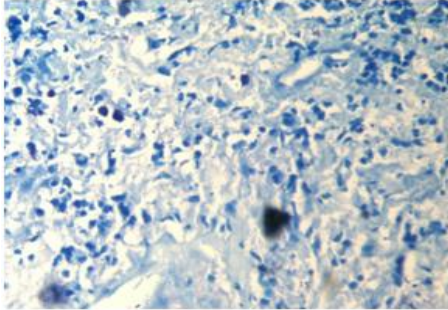


Fig -2 Mast cells in Dentigerous cyst as stained by toluidine blue under 400 x magnification

The distribution of mast cells in radicular cysts -
[Table 3] , [Fig-3]

Zones	Mean	Standard Deviation	Mean \pm S.D	Range
Intraepithelial	4.2000	6.66	4.2000 \pm 6.66	- 19.00
Subepithelial	9.1000	7.17	9.1000 \pm 7.17	2.00- 25.00
Intermediate	3.1000	2.726	3.1000 \pm 2.726	- 7.00
Deep	6.0000	12.44	6.0000 \pm 12.44	- 32.00

The results revealed that mast cells in radicular cyst showed statistically significant difference between intraepithelial vs subepithelial , intermediate vs subepithelial , subepithelial vs deep ($p < 0.05$) and non –significant differences was seen intraepithelial vs deep and intermediate vs deep zones ($p > 0.05$)

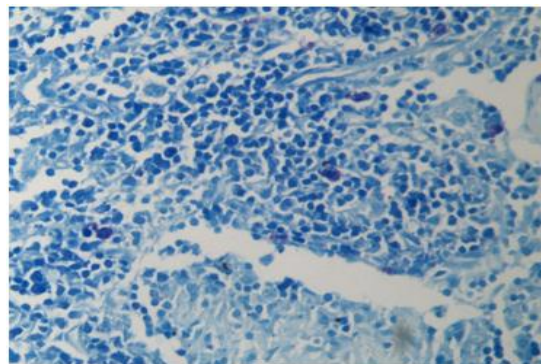


Fig: 3 Mast cells in Radicular cyst as stained by toluidine blue under 400 x magnification.

Comparison of mast cells in all the four zones of odontogenic cysts- [Table -4]

Zones	OKC	DC	RC	OKC VS DC	OKC VS RC	DC VS RC
Intraepithelial	3.60 \pm 5.125	2.60 \pm 2.50	4.20 \pm 6.66	N.S	N.S	N.S
Subepithelial	18.2 \pm 24.28	18.30 \pm 14.28	9.10 \pm 7.17	N.S	N.S	N.S
Intermediate	13.1 \pm 14.77	9.90 \pm 12.520	3.10 \pm 2.726	N.S	N.S	N.S
Deep	5.70 \pm 7.024	5.90 \pm 12.705	6.0 \pm 12.44	N.S	N.S	N.S

Table-4 –The distribution of mast cells when compared zone wise within the cysts, statistically non – significant (N.S) difference was noted.

DISCUSSION

The mast cells are seen widespread in the connective tissue stroma of all the odontogenic cysts and can participate in both acute and chronic inflammatory responses.^[11]

The subepithelial zone of mast cells has highest concentration in all cysts as depicted in our study. The highest concentration of mast cells in subepithelial zone was seen in OKCs, followed

by dentigerous cysts and radicular cysts. This was suggestive of increase in breakdown of subcapsular matrix. Also, the epithelium of OKCs, seems to be fragile and non-keratinized at some areas which creates a passage for breakdown of matrix products to entering into the cystic lumen causing its expansion as suggested by Mervyn Shear. Another possible mechanism for the density of mast cells in subepithelial zone could be glycosaminoglycan's and proteoglycans of connective tissue capsule which are released as a result of proteolytic breakdown as proposed by Smith et al in their study.^[12] This release of glycosaminoglycans contributes to osmotic and hydrostatic pressure of cystic fluid causing their expansion. Thus, contributing the role of mast cells in cystic expansion. In the present study no statistical significant difference was noted in intermediate and deep zones which are in accordance with the study by Shylaja et al. The observations by Smith et al in 1989 notified the mast cell density significant in subepithelial zones of all the cysts, however our study could not find any significant co-relation. The reason could be attributed to specificity of avidian peroxidase staining for mast cells which does not stain other inflammatory components of the stroma. However, toluidine blue in spite of having capacity of metachromasia stain the inflammatory cells of the stroma. The reasons for variability in density of mast cells in different zones is by the fact that toluidine blue does not stain the degranulated mast cells which could otherwise be specified if immunohistochemical methods were applied. Johannsson et al. (1984) assumed that antigen, toxins or other noxious substances from an infected root canal or necrotic pulp would initiate and maintain inflammation in the periapical area. The inflammation would manifest itself as a periapical granuloma or cyst.^[7]

The mast cell, with its specific membrane receptors, is positioned where potentially noxious materials are likely to enter the body. Although many mast cells could be present in the inflamed

regions, other portions within the same tissue might contain few or no mast cells and same was also observed in our case. Degranulation of mast cells releases various mediators which bring about many changes as both humoral and cell mediated immunological reactions have been suggested in the pathogenesis of periapical granulomas and cysts. IgE attaches itself for long periods to receptors on mast cells and the effect of this reaction is liberation of the histamine and other chemical mediators from mast cells, which in turn promotes leukocyte infiltration.^[8] Tryptase and chymase take part in the degradation of the extracellular matrix. Tryptase activates matrix metalloproteinases 1 and 2, takes part in breakdown of proteoglycan in the connective tissue capsule of the cyst. Heparin is involved in the bone resorption and inhibition of collagen synthesis. IL-1, IL-6 and TNF- α intensify the osteoclastic activity.^[9] TNF- α may maintain the migration of leukocytes and promote chronicity in periapical inflammatory lesions. Prostaglandins promote bone resorption to accommodate the growing cyst thereby playing a central role in the pathogenesis of odontogenic cysts.^[10]

CONCLUSION

The present study demonstrated the presence of mast cell density to be higher in subepithelial zone as compared to other three zones. However, before any research could be concluded various immunohistochemical methods needs to warranted to determine their specific role and position within the zones. Further works needs to be devised in this area using newer advances to throw some light regarding the pathogenesis of these cysts.

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