

Immunohistochemical Expression of PCNA in Epithelial Linings of Selected Odontogenic Lesions

TANVEER SHAHELA¹, SYEDA AESHA², KANNAN RANGANATHAN³, ROOBAN T.⁴, UMA DEVI ROA K.⁵, ELIZABETH JOSHUA⁶, AFROZ SYED AHMED⁷, HARIKA CHITTAMSETTY⁸

ABSTRACT

Aim: Though odontogenic lesions have in common origin from the primitive odontogenic epithelium, there is a diversity in their initiation behavior and growth. The aggressive nature of odontogenic keratocyst similar to ameloblastoma in comparison with the other common odontogenic (dentigerous and radicular) cyst could be due to possible differences in their epithelial linings. PCNA (Proliferating cell nuclear antigen) is a cell cycle related antigen, used in the study of cell kinetics of these epithelial linings, to correlate the biological behaviour among these common odontogenic lesions.

Objectives: This paper has intended to study the cell kinetics of 4 selected odontogenic lesions (dentigerous cyst, radicular cyst, odontogenic keratocyst and ameloblastoma) to demonstrate differences in their epithelial linings.

Material and Methods: Sixty samples of paraffin embedded tissue specimens (archival tissues) were included (15 radicularcysts, 15 odontogenic keratocysts; 15 dentigerous cysts and 15 Ameloblastoma).

Results: Among cyst OKC had higher values than DC and RC. When staining results of all the cyst were compared individually with ameloblastoma, the staining results of OKC was similar to ameloblastoma, and ameloblastoma presented higher values than OKC ($p=0.000$).

Conclusion: The results of this study show 1) Though OKC and DC are developmental in origin and radicular cyst is inflammatory, OKC has intrinsic growth potential among these cyst 2). Ameloblastoma has similar proliferative potential as OKC and hence it has been reinforced as KCOT (Keratocystic odontogenic tumour) in the recent classification.

Keywords: PCNA, Immunohistochemistry, Odontogenic tumours, Cell kinetics

INTRODUCTION

Odontogenic lesions originate from multiple sources of odontogenic epithelium [1]. Among the most commonest cystic lesions like dentigerous cyst, radicular cyst and odontogenic keratocyst, the biological behaviour of few odontogenic keratocysts is in every way as a benign neoplasm similar to that of ameloblastoma, and hence OKC is now designated by WHO as a Keratocystic odontogenic tumor (KCOT) [2,3].

The increased cell proliferation in the odontogenic epithelium can result from perturbations in cell cycle regulators, mutations tumour suppressor genes and genetic mutations [4]. Investigating cell kinetics of epithelial lining of these cystic lesions may be helpful in assessing their biological behaviour [5].

Proliferating Cell Nuclear Antigen (PCNA) is a 36kDa acidic non histone nuclear protein important for DNA synthesis. In the presence of replicating factor C, a multi sub unit complex, PCNA allows DNA polymerase to initiate leading strand for DNA synthesis [6]. PCNA expression may be used as a marker of cell proliferation because cells remain longer time in G1 – S phase when proliferating. An increase in PCNA levels may be induced by growth factors or as a result of DNA damage in absence of cell cycle [7].

The aim of the present immunohistochemical study is to investigate the role of PCNA in the epithelial linings of these selected odontogenic lesions to predict their biological behaviour.

AIMS AND OBJECTIVES

To compare and evaluate PCNA labelling index 4 different

odontogenic lesions of (n=15) radicular cyst, (n=15) OKC, (n=15) dentigerous cyst and (n=15) ameloblastoma.

MATERIAL AND METHODS

A retrospective study was done to evaluate the expression of PCNA using immunohistochemistry in formalin fixed, paraffin embedded tissue specimens (archival tissues). The study was conducted in the Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, Chennai, India and was approved by Institutional Review Board (IRB).

The study material comprised of 60 formalin fixed, paraffin embedded tissue specimens archival blocks randomly selected from department of oral pathology with adequate clinically, radiographically and histologically confirmed cases of dentigerous cyst (Group I, n=15), radicular cyst (Group II, n=15) odontogenic keratocyst (Group III, n=15) and ameloblastoma (Group IV, n=15).

Immunohistochemistry (IHC) Procedure

A 5µ sections from paraffin embedded blocks were made and transferred onto the amino propyl epoxy silane (APES) coated slides. The slides with tissue sections were treated with three changes of xylene to remove paraffin wax, followed by descending grades of alcohol and rehydration with water. The slides were then transferred to citrate buffer and autoclaved for antigen retrieval at 15 lbs pressure for 15 minutes. The slides were allowed to cool and washed in phosphate buffer solution (PBS) (pH 7.2) for 5 minutes. Peroxidase and protein blocking were done using 3%

hydrogen peroxide and protein block reagent respectively for 10 minutes each. The slides were incubated with primary antibodies (Anti-Human PCNA, PC10 Prediluted in PBS (Biogenex, USA) at room temperature for overnight and for 2 hours. The secondary antibody used was an enhancer followed by streptavidin solution for 20 and 30 minutes respectively from the secondary polymer kit (Biogenex). A drop of freshly prepared DAB (3' Diaminobenzidine Tetrahydrochloride - a substrate chromogen) was added. Three changes of PBS washes were performed after every step during the immunostaining procedure. The sections were counter stained with hematoxylin and mounted with DPX®.

Criteria for evaluation of PCNA staining

The following parameters were used to evaluate PCNA staining

- The cellular localization of stain in the nucleus.
- Nuclei with brown color regardless of staining intensity were regarded as positive.
- Tissue localization of stain – basal layer or suprabasal layer of the epithelium

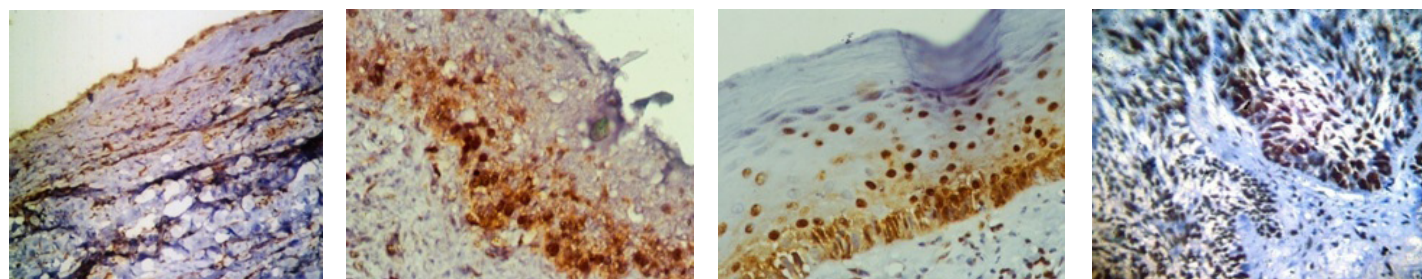
Labelling index (LI)

PCNA positivity was calculated by dividing the number of positive cells by the total number of cells counted in the slide and expressed as percentage for PCNA. The percentage of PCNA was evaluated from a minimum of 1000 nuclei per case. A minimum of thousand cells was counted for each slide (LI = Number of positive cells / Total number of cells counted x100).

RESULTS AND OBSERVATIONS

Distribution of PCNA staining among 4 groups in the epithelium

PCNA revealed positivity in all the Groups I, II, III and IV. In Group



[Table/Fig-1]: PCNA staining in dentigerous cyst

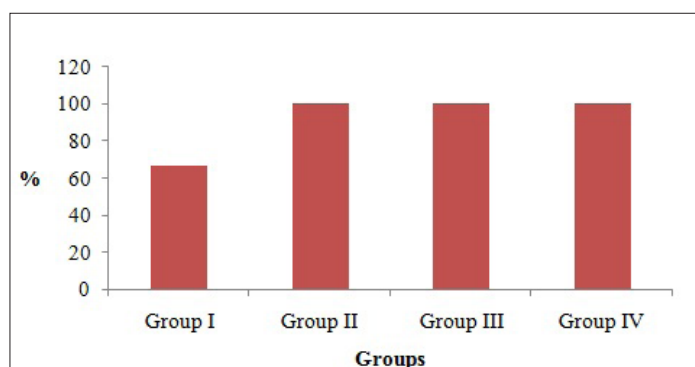
[Table/Fig-2]: PCNA staining in radicular cyst

[Table/Fig-3]: PCNA staining in odontogenic keratocyst

[Table/Fig-4]: PCNA staining Ameloblastoma

Group I		Group II		Group III		Group IV		p-value
n	%	n	%	n	%	n	%	
10	66.6	15	100	15	100	15	100	0.001*

[Table/Fig-5]: PCNA positive staining among study groups



[Table/Fig-6]: PCNA positive staining among study groups

I 66.6% n=10) showed positivity [Table/Fig-1] and (n=5) 33.3% showed negative staining. In Group III 80% (n=12) showed positive staining in the epithelium [Table/Fig-2], and Group II [Table/Fig-3] and Group IV [Table/Fig-4], the cases showed 100% staining. There was significant difference (P=0.001) in the staining pattern. [Table/Fig-5 and 6].

Distribution of expression in the basal layer and suprabasal layers among the cyst.

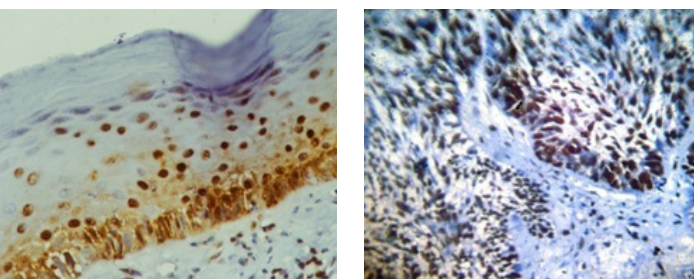
PCNA staining was present in the basal layer among three study groups. In Group II (n=15)100% of cases showed staining in both basal and suprabasal layer. In Group I and II none of the cases have shown suprabasal positivity. There was no significant difference in the basal and suprabasal layers among the study groups [Table/ Fig-7 and 8].

Comparison of mean labelling index among the study groups

In Group I, 10/15 cases showed nuclear staining and their mean labelling index were 2.3 ± 1.6. In Group II, 15/15 cases showed nuclear staining which had the mean labelling index of 9.5 ± 4.4. In Group III, 12/15 case showed nuclear staining and its mean labeling index is 3.9 ± 1.8. The mean labelling index for ameloblastoma were 12.2 ± 12.6. There was significant difference in the mean labeling index among the groups (p=0.00) [Table/Fig-9 and 10].

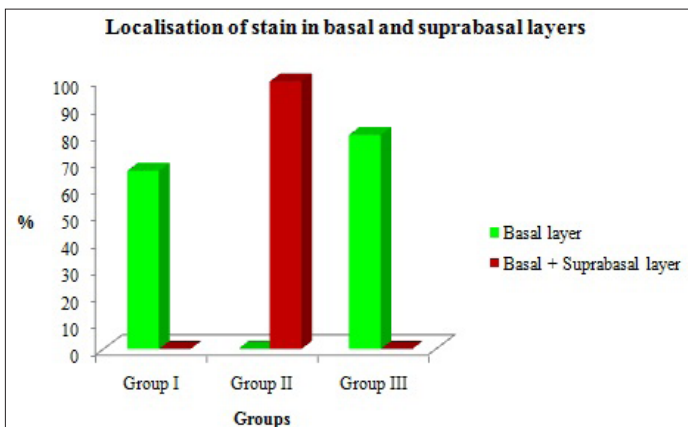
DISCUSSION

Various studies on molecular alterations are now proposed to be responsible for the development and biological behavior of these cyst in particular the OKC [2,3]. The role of PCNA is a marker of normal cellular proliferation, and its overexpression suggests deregulated DNA synthesis and genomic instability [7]. Cell proliferation in odontogenic lining epithelium may be induced in response to various growth factors, cells tress [8].



Groups	Localization						p-value
	Basal Layer		Basal and Supra-basal		No stain		
	n	%	n	%	n	%	
Group I	10	66.6	0	0	5	33.3	0.131
Group II	0	0	15	100	0	0	
Group III	12	80	0	0	3	20	

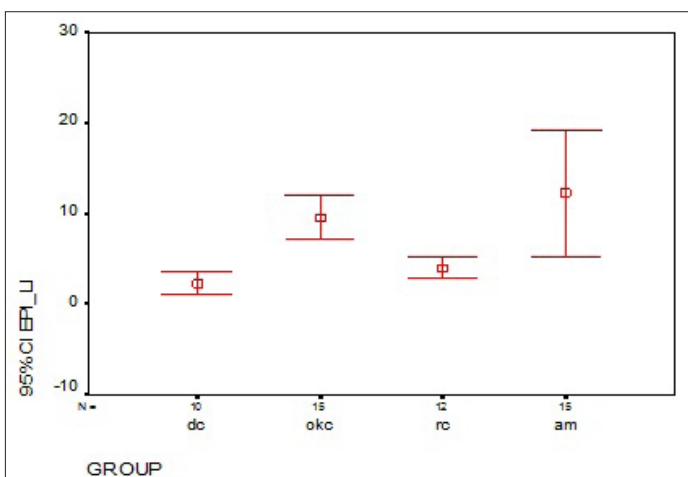
[Table/Fig-7]: Localization of stain in basal and suprabasal layers among odontogenic cysts



[Table/Fig-8]: Localization of stain in basal and suprabasal layers in odontogenic cysts. (Pearson Chi-square value test)

Groups	s			p-value
	Mean	SD	Median	
Group I (n=10)	2.31	1.63	1.75	0.000*
Group II (n=15)	9.58	4.42	9.30	
Group III (n=12)	3.99	1.86	3.80	
Group IV (n=15)	12.25	12.64	4.30	

[Table/Fig-9]: PCNA labelling index in the odontogenic epithelium among the study groups. (Mann-Whitney U-Test)



[Table/Fig-10]: PCNA labelling index in the odontogenic epithelium among the study groups

In the present study, our results are consistent with the studies of A. Patelli et al., Browne et al., [1,9], which shows lower PCNA expression in radicular and dentigerous cyst although they have different origin. Marcia Gaiger et al., [10], suggested the plausible cause of minimal PCNA positivity in dentigerous cyst is because of the inflammatory stimuli as a result of eruptive process, this may make cells to proliferate, but may be inconstant and present only for a short time.

Bando et al., [11], suggested that in radicular cyst the inflammatory stimulus (cytokines) increases cell proliferation from the continuous aggression caused by the bacterial contamination of the root canal, which may inhibit degradation of p53 protein and increase the level of PCNA in the absence of cell cycling, in contrast to which the stimuli is only for short periods in dentigerous cyst.

Distribution of PCNA positive cells within OKC linings differed significantly from that in other odontogenic cyst, with greater than 95% of stained cells located in suprabasal layers, this could probably suggest increased growth factors (Egfr) in OKC [12,13], and higher level of cytokines(IL-1) stimulates epithelial cell proliferation directly and/or indirectly by inducing the secretion of some factors like keratinocyte growth factor with increased activity

of keratin layers [14], which may contribute to the higher thickness of the epithelium in OKC.

In our study, PCNA staining was 100% in both OKC and ameloblastoma. In ameloblastoma cases our results were consistent with other studies in which cells at the periphery stained intensely than the central cells, which could be because these cells may be similar to ameloblast of enamel organ which are metabolically active (increased PCNA), in comparison to central stellate reticulum like cells [15,16].

In our study comparing PCNA indices among the OKC and ameloblastoma, the OKC had no higher labelling index (mean 9.58) than ameloblastoma (mean 12.25), but had higher labelling indices than DC (mean 2.31) and RC (mean 3.99). Our results were not in accordance with findings of Takahashi and Fujita [17], which suggests that OKC has higher labelling indices than Ameloblastoma.

CONCLUSION

The results of this study shows that although these cyst are classified as lesions of different origin, the cellular proliferation is governed by many molecular factors (growth factors, P53) which influences the PCNA expression. Among the cyst OKC has higher cellular kinetics in comparison to DC and RC, which suggest that OKC has intrinsic growth potential, similar to ameloblastoma and hence it has been reinforced as KCOT (Keratocystic odontogenic tumour) in the recent classification. However larger sample size and further studies are needed using DNA assays to analyse if any cell cycle perturbations or gene mutations play a role in pathogenesis of these tumours.

REFERENCES

- [1] Browne R M. The pathogenesis of odontogenic cysts: a review. *Journal of Oral Pathology*. 1975; 4: 31-46.
- [2] Mendes R A, Carvalho F C Biological pathways involved in the aggressive behavior of the keratocystic odontogenic tumor and possible implications for molecular oriented treatment. An overview. *Oral Oncology*. 2010; 46: 19-24.
- [3] Mervyn Shear. The aggressive nature of the odontogenic keratocysts: is it a benign cystic neoplasm? Part 2. Clinical and early experimental evidence of aggressive behavior. *Oral oncology*. 38(2002) 323-31.
- [4] Philipsen HP, Barnes L, Everson JW, Reichart P, Sidransky D. WHO classification of tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press; 2005. p.306-07.
- [5] Reichart PA, Phillipsen HP. Odontogenic Tumors and Allied Lesions. London: Quintessence; 2004.
- [6] Kelmana Z PCNA structure, functions and interactions. *Oncogene*. 1997;14, 629-40.
- [7] Giovanni M, Hubscher U. Proliferating cell nuclear antigen (PCNA); a dancer with many partner. *Journal of cell science*. 2003; 116: 3051-60.
- [8] Ninomiya T, Kubota Y, Koji T, Shirasuna K. Marsupialization inhibits interleukin 1 alpha expression and epithelial cell proliferation in odontogenic keratocyst. *J Oral Pathol Med*. 2002; 31: 526-33.
- [9] Piattelli A, Fioroni M, Santinelli. Expression of Proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. *Oral Oncology*. 1998;34:408-412
- [10] Marcia G, Isabel S Immunohistochemical analysis of the patterns of p53 and PCNA expression in odontogenic cystic lesions. *Med Oral Pathol Oral Cir Bucal*. 2008; 3: 275-80.
- [11] Bando Y, Handerson B, Meghji S, Poole S, Harris M. Immunocytochemical localization of inflammatory cytokines and vascular adhesion receptors in radicular cyst. *J Oral Pathol Med*. 1993; 22: 221-7.
- [12] Shetty DC, Aadithya M Classifying odontogenic keratocysts as benign cystic neoplasms: a molecular insight into its aggressiveness. *J Maxillofac Oral Surg*. 2010; 9: 30-34.
- [13] Odgen GR, Chisholm DM, Kiddie RA, Lane DP. p53 protein in odontogenic cyst: an increased expression in some odontogenic keratocyst. *J Clinical Pathol*. 1992; 45, 1007-10.
- [14] Mass- Szawowski. N, Shimotoyodome A, Fusenig N E. keratinocyte growth regulation in fibroblasts co-cultures via a double paracrine mechanism. *Journal of cell science*. 1999; 112: 1843-53.
- [15] Carolina Cavalieri Gomes, Alessandra Pires Duarte, Marina Goncalves Diniz, Ricardo Santiago Gomez. Current concepts of ameloblastoma pathogenesis. *J Oral Pathol Med*. 2010 39: 585-91.
- [16] Shabnum Meer. Proliferating cell nuclear antigen and Ki67 immunoreactivity in Ameloblastomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003;95
- [17] Takahashi H, Fujita S, Yambe S, Moriishi T, Okabe H, Tajima T. Comparison of PCNA in odontogenic keratocyst and ameloblastoma: An immunohistochemical study. *Anal Cell Pathol*. 1998;16:185-92.

PARTICULARS OF CONTRIBUTORS:

1. Senior Lecturer, Department of Oral Pathology, Sri Sai College of Dental Sugery, Vikarabad, India.
2. Senior Lecturer, Department of Oral Pathology, Chettinad Dental College, Chennai, India.
3. Professor and Head of Department, Department of Oral Pathology, Ragas Dental College and Hospital, Chennai, India.
4. Assistant Professor, Department of Oral Pathology, Ragas Dental College and Hospital, Chennai, India.
5. Professor, Department of Oral Pathology, Ragas Dental College and Hospital, Chennai, India.
6. Professor, Department of Oral Pathology, Ragas Dental College and Hospital, Chennai, India.
7. Professor and Head of Department, Department of Oral Pathology, Sri Sai College of Dental Surgery, Vikarabad, India.
8. Senior Lecturer, Department of Oral Pathology, Sri Sai College of Dental Surgery, Vikarabad, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Tanveer Shahela,
H.N. 0-9-4-87/b/43, Minarcolony, Tolichowki, Hyderabad-500008, India.
Phone: 8790144486, E-mail: drshahela17@gmail.com

Date of Submission: **Feb 07, 2013**Date of Peer Review: **Jul 04, 2013**Date of Acceptance: **Sep 18, 2013**Date of Publishing: **Nov 10, 2013****FINANCIAL OR OTHER COMPETING INTERESTS:** None.