# **Evaluation of Infiltration of Immunological Cells (Tissue Eosinophil and Mast Cell) in Odontogenic Cysts by Using Special Stains**

#### Priyanka Debta<sup>1\*</sup>, Fakir Mohan Debta<sup>2</sup>, Minal Chaudhary<sup>3</sup> and Vijay Wadhwan<sup>3</sup>

<sup>1</sup>Department of Oral Pathology and Microbiology, Chhattisgarh Dental College and Research institute, PB No.25, Sundra, Rajnandgaon, Chhattisgarh, India <sup>2</sup>Department of Oral Medicine & Radiology, C.D.C.R.I. Sundra, Rajnandgaon, Chhattisgarh, India <sup>3</sup>Department of Oral Pathology and Microbiology, S.P.D.C.,Sawangi, Wardha, Maharashtra, India

## Abstract

Cells of immune system comprised of lymphoid series and myeloid progenitor series cells. Mast cells and tissue eosinophils both are granulocytes which come under myeloid progenitor series of immune cells system. The presence of mast cells in odontogenic cyst could contribute to their pathogenesis in several ways. Mast cells liberate ECF (Eosinophils chemoattractant factor) and histamine, which attract eosinophils in tissue. Mast cells and eosinophils both have also been implicated in stimulating the production of prostaglandins, important in bone resorption for odontogenic cyst growth. The mast cells and eosinophils are features of both keratinizing and non-keratinizing cysts (OKC, DC, RC) but their number does not necessarily correlate with degree of inflammation. Special stains to demonstrate mast cells and eosinophils were used. Carbol Chromotrope staining method to demonstrate tissue eosinophil was found to be better than Congo red. The staining intensity for mast cells was equally good with thionin and toluidine blue.

# Keywords: Mast cells; Eosinophils; Special stain; Odontogenic cyst Introduction

Odontogenic cysts represent the commonest form of cystic lesions that affect the human skeleton. Despite this, it has to be said at the outset that our knowledge of their pathogenesis is at best rudimentary. Cystic lesion with its central fluid, reservoir of non-physiological composition, is in itself likely to provoke an inflammatory response in the surrounding host tissues. There is often, therefore, at the time of microscopical examination of a cyst, some degree of inflammatory change present in its wall. Thus there are some common factors contributing to the pathogenesis of cystic lesion and in their subsequent enlargement [1]. The presence of mast cells has been recognized in odontogenic cysts earlier also [2] interestingly, glycosaminoglycan appears more prevalent in the fluid of the odontogenic keratocyst than non-keratinizing cysts [3]. It would seem probable this arose from degranulation of the mast cells and subsequent passage of heparin from the extracellular matrix of capsule into the luminal fluid could then account for the observed presence of this glycosaminoglycan in cyst fluid [3]. On degranulation of the mast cell, dissociation of heparin proteoglycan may provide a mechanism for activation of the granule associated proteases [4]. A variety of hydrolytic enzymes are released on degranulation of mast cells [5-7] and these enzymes would be expected to degrade components of the connective tissue capsule of odontogenic cysts. Because of poor lymphatic drainage in the cyst wall, these released components would then largely diffuse into the luminal fluid where they would be expected to contribute to increase the osmotic pressure. Mast cells have also been suggested to promote collagenolytic activity [8], which in view of the reported collagenase activity in odontogenic cysts [9,10], may be pertinent to their pathogenesis. Mast cells liberate ECF (Eosinophils chemoattractant factor) and histamine, which attract eosinophils in tissue [11]. Mast cells and eosinophils have also been implicated in stimulating the production of prostaglandins, important in bone resorption for odontogenic cyst growth.

Thus there is several fundamental ways in which mast cells and eosinophils could exert an influence and be important in the pathogenesis of odontogenic cysts. So because of their possible importance in the pathogenesis of odontogenic cysts, our study is aimed at evaluation of the presence of mast and eosinophils in odontogenic cysts and to compare the different special stains which can be use to demonstrate the mast cells and eosinophils. We have also correlated the infiltration of mast cell and eosinophils in odontogenic cyst (OKC, DC, RC).

# Materials and Methods

Cases of odontogenic cyst for study are taken from archive of Department of Oral Pathology, S.P. Dental College, Sawangi, (M), and Wardha (M.H.). The study comprised of thirty cases and they were grouped in to three categories; OKC, Dentigerous cyst and Radicular cyst. Each group comprised of 10 cases. All the cases had been diagnosed on the basis of clinical, radiological and histopathologic correlation. Parakeratinized odontogenic keratocysts cysts has been included in our study and excluded orthokeratinized odontogenic keratocysts.

Special stains were used for evaluation of presence of mast cells and eosinophils in odontogenic cysts. Two special stains; Toluidine blue & Thionin stains were used for mast cells staining and for tissue eosinophils staining Carbol Chromotrope & Congo red stains were used. High density areas were selected in section and cells were counted in randomly choosen ten high power fields.

\*Corresponding author: Priyanka Debta, Lecturer, Oral Pathology and Microbiology, Staff Quarter No. 8/16, College campus, Chhattisgarh Dental College and Research institute, PB No.25, Sundra, Rajnandgaon, (C.G.) 491441, India, Tel: 09685714325; E-mail: <a href="https://drediffmail.com">drepriyanka\_1234@rediffmail.com</a>

Received October 07, 2010; Accepted October 19, 2010; Published October 29, 2010

**Citation:** Debta P, Debta FM, Chaudhary M, Wadhwan V (2010) Evaluation of Infiltration of Immunological Cells (Tissue Eosinophil and Mast Cell) in Odontogenic Cysts by Using Special Stains. J Clin Cell Immunol 1:103. doi:10.4172/2155-9899.1000103

**Copyright:** © 2010 Debta P, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

#### Common steps for staining methods

Study was performed on paraffin embedded tissue which was fixed in 10% neutral buffered formalin and routinely processed. Paraffin wax blocks were cut and the sections of  $5\mu m$  were used for staining. All sections were dewaxed thoroughly in xylene and hydrated through descending grades of alcohol to water.

## [14] For mast cells staining

Toluidine blue staining method -0.5% toluidine blue solution was prepared. Section were stained in this solution for 30 seconds (Figure 1). Thionin staining method -0.6% aqueous thionin solution was prepared. Sections were stained in this solution for 30 minutes & then differentiate the washed section with 0.2% acetic acid (Figure 2).

#### [15] For eosinophils staining

Carbol Chromotrope staining method - 0.5% chromotrope solution was prepared & added 1gm carbol to it. Section were stained with Harries Haematoxilin then differentiate with 1% acid alcohol. After this counterstaining with Carbol Chromotrope is done & section were stained in this solution for 30 minutes (Figure 3). Congo Red staining method -1% congo red solution was prepared. Section were stained in this solution for 5 minutes, then washed in water then dip in 2.5% KOH solution then counterstaining with haematoxilin was done (Figure 4). Common steps after staining - Sections were washed in tap water then dehydrated through ascending grades of alcohol, cleared and mounted in DPX. Slides were examined in 40 X magnification. High density areas were selected in tissue section and cells were counted in randomly in chosen ten high power fields. The mean and SD for each cyst group were determined. As mast cells and eosinophils are inflammatory cells so the degree of inflammation present in the cyst wall was also assessed to see if there was any correlation of number infiltration of mast cells and eosinophils with degree of inflammation. Inflammation was scored as +1 = mild, +2= moderate, +3 = severe inflammation.

#### Results

In our study we found that mean±SD value of mast cells in

Odontogenic Cysts	Mast Cells	Eosinophils
OKC	14.20±2.61	07.10±01.72
DC	7.50±01.56	06.10±01.83
RC	18.20±03.42	22.20±02.19

Table 1: Descriptive Statistics for OKC, DC and RC.

Odontogenic Cysts	Correlation 'r'	p-value
OKC	0.12	0.37, NS,p>0.05
DC	0.38	0.27, NS,p>0.05
RC	0.74	0.11, NS,p>0.05
110	0.74	0.11, 100,0+ 0.00

Table 2: Correlation of Mast Cells and Eosinophils in Odontogenic Cysts.

Odontogenic Cysts (no. of cases)	Mild	Moderate	Severe
OKC	5	4	1
DC	8	2	0
RC	4	3	3

Table 3: Degree of inflammation in odontogenic cysts.

Odontogenic	Mild		Moderate		Severe	
Cysts	Mast Cells	Eosinophils	Mast Cells	Eosinophils	Mast Cells	Eosinophils
OKC	8.80±0.26	6.40±1.36	26.21±3.25	9.00±1.75	8.00±0.00	6.00±0.00
DC	2.87±0.14	6.62±2.34	22.00±4.97	7.25±1.89	0.00±0.00	0.00±0.00
RC	10.52±2.51	12.50±4.80	30.66±5.81	33.66±7.50	26.66±2.52	23.66±2.89

 Table 4: Mean and SD of Mast cells and Eosinophils correlated with degree of inflammation in odontogenic cysts.









radicular cyst is more in comparison to developmental cyst and among developmental cyst more mast cells infiltration is seen in odontogenic keratocyst as compared to dentigerous cyst whereas tissue eosinophils are more infiltrated in radicular cyst cases (Table 1). There is positive correlation between infiltration of mast cells and tissue eosinophils in odontogenic cysts but their infiltration is not statistically significant (Table 2). Assessment of inflammation in the cvst walls indicated rather more inflammation in the radicular cyst cases than dentigerous and keratocysts (Table 3). Mast cells and eosinophils appear to be a feature common to both keratinizing and nonkeratinizing cysts and their infiltration does not necessarily correlate with the degree of inflammation (Table 4), (Chart 1a and Chart 1b). Carbol Chromotrope staining method to demonstrate tissue eosinophil is better than Congo red whereas staining intensity of toluidine blue for mast cell was equally good when compared with thionin. (Figure 1-4)

# Discussion

The presence of mast cells in both keratinizing and nonkeratinizing cysts is in accordance with studies of Goldsmith et al. [2,3,16]. Release of numerous mediators on degranulation of mast cells, play an important role in the pathogenesis of odontogenic cysts. 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 at least three ways: - 1) By direct release of heparin into the luminal fluid [3]. 2) By release of

Page 2 of 4

Citation: Debta P, Debta FM, Chaudhary M, Wadhwan V (2010) Evaluation of Infiltration of Immunological Cells (Tissue Eosinophil and Mast Cell) in Odontogenic Cysts by Using Special Stains. J Clin Cell Immunol 1:103. doi:10.4172/2155-9899.1000103

Page 3 of 4

hydrolytic enzymes [5-7] which could degrade capsular extracellular matrix components thereby facilitating their passage into the fluid. 3) By the action of histamine on smooth muscle contraction and vascular permeability encouraging transudation of serum protein [13] and their subsequent entry into the luminal fluid. A major component of mast cell granules is the glycosaminoglycan. In term of the total glycosaminoglycan content of cyst capsular connective tissue, heparin is an important component of non keratinizing (radicular: 31.4%; dentigerous: 22.2% of total glycosaminoglycan) and keratinizing (odontogenic keratocyst: 28.3% of total glycosaminoglycan) cyst [17]. In our study we have also found increased infiltration of mast cells and tissue eosinophil in radicular cyst in comparison to developmental odontogenic cysts. Mast cells [5] and eosinophils [13], have also been implicated in stimulating the production of prostaglandins, important in bone resorption for odontogenic cyst growth.

#### Conclusion

Mast cells and eosinophils infiltration is not directly proportional to degree of inflammatory response in odontogenic cysts. Mast cells and eosinophils may play important role in the pathogenesis of odontogenic cysts. There is positive correlation between infiltration



Figure 1: Toluidine blue stained section showing mast cells in cyst capsule.



Figure 2: Thionin stained section showing mast cells in cyst capsule.



Figure 3: Carbol Chromotrope stained section showing tissue eosinophils in cyst capsule.



Figure 4: Congo Red stained section showing tissue eosinophils in cyst capsule.

of mast cells and tissue eosinophils in odontogenic cysts but their infiltration is not statistically significant. Carbol Chromotrope staining method to demonstrate tissue eosinophil is better than Congo red. The staining intensity of toluidine blue for mast cell was equally good as well as with thionin. Special stains are wonderful they allow us to see which we can not see with routine H&E stain. The special stains, we used in our study are inexpensive and gives rapid and results reproducible. So microscopic evaluation of mast cells and eosinophils are one of the important aspect to understand the pathogenesis of odontogenic cyst.

#### Future scope

Further studies with larger sample size are required to better understand the role of mast cells and eosinophils in odontogenic cysts & to correlate these cells with enlargement of cysts. Citation: Debta P, Debta FM, Chaudhary M, Wadhwan V (2010) Evaluation of Infiltration of Immunological Cells (Tissue Eosinophil and Mast Cell) in Odontogenic Cysts by Using Special Stains. J Clin Cell Immunol 1:103. doi:10.4172/2155-9899.1000103

Page 4 of 4

#### References

- Roger M Browne (1991) Investigative pathology of odontogenic cysts. CRC Press: 87-88.
- Smith G, Smith AJ, Browne RM (1988) Histochemical studies on glycosaminoglycans of odontogenic cysts. J Oral Pathol 17: 55-59.
- Smith G, Smith AJ, Browne RM (1984) Glycosaminoglycans in fluid aspirates from odontogenic cysts. J Oral Pathol 13: 614-621.
- Galli SJ, Dvorak HF (1979) Cellular, molecular and clinical aspect of allergic disorders. New York: plenum medical 1.
- 5. McKenna J, Frame B (1985) The mast cell and bone. Clin Orthop Relat Res 20: 226-233.
- Wassermann SI (1979) The mast cell and the inflammatory response. In: Pepys J, Edwards Am,eds. The mast cell – its role in health and disease. Bath: pitman Medical 9.
- Yanagisawa S (1980) Pathological study of periapical lesions. I. Periapical granulomas in chemical, histopathological and innunohistopathologic studies. J Oral Pathol 9: 288-300.
- Taylor AC (1971) Collagenolysis in culture tissue. II. Role of mast cells. J. Dent Res 50: 1301-1306.

- 9. Danoff RB, Harper E, Guralnich WC (1972) Collagenolytic activity in keratocysts. J Oral Surg 30: 879-884.
- Uitto VJ, Ylipaavalniemi P (1977) Alterations in collagenolytic activity caused by jaw fluids. Proc Finn Dent Soc 73: 197-202.
- 11. Stewart S, Edward EM (2001) Immunology, immunopathology and immunity. (6<sup>th</sup> Edition), ASM, Washington D.C.
- Majno G, Shea SM, Leventhal M (1969) Endothelial contraction induced by histamine-type mediators; An electronic microscopy study. J Cell Biol 42: 647-72.
- Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, et al. (1999) Wintrobe's clinical Haematology. (10<sup>th</sup> Edition), Williams & Wilkins. London.
- 14. Bancraft JD, Gamble M (2002) Theory and Practice of histological techniques. (4<sup>th</sup> Edition) Churchill Livingstone.
- Culling CFA, Allison RT, Barr WT (1985) Cellular pathology technique. (4<sup>th</sup> Edition) Butterworth and Co. Ltd.
- Smith G, Smith AJ, Basu MK (1989) Mast cells in human odontogenic cysts. J oral pathol med 18: 274-278.
- Smith G. Smith AJ, Browne RM (1988) Quantification and analysis of the glycosaminoglycans in human odontogenic cyst linings. Arch Oral Biol 33: 623-626.