

# Expression of Interleukin-1 Alpha and Interleukin-6 Expression in Keratocystic Odontogenic Tumors and Ameloblastomas.

Kaluram Khande<sup>1</sup>, Abhishek Pathak<sup>2</sup>, Atul kushwah<sup>2</sup>

<sup>1</sup>Oral medicine, Department of Dentistry, J.A. hospital, G.R. Medical College, Gwalior, M.P., India.

<sup>2</sup>Oral surgery, Department of Dentistry, J.A. hospital, G.R. Medical College, Gwalior, M.P., India.

Received: March 2019

Accepted: March 2019

**Copyright:** © the author(s), publisher. Annals of International Medical and Dental Research (AIMDR) is an Official Publication of "Society for Health Care & Research Development". It is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Background:** Both Ameloblastomas and odontogenic keratocysts share many clinical features in common such as local aggressiveness, high recurrence rates and radical management options. Understanding the pathogenesis and biological behavior of these would improve our success at diagnosis and treatment. Despite efforts focused on understanding the pathogenesis of these lesions still little is known about them. **AIM:** To evaluate the expression of IL-1 $\alpha$  and IL-6 by immunohistochemistry in ameloblastomas and KCOTs and correlate their expression with their increase in size and extent of bone destruction. **Methods:** A total of 25 cases of ameloblastomas and 25 cases of keratocystic odontogenic tumors were included in the study. All histological slides were stained immunohistochemically to show the expression of IL-1 $\alpha$  and IL-6. **Results:** Immunohistochemical expressions of IL-1 $\alpha$  and IL-6 in ameloblastoma was observed in only stellate reticulum-like cells While in KCOT the immunohistochemical expression of both the antibodies in comparison to ameloblastoma was observed only in the lining epithelial cells. The higher expression rates of IL-1 $\alpha$  and IL-6 were associated with increases in tumor size in ameloblastomas and connective tissue cyst wall thickness in keratocystic odontogenic tumors. **Conclusion:** The higher expression rates of IL-1 $\alpha$  and IL-6 were associated with increased tumor size in ameloblastomas and with connective tissue cyst wall thickness in KCOT. Thus we can suggest that the cytokines play a role on aggressive behaviour of ameloblastomas and keratocystic odontogenic tumors by facilitating increased bone resorption.

**Keywords:** Ameloblastoma, keratocystic odontogenic tumor, interleukin, aggressiveness, bone resorption.

## INTRODUCTION

Odontogenic tumors are a heterogeneous group of lesions that originate from the tissue that form teeth. Ameloblastoma is a benign tumor originating in the odontogenic epithelium without ectomesenchyme, affecting the maxillo-mandibular complex.<sup>[1]</sup> It is an asymptomatic lesion, and it presents locally invasive behavior, and higher recurrence rates.<sup>[2]</sup> The keratocystic odontogenic tumor (KCOT), according to the most recent classification of tumors of the head and neck of the World Health Organization (WHO), has been categorized as benign neoplasm derived from odontogenic epithelium due to its aggressive clinical behavior, high recurrence and proliferation rate. But still there are disagreements, questioning whether this odontogenic lesion indeed is a

neoplasm or a cyst of odontogenic nature.

Both Ameloblastomas and odontogenic keratocysts share many clinical features in common such as local aggressiveness, high recurrence rates and radical management options. Understanding the pathogenesis and biological behavior of these would improve our success at diagnosis and treatment. Despite efforts focused on understanding the pathogenesis of these lesions still little is known about them.

It has been established during the past few years that inflammatory cytokines like interleukin(s)-1, -6, -11 and tumor necrosis factor (TNF) can stimulate osteoclast development and thereby the process of bone resorption. Moreover, upregulation of the production and/or action of IL-6 has been implicated in the pathogenesis of disease states characterized by excessive osteoclastic bone resorption. It has been reported that Cytokine levels, especially osteolytic cytokines found in intracystic fluids of cysts or expressed by the cells may play an important role on growth of ameloblastomas and keratocystic odontogenic tumors in jaws.<sup>[5-7]</sup> Also, it is suggested that in

### Name & Address of Corresponding Author

Dr. Abhishek Pathak  
Oral surgery, Department of dentistry  
J.A. hospital, G.R. Medical College,  
Gwalior, M.P., India.

ameloblastoma the IL-1 $\alpha$  and IL-6 cytokines play a role in the aggressive behavior of ameloblastomas by increasing bone resorption. Several studies have indicated that in KCOT proliferating epithelial cells expressed various genes, such as IL-1 $\alpha$ , IL-6, TNF $\alpha$ , and RANKL related to osteoclastogenesis and bone resorption.<sup>[8]</sup>

Therefore we aimed the present study to evaluate the expression of IL-1 $\alpha$  and IL-6 by immunohistochemistry in ameloblastomas and KCOTs and correlate their expression with their increase in size and extent of bone destruction.

## MATERIALS AND METHODS

A total of 50 sample including 25 ameloblastomas and 25 KOTs were selected from the archive of the department of ..... as control we used 10 tissues of dental follicles and 10 tissues of non inflamed dentigerous cysts. The selected tissues were stained Immunohistochemically. Sections were incubated with rabbit polyclonal anti-IL-1 $\alpha$  and goat polyclonal anti-IL-6 antibodies overnight at 40 C. The dilutions of antibodies were 1:25 and 1:50 in PBS, respectively. Positive control tissues were tonsil for both antibodies. The red-brown cytoplasmic staining was considered as positive. The staining intensity for antibodies was regarded intense (+++), moderate (++) , mild (+) and negative (-) as suggested by Kubota et al.<sup>9</sup>

### Statistical analysis

The statistical analyses were carried out using SPSS (Version 21.0, SPSS Inc., and Chicago, USA) software program. The level of significance set at P value  $\leq$  0.05. Univariate analysis was performed by using logistic regression analysis to identify possible predictors of ameloblastoma or keratocystic odontogenic tumor.

## RESULTS

Our results show that immunohistochemical expressions of IL-1 $\alpha$  and IL-6 in ameloblastoma was observed in only stellate reticulum-like cells while it was absent in basal cells of the epithelial islands. Staining intensity of IL-1 $\alpha$  was mild (+) in 4 out of 25 cases, moderate (++) in 12 out of 25 cases and intense (+++) in 9 out of 25 cases. Immunohistochemical expression of IL-6 was observed positive in only 12 cases (7 cases showing mild staining and 5 cases showing moderate staining). [Table 1].

Whereas, in KCOT the immunohistochemical expression of both the antibodies in comparison to ameloblastoma was observed only in the lining epithelial cells. Distribution of number of cases according to staining intensities in KCOT are tabulated in [Table 2]. Apart from this, staining was

also seen in the inflammatory cells and capillary endothelial cells in. Negative the staining was observed in the control tissues for both cytokines. Though there was a difference in staining intensity between both lesions, but the results were not statistically significant.

On further comparison a positive correlation was observed with the size of tumor and connective tissue wall with the expression of both cytokines in both amelolastoma and KCOT respectively.

**Table 1: Results of Immunostainig of Il-1 $\alpha$  in Ameloblastoma and KCOT**

Immunostaining	Intensity Score	Ameloblastoma (N=25)	KCOT (N=25)
IL-1 $\alpha$	Negative	0	3
	mild	4	14
	moderate	12	8
	intense	9	0

**Table 2: Results of immunostainig of il-6 in ameloblastoma and KCOT**

Immunostaining	Intensity Score	Ameloblastoma (n=25)	KCOT (N=25)
IL-6	Negative	13	6
	mild	7	6
	moderate	5	11
	intense	0	2

## DISCUSSION

The present study was aimed to evaluate the expression of of IL-1 $\alpha$  and IL-6 by immunohistochemistry in ameloblastomas and KCOTs and correlate their expression with their increase in size and extent of bone destruction.

The results of the present study shows that both IL-1 $\alpha$  and IL-6 are expressed in stellate reticulum-like cells in ameloblastomas. Previous literature have shown that Cytokines, including IL1 $\alpha$ , IL-1 $\beta$ , IL-6 and TNF- $\alpha$ , have proven osteolytic activity and can also stimulate cell growth. The activities of these cytokines are consistent with them having a role in growth and intraosseous expansion in both ameloblastoma and KCOT. The results of the present study are consistent with those of Pripatnanont et al,<sup>[10]</sup> and Sengüven B et al.<sup>[11]</sup> Further Sengüven B et al,<sup>[11]</sup> also showed through mRNA hybridization that the source of IL-1 $\alpha$  and IL-6 in ameloblastomas is stellate reticulum-like cells. In studies by various authors, IL-1 $\alpha$  protein has been shown to be associated with epithelial stellate reticulum cells in tooth germs and also associated with osteoclastic activity in developing tooth germs.<sup>[12,13]</sup>

Further in our study, we observed that expressions of IL-1 $\alpha$  and IL-6 were in lining epithelial cells of KCOTs. Such findings were consistent with those reported by as Pripatnanont et al.<sup>[10]</sup> and Ninomiya et al.<sup>[14]</sup> earlier as well. Contrasting results were obtained with the inflammatory radicular cysts,

probably due to the inflammatory cell infiltrate present.<sup>[15]</sup> In the control tissues, the epithelial surface cell layers demonstrated no reactivity with antibodies to both IL-1 $\alpha$  and to IL-6. Dentigerous cysts and dental follicles rarely reach significant sizes in jaw bones unlike ameloblastomas and KCOTs. Weak expression of IL-1 $\alpha$  and IL-6 could be an additional reason. Thus, our results justifies the concept that epithelial proliferation is efficient in KCOT growth rather than the osmotic pressure. Like most of other cytokines IL-1 $\alpha$  and IL-6 play role in epithelial cells as growth factor.

Another significant finding of our study was a positive correlation between expression of these cytokines and tumor size in ameloblastoma and connective tissue wall thickness in KCOT. This suggest that main osteolytic cytokines expressed in these lesions help in tumor growth by increased bone resorption. Based on this finding we can say that both IL-1 $\alpha$  and IL-6 proteins play important role on the aggressive behavior of ameloblastoma and KCOT.

## CONCLUSION

The higher expression rates of IL-1 $\alpha$  and IL-6 were associated with increased tumor size in ameloblastomas and with connective tissue cyst wall thickness in KCOT. Thus we can suggest that the cytokines play a role on aggressive behaviour of ameloblastomas and keratocystic odontogenic tumors by facilitating increased bone resorption.

## REFERENCES

- Rastigi S, Nijhawan S, Modi M, et al. A Large Mixed Radiolucent-Radiopaque Lesion In The Mandible- A Nobel Diagnostic Approach. J Clin Diagn Res. 2010;4:2300-7.
- Fregnani ER, da Cruz Perez DE, de Almeida OP, et al. Clinicopathological study and treatment outcomes of 121 cases of ameloblastomas. Int J Oral Maxillofac Surg. 2010;39:145-9.
- de Oliveira MG, Lauxen Ida S, Chaves AC, et al. Immunohistochemical analysis of the patterns of p53 and PCNA expression in odontogenic cystic lesions. Med Oral Patol Oral Cir Bucal. 2008;13:E275
- Thosaporn W, Iamaroon A, Pongsirivet S, et al. A comparative study of epithelial cell proliferation between the odontogenic keratocyst, orthokeratinized odontogenic cyst, dentigerous cyst, and ameloblastoma. Oral Dis. 2004;10:22-6.
- Sengüven B, Oygür T. Investigation of interleukin-1 alpha and interleukin-6 expression and interleukin-1 alpha gene polymorphism in keratocystic odontogenic tumors and ameloblastomas. Med Oral Patol Oral Cir Bucal 2011; 16: e467-e472.
- Pripatnanont P, Song Y, Harris M, Meghji S. In situ hybridisation and immunocytochemical localisation of osteolytic cytokines and adhesion molecules in ameloblastomas. J Oral Pathol Med. 1998;27:496-500.
- Ninomiya T, Kubota Y, Koji T, Shirasuna K. Marsupialization inhibits interleukin-1alpha expression and epithelial cell proliferation in odontogenic keratocysts. J Oral Pathol Med. 2002;31:526-33
- Mendes RA, Carvalho JFC, van der Waal I (2010a). Biological pathways involved in the aggressive behavior of the keratocystic odontogenic tumor and possible implications for molecular oriented treatment - An overview. Oral Oncol 46: 19-24.
- Kubota Y, Nitta S, Oka S, Nakagawa S, Ninomiya T, Shirasuna K. Discrimination of ameloblastomas from odontogenic keratocysts by cytokine levels and gelatinase species of the intracystic fluids. J Oral Pathol Med. 2001;30:421-7
- Pripatnanont P, Song Y, Harris M, Meghji S. In situ hybridisation and immunocytochemical localisation of osteolytic cytokines and adhesion molecules in ameloblastomas. J Oral Pathol Med. 1998;27:496-500.
- Sengüven B, Oygür T. Investigation of interleukin-1 alpha and interleukin-6 expression and interleukin-1 alpha gene polymorphism in kerato- and interleukin-1 alpha gene polymorphism in keratocystic odontogenic tumors and ameloblastomas. Med Oral Patol Oral Cir Bucal. 2011 Jul 1;16 (4):e467-72
- Wise GE, Lin F, Zhao L. Immunolocalization of interleukin-1 alpha in rat mandibular molars and its enhancement after in vivo injection of epidermal growth factor. Cell Tissue Res. 1995;280:21-6.
- Marks SC Jr, Gorski JP, Wise GE. The mechanisms and mediators of tooth eruption--models for developmental biologists. Int J Dev Biol. 1995;39:223-30
- Ninomiya T, Kubota Y, Koji T, Shirasuna K. Marsupialization inhibits interleukin-1alpha expression and epithelial cell proliferation in odontogenic keratocysts. J Oral Pathol Med. 2002;31:526-33.
- Bando Y, Handerson B, Meghji S, Harris M. Immunocytochemical localisation of cytokines and adhesion receptor in radicular cyst. Immunocytochemical localisation of cytokines and adhesion receptor in radicular cysts. J Oral Pathol Med 1993; 22: 221-7

**How to cite this article:** Khande K, Pathak A, Kushwah A. Expression of Interleukin-1 Alpha and Interleukin-6 Expression in Keratocystic Odontogenic Tumors and Ameloblastomas. Ann. Int. Med. Den. Res. 2019; 5(3):DE04-DE06.

**Source of Support:** Nil, **Conflict of Interest:** None declared