

## Original Article

### Comparison of Mast Cells Count in Odontogenic Cysts Using Histochemical Staining

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#### Abstract

**Background & Objectives:** Odontogenic cysts are among the most frequent destructive lesions of jaws which their pathogenesis and growth mechanism are not cleared. With respect to different roles of mast cells, they may play a role in the pathogenesis and growth of odontogenic cysts. The aim of present study was to evaluate mast cells in the most common odontogenic cyst.

**Methods:** Thirty paraffin-embedded tissue blocks including 10 radicular cysts, 10 dentigerous cysts and 10 odontogenic keratocysts were used and 5 micron sections stained with toluidine blue and observed by light microscope under  $\times 400$  magnification to evaluate mast cells within these cysts. For each case, 5 high-power field areas, selected from hot-spot areas, were considered and each area divided into 3 zones: intra-epithelial zone, sub-epithelial zone and deep zone.

**Results:** Most of the studied cyst showed presence of mast cells. There was not any significant difference in mast cell count between studied cysts ( $P$ -values  $> 0.05$ ). With respect to intra-epithelial, sub-epithelial and deep zones, there was not any significant difference between three studied cysts. There was not any significant difference between sub-epithelial zone and deep zone within each of these cysts. There was only significant difference between intra-epithelial zone and sub-epithelial zone within dentigerous cysts and odontogenic keratocysts ( $P$ -value  $< 0.05$ ).

**Conclusion:** Prevalence of mast cells in fibrous wall of odontogenic cysts suggests their activity in these cysts. Mast cells may not be directly involved in the pathogenesis of odontogenic keratocysts.

**Keywords:** Mast Cells, Odontogenic Cyst

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## Introduction

Odontogenic cysts are among the most frequent destructive benign lesions of jaws. The most common odontogenic cysts are radicular cysts, dentigerous cysts and odontogenic keratocyst. Many efforts have been carried out to understand the pathogenesis of jaw cysts, but many of those have been unsuccessful (1). Several mechanisms have been proposed for growth and expansion of odontogenic cysts, but the exact mechanism of their enlargement still remains to be cleared. One of the unknown questions is the participation of immune and inflammatory cells in formation and growth of these lesions (2).

Mast cells are one of the defense cells of immune system with metachromatic cytoplasmic granules (3). Degranulation of mast cells plays an important role in initiation of inflammatory response and is of great importance in the pathogenesis of different diseases such as oral lichen planus and periodontal diseases (4). Furthermore, mast cells are a rich source of heparin and proteolytic enzymes that participate in degradation of connective tissue (5-6). Mast cells have been implicated to stimulate production of prostaglandins that is considered important in bone resorption (1).

With respect to several roles of mast cells such as participation in inflammation, degradation of extracellular matrix and bone resorption, these cells may play a role in the pathogenesis of jaw cysts and mechanism of their enlargement.

On the other hand, WHO has classified odontogenic keratocyst as an odontogenic tumor because of its growth potential. There is this hypothesis that the more aggressive behavior of odontogenic keratocysts is related at least, partly, to distribution of mast cells.

The aim of this study was evaluation of presence and distribution of mast cells in epithelial lining and connective tissue wall of radicular cysts, dentigerous cysts, and odontogenic keratocysts to achieve better understanding of their pathogenesis and mechanism of expansion and enlargement.

## Materials and Methods

In this cross-sectional and descriptive-analytical study, 30 paraffin-embedded tissue blocks including 10 cases of radicular cyst, 10 cases of dentigerous cyst and 10 cases of odontogenic keratocyst were retrieved from archive of Oral and Maxillofacial Pathology Department of Dental College of Babol University of Medical Sciences, Babol, Iran. Cases were included in the study that their diagnosis were confirmed by pathologist and had enough tissue for special staining. All cases that underwent decalcification were excluded from study (2).

Five  $\mu$  sections were prepared from these tissue blocks and stained with toluidine blue solution to observe the presence of mast cells by standard method of toluidine blue staining (Bancroft and Gamble 2007) (7). Neurofibroma was used as positive control and omission of toluidine blue solution and replacement by tris-buffered saline as negative control (2). On microscopic examination, intact and degranulated mast cells were taken into account; round, ovoid or fusiform cells containing metachromatic granules in their cytoplasm and around the boundary of cytoplasmic membrane were considered as mast cell. Mast cell counting was performed under 400 $\times$  magnification using Olympus C $\times$ 21 light microscope (Olympus corporation, Tokyo, Japan). For each case, 5 high-power field areas, selected from hot-spot areas (areas with greatest number of mast cells), were considered and each area divided into 3 zone: intra-epithelial zone, sub-epithelial zone (superficial connective tissue zone) and deep zone (deep connective tissue zone) (Three zones were defined because different sites of mast cells may be relevant to mechanism of cystic growth); the sub epithelial zone was the area just below the epithelium and the next consecutive microscopic field was the deep zone (4). Therefore, mast cell counting was ultimately performed in 15 high-power field microscopic fields (5 fields representing intra-epithelial zone, 5 fields representing sub-

epithelial zone, 5 fields representing deep zone). Two pathologists carried out mast cell counting; the final mast cell count for each zone equaled with mean of the two resultant numbers.

Data were analyzed by SPSS20 statistical software using ANOVA and paired samples tests. A probability ( $P$ ) value less than 0.05 was considered statistically significant.

## Result

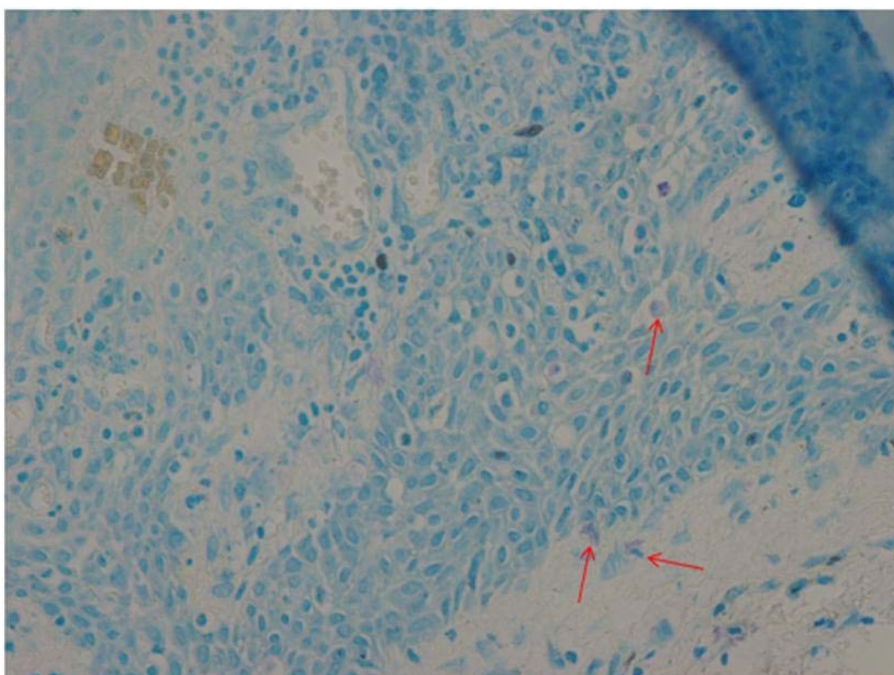
Most of the studied cyst showed presence of mast cells, either within intra-epithelial zone, sub-epithelial zone of deep zone. Figure 1-3 shows mast cells in different zones of the studied cysts. Table 1 shows the mean mast cell count for each zone in radicular cyst (RC), dentigerous cyst (DC) and odontogenic keratocyst (OKC). Data showed that there was not any significant difference in mast cell count

between studied cysts ( $P > 0.05$ ). In detail, there was not any significant difference between intra-epithelial zone in three studied cysts ( $P = 0.602$ ). Moreover, with respect to sub-epithelial and deep zones, there were no significant difference between these cysts ( $P = 0.622$  and  $P = 0.875$ , respectively). Figure 4 shows the distribution of mast cells within different zones of the studied cysts. Analyze shows that within each of these cysts, there was not any significant difference between sub-epithelial zone and deep zone ( $P > 0.05$ ). Besides, there was not any significant difference between intra-epithelial zone and deep zone within each of these cysts. There was only significant difference between intra-epithelial zone and sub-epithelial zone within dentigerous cysts and odontogenic keratocysts ( $P = 0.009$  and  $P = 0.023$ , respectively).

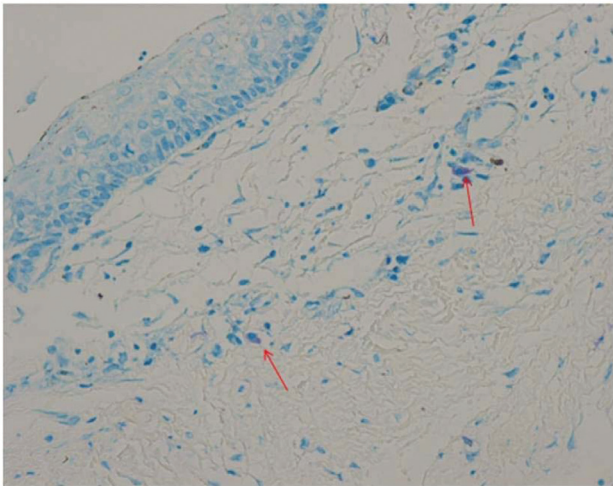
**Table 1-** Mean of mast cell count in different zones of studied cysts

Cyst type zone	Radicular cyst (Mean $\pm$ SD)*	Dentigerous cyst (Mean $\pm$ SD)	Odontogenic keratocyst (Mean $\pm$ SD)	$P$ -value
Intra-epithelial zone	0.14 $\pm$ 0.25	0.08 $\pm$ 0.139	0.06 $\pm$ 0.134	0.602
Sub-epithelial zone	1.44 $\pm$ 2.851	2.52 $\pm$ 2.381	2.3 $\pm$ 2.53	0.622
Deep zone	2.94 $\pm$ 4.692	2.62 $\pm$ 3.741	2.06 $\pm$ 2.918	0.875

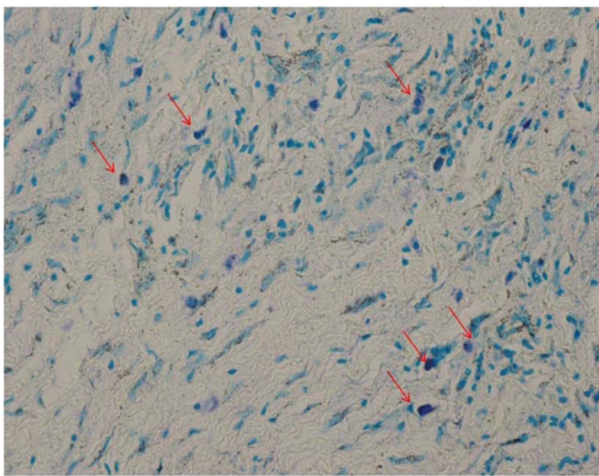
\* Standard Deviation



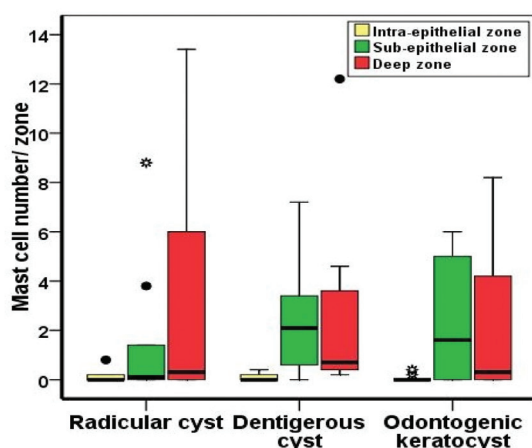
**Fig.1:** Mast cells in intra-epithelial zone of a radicular cyst ( $\times 400$ )



**Fig.2:** Mast cells in sub-epithelial zone of an odontogenic keratocyst ( $\times 400$ )



**Fig.3:** Mast cells in deep zone of a dentigerous cyst ( $\times 400$ )



**Fig.4:** Mast cell distribution within different zones of studied cysts

## Discussion

The participation of mast cells in host defense as effector cells in innate immunity and in responses to allergic, chemical, and biological factors such as microorganisms and parasites has been widely assessed. There is increased data about mast cell functions and interactions in the immune response, including modulating humoral and cellular events. As a source of histamine, serotonin, and other vasoactive amines, these cells can control vascular tone and permeability. With respect to different roles of mast cells such as extracellular matrix degradation and bone resorption, they may play a role in the pathogenesis and growth of odontogenic cysts. So, the aim of present study was to evaluate mast cells in radicular cysts, dentigerous cysts and odontogenic keratocysts.

In our study, with respect to intra-epithelial, sub-epithelial and deep zones, there was not any significant difference between three studied cysts. This is in contradiction with Patidar *et al.* study that found greatest number of mast cells in radicular cysts and the lowest number of mast cells in odontogenic keratocyst. They found that the sub-epithelial zones of all cysts contained more mast cells than deeper zones, while we found that there is not any significant difference between sub-epithelial zone and deep zone (1). de Noronha Santos Netto *et al.* (2) found that inflamed dentigerous cysts showed the highest number of mast cells and the deep region from all these cysts showed the highest mean number of degranulated mast cells. These findings are in contradiction with our findings because we found that there was not any significant difference between the three studied cysts when considering different zones and deep zone in all cysts do not devote the highest mean number of mast cells to itself.

Fonseca-Silva *et al.* (8) described the presence of mast cells in the peripheral regions of both radicular cyst and periapical granulomas. Presence of mast cells in radicular cysts of

their study is consistent with our study, but distribution of mast cells within radicular cysts is in contradiction to our study, because we found that mast cells distributed in the stroma not only in the periphery. Seifi *et al.* (3) and Lima SCA *et al.* (9) described the presence of mast cells in all of studied periapical cysts and granulomas. Presence of mast cells in their studies is consistent with our study, but we found mast cells in most of the studied radicular cysts not all of them.

Shylaja (4) found maximum number of mast cells in subepithelial zones of the studied cysts, while we found more mast cells in deep zone of two cysts and more mast cells in sub-epithelial zone of the third cyst. In that study, significant difference was found between subepithelial and deep zones in periapical cysts, dentigerous cysts and odontogenic keratocysts, while we did not find any significant difference between those zones in none of the cysts.

In study of Chatterjee *et al.* (6), mast cells had more concentration in sub-epithelial zone of studied cysts, specially odontogenic keratocysts; As previously mentioned, in our study deep zone had more mast cells in two studied cysts; also, although in our study sub-epithelial zone in odontogenic keratocysts had more mast cells than the other two zones, distribution of mast cell did not differ significantly between sub-epithelial and deep zones and with respect to sub-epithelial zone, there was no significant difference between three studied cysts; In their study, there was not any significant difference between intra-epithelial and sub-epithelial zones in odontogenic keratocysts that is in contradiction to our findings. On the other hand, in dentigerous cyst, the difference between intra-epithelial and sub-epithelial zones was significant that is in coordination with our findings. Chatterjee *et al.* suggested that more mast cell concentration in sub-epithelial zone, specially in odontogenic keratocyst, indicates these cells could cause more degradation of glycosaminoglycans in fibrous wall and at least somewhat demonstrate

aggressiveness of odontogenic keratocysts. According to our findings, mast cells probably do not contribute to more aggressive behaviour of odontogenic keratocysts.

The prevalence of mast cells in the fibrous wall that has been seen in the present study and previous studies suggest intense activity of these cells within capsular connective tissues of the odontogenic cysts that is possibly related to growth of these cystic lesions. Increase of hydrostatic pressure of the luminal fluid have an important role in enlargement of cysts and mast cells may contribute to increase the osmotic pressure of the luminal fluid by three ways: 1) by direct release of heparin into luminal fluid 2) by release of hydrolytic enzymes that degrade extracellular matrix and subsequent transfer of resultant component into the luminal fluid 3) by release of histamine and increasing vascular permeability that result in transduction of serum proteins. Besides, by stimulating the production of prostaglandins, mast cells have been implicated in bone resorption. These cells may contribute to cystic growth by bone resorption in deep regions or mast cell activity within sup-epithelial layer may assist in increasing the osmotic pressure of cystic fluid and subsequently cyst expansion by releasing heparin, hydrolytic enzymes and histamines.

## Conclusion

Presence of mast cells within these cysts could indicate the participation of these cells in the inflammatory response in odontogenic cysts. Prevalence of mast cells in fibrous wall of the studied cysts suggests intense activity of these cells. Mast cells may not be directly involved in the pathogenesis of odontogenic keratocysts.

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