Analysis of mast cells in dental follicle and dentigerous cyst: A histopathological study

Jayaraman Sindhumati¹, Sanjai Karpagaselvi², Kumaraswamy Jayalakshmi², Papaiah Lokesh², Keshavaiah Roopavathi², Pandey Bhavna³

¹Department of Oral Pathology and Microbiology, Rajiv Gandhi College of Dental Sciences and Research Centre, Cholanagar, Hebbal, R T Nagar Post, Bengaluru, Karnataka, ²Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, Nallurahalli, Whitefield, Bengaluru, Karnataka, ³Senior Medical Writer, Pitchman Communications, Mumbai, India

Abstract

Introduction: Mast cells are large granular cells that arise from multipotent CD 34+ precursors in the bone marrow normally distributed throughout the connective tissues. Following activation of immunologic or nonimmunologic stimuli, mast cells release secretory granules which give the characteristic metachromatic appearance with toluidine blue stain. Release of numerous mediators on degranulation of mast cells plays an important role in the pathogenesis of odontogenic cysts.

Context: Odontogenic cysts, such as dentigerous cysts, arise due to the accumulation of fluid between the crown of an unerupted tooth and the reduced enamel epithelium. Dental follicles, which surround developing teeth, can also undergo cystic transformation. Mast cells activity might contribute to cyst expansion and bone resorption, highlighting their potential role in cystic pathology.

Aims: To study the presence of mast cells in the dental follicle and dentigerous cyst. To quantify the mast cells in the abovementioned subjects. To study the pattern of distribution of mast cell distribution in different zones of the study groups.

Settings and Design: This was histopathological study conducted at the Department of Oral Pathology and Microbiology, Vydehi College of Dental Sciences and Research Centre, Bengaluru, between 2012 and 2015. Methods and Material: Our study was conducted in the Department of Oral Pathology and Microbiology at Vydehi College of Dental Sciences and Research Centre in the year 2012 to 2015. Histopathologically analyzed 30 cases each of dental follicle, and dentigerous cysts were taken and 4–5 micron sections were stained with toluidine blue. Counting of mast cells was done in three different zones which included subepithelial, intermediate, and deep zone. The results were tabulated and statistically analyzed.

Results: Both dental follicles and dentigerous cysts showed the presence of mast cells, and highest numbers of mast cells were seen in subepithelial zone followed by intermediate and deep zones. There was statistically significant relation in the number of mast cells in dentigerous cysts and dental follicle

Address for correspondence: Dr. Jayaraman Sindhumati, Department of Oral Pathology and Microbiology, Rajiv Gandhi College of Dental Sciences and Research Centre, Cholanagar, Hebbal, R T Nagar Post, Bengaluru - 560 032, Karnataka, India.

E-mail: sindhumati@gmail.com

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Statistical Analysis Used: Kruskal–Wallis Chi-squared test.

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along subepithelial and intermediate zone with a P value of <0.05. In our study, we also found increased mast cell count in inflamed cases of dental follicle and dentigerous cyst compared with noninflamed cases with a P value of <0.01.

Conclusions: It is well known that mast cells play a role in the initiation of inflammation, and this inflammatory process may be associated with pericoronal follicle enlargement, a process that could result in cystic transformation of the follicle. Hence, regular radiographic follow-up is necessary especially for teeth with a maximum dental follicle width of 2–3 mm.

Keywords: Dental follicle, dentigerous cyst, mast cells, toluidine blue

INTRODUCTION

Mast cells are innate immune cells, named by Paul Ehrlich in 1878 on the basis of their granular histological staining with aniline dyes. [1] These cells are round or oval in outline measuring about 12 to 15 micron meter in diameter, containing numerous basophilic granules in their cytoplasm. [2] Human mast cells originate from hematopoietic stem cells in the bone marrow, which give rise to common myeloid progenitors that can subsequently differentiate into mast cell precursors. It is also suggested that mast cells could arise from a shared basophil or mast cell progenitor. [1] In humans, there are three subsets of mast cells that have been characterized as [3]:

- MCT, which expresses tryptase and resides primarily in the alveolar septa of the lung and in the small intestinal mucosa.
- 2. MCTC, which expresses tryptase, chymase, carboxypeptidase A (CPA), and cathepsin G and resides primarily in the skin and in the small intestinal submucosa.
- 3. MCc, which expresses chymase but lacks tryptase.

Mast cells are found in almost all major organs and vascularized tissues of the body. [4]

Following activation of immunologic or nonimmunologic stimuli, mast cells release secretory granules which give characteristic metachromatic appearance with toluidine blue stain.^[5]

Release of numerous mediators on degranulation of mast cells plays an important role in the pathogenesis of odontogenic cysts. [6] They also play a role in the development of fibrotic conditions. [7] In addition to preformed granule contents, activated mast cells can also synthesize vasoactive mediators, for example, platelet activating factor, chemotactic mediators, and several proinflammatory cytokines such as IL-1, IL-3, IL-6, and TNF. Furthermore, mast cells are a rich source of heparin

and proteolytic enzymes, such as tryptase, chymase and hyaluronic acid, which participate in connective tissue breakdown in the capsule during normal metabolic turnover, as well as in inflammation.^[8]

Because of poor lymphatic drainage in the cyst wall, ^[6] breakdown products of connective tissue elements are extruded into the cystic lumen raising the hydrostatic pressure and leading to their enlargement. It has also been stated that mast cells may enhance bone resorption by heparin production. ^[9] Mast cells have also been regarded as the initiators of inflammation. ^[10]

The dental follicle (DF), a loose ectomesenchymally derived connective tissue surrounding the tooth germ, participates in tooth eruption and contributes extensively to the periodontium by producing osteoblasts, cementoblasts, and periodontal ligament cells (PDLCs) in tooth development. Dental follicles are most frequently misdiagnosed entities. Age-related morphological changes in dental follicles have been investigated for almost a century, in association with unerupted and impacted teeth because of its potential to undergo cystic degeneration yet is the most common odontogenic lesion associated with unerupted teeth, followed by keratocyst, odontomas, and ameloblastoma. Control of the participates of the production of the

The dentigerous cyst is a cystic lesion which arises when fluid or inflammatory exudates accumulate between the crown of an unerupted tooth and the reduced enamel epithelium.^[16] The dentigerous cyst may be of either extra follicular or intrafollicular origin.^[17]

The hydrostatic pressure of the luminal fluid is important in cyst enlargement and mast cell activity might contribute to this by increasing the osmotic pressure of the fluid in ways such as by direct release of heparin into the luminal fluid, by release of hydrolytic enzymes which could degrade capsular extracellular matrix components, thereby

facilitating their passage into the fluid and by the action of histamine on smooth muscle contraction and vascular permeability. [18] Mast cells are also implicated to stimulate production of prostaglandins which is considered important in bone resorption. [19] Tryptase also aid in bone resorption which is a feature in cyst enlargement at cyst bone interface. [8]

Hence, the purpose of the present study was to examine the role of mast cell activity in dental follicle and dentigerous cyst.

SUBJECTS AND METHODS

Study population consists of patients with impacted teeth and histopathologically diagnosed cases of dental follicle and dentigerous cyst reporting to the Department of Oral Medicine and Radiology, Department of Oral and Maxillofacial Surgery, and Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, Bangalore. The study population consisted of histopathologically diagnosed, 30 cases each of dental follicle and dentigerous cyst and specimens that had undergone decalcification were excluded (as mast cell granules are obscured by this method).

- Paraffin blocks of cases were retrieved and sections varying from 4–5 micron in thickness was cut and stained with toluidine blue.
- Stained sections were analyzed under light microscopy.
- Counting was done in 3 different zones: subepithelial, intermediate, and deep.
- Subepithelial zone is the areas just beneath the epithelium. The next two consecutive microscopic fields are intermediate and deep zones.
- Randomly selected 10 areas under 10 X magnifications were considered.
- In each area, all 3 zones were considered.

RESULTS

Statistical Analysis

Both dental follicles and dentigerous cysts showed the presence of mast cells, and highest numbers of mast cells were seen in subepithelial zone followed by intermediate and deep zones. There was statistically significant relation in the number of mast cells in dentigerous cysts and dental follicle along subepithelial and intermediate zone with a P value of <0.05. In our study, we also found increased mast cell count in inflamed cases of dental follicle and dentigerous cyst compared with noninflamed cases with a P value of <0.01.

Table 1: Age distribution in the study sample

Age Group	Dental	follicle	Dentigerous Cyst		
	n	%	n	%	
<20 years	6	20%	7	23%	
20-29 years	18	60%	9	30%	
30-39 years	5	17%	6	20%	
≥40 years	1	3%	8	27%	
Total	30	100%	30	100%	

Table 2: Sample distribution according to site of origin

Site	Dental	follicle	Dentigerous cyst		
	n	%	n	%	
Maxilla	3	10%	10	33%	
Mandible	27	90%	20	67%	
Total	30	100%	30	100%	

Table 3: Gender distribution in the study sample

Lesion	N	/lale	Female		
	n	%	n	%	
Dental follicle	18	60%	12	40%	
Dentigerous cyst	21	70%	9	30%	
Total	39	100%	21	100%	

Table 4: Comparison of mast cells within different layers in dental follicle

Layer Mean St		an Std SE of		95% CI	for Mean	Kruskal-	P
	Dev Mean		Lower Bound	Upper Bound	Wallis chi-square		
Subepithelial	6.40	8.69	1.59	3.16	9.64	0.878	0.645
Intermediate	6.30	8.61	1.57	3.08	9.52		
Deep	6.07	11.76	2.15	1.67	10.46		

Table 5: Comparison of mast cells within different layers in dentigerous cyst

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Layer	Mean	Mean Std Dev			CI for ean	Kruskal- Wallis Chi-sq	P
			Lower Bound	Upper Bound			
Subepithelial				7.94	17.59	0.860	0.651
Intermediate Deep			2.28 2.50	7.41 5.48	16.73 15.72		

Table 6: Comparison of mast cells in subepithelial layer between dental follicle and dentigerous cyst

Lesion	Mean		SE of mean	Mean difference	Z	Р
Dental follicle Dentigerous cyst		8.69 12.92	,	-6.367	-2.043	0.041*

DISCUSSION

Cysts of the jaws are probably the most common destructive bone lesions in the human maxillofacial skeleton. Odontogenic cysts are derived from the epithelium, which is associated with the development of the dental apparatus and can be either developmental or inflammatory in origin.^[18] Mean eruption age of third

Table 7: Comparison of mast cells in intermediate layer between dental follicle and dentigerous cyst

		0	_			
Lesion	Mean	Std Dev	SE of Mean	Mean difference	Z	Р
Dental follicle Dentigerous cyst	6.30 12.07	8.61 12.48	1.57 2.28	-5.767	-2.067	0.039*

Table 8: Comparison of mast cells in deep layer between dental follicle and dentigerous cyst

	_	_				
Lesion	Mean	Std Dev	SE of mean	Mean difference	Z	Р
Dental follicle Dentigerous cyst	0.07	11.76 13.71	2.15 2.50	-4.533	-1.807	0.071

Table 9: Comparison of number of mast cells in inflamed and noninflamed cases of dental follicle

Dental follicle	Mean t		95% cor interval of s	Р	
			Upper	Lower	
Inflamed cases Noninflamed cases	18.16667 0.60000	3.881 1.369	8.5922 -0.2966	27.7411 1.4966	<0.01

Table 10: Comparison of number of mast cells in inflamed and noninflamed cases of dentigerous cyst

Dentigerous cyst	Mean	t	95% confidence interval of significance		P
			Upper	Lower	
Inflamed cases Noninflamed cases	34.70000 0.73333		21.3237 -0.7665	48.0763 2.2332	<0.01

molars varies in the range of 17–21 years Table 1. The mandibular third molar is the most frequently impacted tooth. The incidence varies from 9.5% to 68% in different populations [Table 2],^[20] followed by maxillary third molars with a reported frequency which varies from 16.7% to 73.82%. Maxillary canines also have reported with a high prevalence rate of followed by second premolars.^[21]

Dental follicular space radiographically appears as thin pericoronal radiolucency, considered normal by some authors when the thickness is within 2.5–3 mm. The dental follicle may show various histopathological changes during tooth development, which may lead to the development of odontogenic tumors or cysts. [22] According to Saravana, if odontogenic epithelial lining of the dental follicle, changes from reduced enamel epithelium to stratified epithelium, it is indicative of dentigerous like changes. [23]

Because of the possible importance of mast cells in the pathogenesis of odontogenic cysts, an attempt was made by us to evaluate the presence and distribution of mast cells in different zones of the connective tissue.

Various stains are being used for staining mast cells like Romanowsky combinations (Wright, Giemsa,

May-Grünwald Giemsa, and Leishman),^[24] Azur A, Bismarck brown, Thionin Alcian blue, and toluidine blue.^[18]

Hematoxylin and eosin staining is not a specific or reliable method for detecting mast cells in tissue sections because of variable cellular morphology [Figures 1a and 2a]. [25] Hence, we choose to evaluate these cells by means of histochemistry through toluidine blue staining, which stains, mast cell granules purple in color due to the presence of heparin and histamine. [26]

Toluidine blue is also known as tolonium chloride and is an acidophilic metachromatic dye that selectively stains acidic tissue components. [26] Metachromatic staining of mast cells significantly depends on the number of factors such as type and maturity of mast cells, a test tissue type, animal species, a used dye, incubation solution pH and the staining duration, fixation solution type, fixation time, and the final processing technique of stained preparations. [27]

In our present study, we included 30 cases each of dental follicle and dentigerous cyst. In cases of dental follicle, we found greater predilection for males compared with females [Table 3]. The results were in accordance with a study done by Syed *et al.*^[28] who found that males had a higher incidence of third molar impactions as compared to females.

Mast cells are found widespread throughout the connective tissue wall of all the cysts mainly in the subepithelial zone and are source of a variety of proteolytic enzymes found in the cystic fluid.^[8]

The present study included evaluation of mast cells in subepithelial, intermediate, and deep layers in dental follicle; highest mean number of mast cells was recorded in subepithelial layer followed by intermediate layer and deep layer, respectively [Figure 1b-d] [Table 4]. The difference in mean number of mast cells among the groups was not statistically significant (P > 0.05). In dentigerous cyst, higher mean number of mast cells was recorded in subepithelial layer, followed by intermediate layer and deep layer, respectively [Figure 2b-d]. The difference in mean number of mast cells among the groups was not statistically significant (P > 0.05) [Table 5].

A study was conducted by Shylaja S *et al.*^[18] using cases of radicular cyst, odontogenic keratocyst, and dentigerous cysts to evaluate the distribution of mast cells in subepithelial, intermediate, and deep zones. In all the abovementioned cysts, the maximum numbers of mast cells were seen in subepithelial zone compared to intermediate and deep zones. They stated that mast cell degranulation

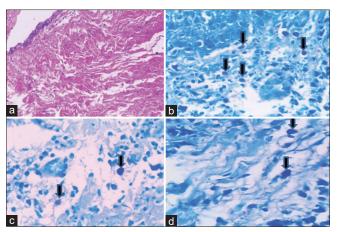


Figure 1: (a) Hematoxylin and Eosin stained tissue section of dental follicle. (b) Mast cells in toluidine blue stained tissue section of dental follicle in subepithelial layer. (c) Mast cells in toluidine blue stained tissue section of dental follicle in intermediate layer. (d) Mast cells in toluidine blue stained tissue section of dental follicle in deep layer

plays an important role in the inflammatory response and alteration in their number and distribution could contribute to the pathogenesis of odontogenic cysts.

Another study conducted by Chatterjee S *et al.*^[8] to evaluate the mast cell distribution and cystic lining and capsule along 4 zones: intraepithelial, subepithelial, intermediate, and deep and found that mast cells had a greatest concentration in the subepithelial zone.

The results of our study showed that less number of mast cells was recorded in dental follicle compared with dentigerous cyst. Higher mean numbers of mast cells were recorded in the subepithelial layer followed by intermediate layer and deep layer, respectively, in both the lesions. There was statistically significant value (P = 0.041), between the number of mast cells in the subepithelial zones of dental follicle and dentigerous cyst [Table 6]. Significant association in the number of mast cells in the intermediate layer of dental follicle and dentigerous cyst was also noted (P = 0.039) [Table 7]. Although higher mean number of mast cells was recorded in the deep layer of dentigerous cyst compared with dental follicle, the difference between them was not statistically significant (P > 0.05) [Table 8].

Teronen *et al.*^[29] stated that activated mast cells can synthesize vasoactive and chemotactic mediators (e.g., platelet activating factor) as well as several proinflammatory cytokines such as IL-3, IL-6, and TNF- α de novo. These chemical mediators increase vascular permeability, thereby facilitating influx of highly osmolar substances in cystic lumen.^[8]

A study by Li et al.^[30] found that the maximum width of the dental follicle was positively correlated with the incidence

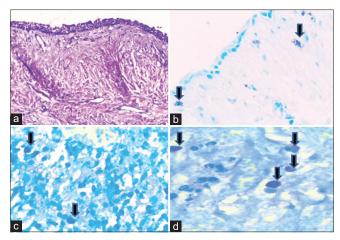


Figure 2: (a) Hematoxylin and Eosin stained tissue section of dentigerous cyst. (b) Mast cells in toluidine blue stained tissue section in subepithelial layer of dentigerous cyst. (c) Mast cells in toluidine blue stained tissue section of dentigerous cyst in intermediate layer. (d) Mast cells in toluidine blue stained tissue section of dentigerous cyst in deep layer

of inflammation in the dental follicle. In addition, it is well known that mast cells (MCs) initiate inflammation.^[10]

In our study, in cases of dental follicle and dentigerous cyst, maximum inflammation was seen in the subepithelial layer followed by intermediate and deep layers. There was statistically significant increase in the number of mast cells in cases of inflamed dental follicle and inflamed dentigerous cyst with a P value of <0.01 [Table 9 and 10].

According to Shylaja S, the cysts of the developmental origin may show inflammatory changes secondary to infection. Mast cell histamine plays an important role in the inflammatory reaction, and they degranulate in response to antigen—antibody reaction on their surface. In addition, it is speculated that alterations in their number could contribute to the pathogenesis of odontogenic cysts.^[18]

CONCLUSION

Histopathological analysis of mast cells in dental follicle and dentigerous cyst was conducted in our study. This type of analysis is important since high rate of pathosis in follicular tissues were found in many cases with the absence of any radio graphically detectable sign.

Analysis of mast cells was conducted using toluidine blue, which was simple and inexpensive method with less time consumption. Various studies have been conducted where they have proved that toluidine blue is a better stain compared with other stains like thionine in visualizing mast cells.

In the present study, the presence of mast cells was seen both in dental follicle and dentigerous cyst with higher mean number of mast cells in dentigerous cyst. Highest concentrations of mast cells were seen in subepithelial zone of both the lesions which has been suggested to be due to a chemotactic stimulus attracting them to the epithelial lining or luminal fluid contents. Maximum numbers of mast cells were also seen in inflamed cases compared with noninflamed cases in both the lesions. Mast cells are considered to contribute towards cyst enlargement by increasing the osmotic pressure of the fluid in three ways: direct release of heparin, hydrolytic enzymes, and histamine.

In the present study, analysis of mast cells supports the existing view that mast cell may be considered one of the factor in the pathogenesis of odontogenic cysts.

Hence, we infer that the presence of inflammation may be associated with pericoronal follicle enlargement, a process that could result in cystic transformation of the follicle.

Hence, regular radiographic follow-up is necessary especially for teeth with a maximum dental follicle width of 2–3 mm, and combined team effort of the radiologist, pathologist, and the surgeon is important on arriving at a correct diagnosis.

Key messages

Mast cells play a significant role in the pathogenesis of dentigerous cyst, with their increased presence suggesting a potential to cystic transformation understanding their distribution in dental follicle and dentigerous cyst may provide insights into disease progression and early therapeutic interventions.

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Conflicts of interest

There are no conflicts of interest.

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