Journal of Clinical Imaging Science

Editor-in-Chief: Vikram S. Dogra, MD
Department of Imaging Sciences, University of Rochester Medical Center, Rochester, USA

OPEN ACCESS
HTML format

For entire Editorial Board visit : www.clinicalimagingscience.org/editorialboard.asp

www.clinicalimagingscience.org

RADIOLOGIC–PATHOLOGIC CORRELATION

Are All Odontogenic Keratocysts Keratocystic Odontogenic Tumors? Correlation between Imaging Features and Epithelial Cell Proliferation

Harkanwal Preet Singh, Amit Nayar, Asha Raj[1], Prince Kumar[2]

Department of Oral and Maxillofacial Pathology and Microbiology, Swami Devi Dyal Hospital and Dental College, Panchkula, Haryana, India, [1]Departments of Biochemistry and [2]Prosthodontics, Shree Bankey Bihari Dental College and Research Centre, Ghaziabad, Uttar Pradesh, India

Address for correspondence:
Dr. Harkanwal Preet Singh
Department of Oral and Maxillofacial Pathology and Microbiology, Swami Devi Dyal Hospital and Dental College, Panchkula, Haryana, India.
E-mail: hkps0320@gmail.com

Recieved : 10-09-2012
Accepted : 25-10-2012
Published : 30-10-2012

ABSTRACT

This study was to correlate and analyze the imaging features and epithelial cell proliferation pattern in different cases of keratocystic odontogenic tumors (KCOT) and study the role of inflammation using proliferative markers and different radiographic patterns of KCOT to determine its biological behavior. One hundred and eighty-six cases of KCOT were taken together and grouped based on radiographic patterns. Forty cases were randomly selected and stained using a proliferating cellular nuclear antigen marker. The correlation between imaging and epithelial proliferation with and without inflammation was determined. Unilocular variety is the most common type of KCOT, showing least epithelial proliferation of all the patterns. More than 50% of the multilocular KCOTs were associated with inflammation, showing an enhanced rate of epithelial proliferation. Results were subjected to statistical analysis. Different rates of epithelial proliferation of the different patterns suggested that all odontogenic keratocysts do not behave like tumors and that aggressive treatment should be reserved for selective cases only depending on radiographic and other histopathological parameters such as inflammation.

Key words: Cyst, inflammation, proliferation, radiograph, tumor

Access this article online	
Quick Response Code:	Website: www.clinicalimagingscience.org
	DOI: 10.4103/2156-7514.106616

INTRODUCTION

Odontogenic keratocyst (OKC) is a clinicopathologically distinct form of odontogenic cyst known for its pathognomonic features, i.e., aggressiveness and high rates of recurrence, which have been of interest and debate since the first description of this entity by Philipsen in 1956. Recently, the World Health Organization (WHO)

This article may be cited as:
Singh H, Nayar A, Raj A, Kumar P. Are All Odontogenic Keratocysts Keratocystic Odontogenic Tumors? Correlation between Imaging Features and Epithelial Cell Proliferation. J Clin Imaging Sci 2013;3:3.
Available FREE in open access from: http://www.clinicalimagingscience.org/text.asp?2013/3/1/3/106616

Working Group on Odontogenic Tumors recommended the term "Keratocystic Odontogenic Tumors" (KCOT) for these lesions to address their neoplastic nature, which indicated that the OKC epithelial lining may have some intrinsic growth potential but reclassification has not yet been universally accepted.[1-3] Therefore, treatment of this entity is still contentious.

Imaging of the lesions can help narrow the differential diagnosis, thereby helping to guide patient treatment. However, the radiographic appearances of KCOTs are varied and it is difficult to distinguish KCOTs from other cysts or neoplastic lesions on the basis of their radiographic features alone.[4]

Over the years, many antibodies have been developed against relevant proteins associated with the cell cycle. Among these, monoclonal antibodies against proliferating cellular nuclear antigen (PCNA) and Ki-67 are probably the most widely used.[5] PCNA expression may be used as a marker of cell proliferation because the cell remains for a longer time in the G1/S phase during proliferation. As PCNA is one of the several markers of cellular proliferation, high PCNA activity is indicative of higher proliferative activity.[5-7]

Nonetheless, whether the pattern of KCOT proliferation and its radiographic appearances have any correlation is still not completely understood. This study investigates the relationship between radiographic patterns of KCOT and immunohistochemical expression of PCNA in its epithelial cells. In addition, correlation between inflammation in the cyst wall and radiographic appearances and PCNA expression was evaluated to determine the behavior of this controversial entity and define the line of treatment.

Radiographic features

Radiographs of 186 patients (112 males and 74 females, 1.51:1 ratio) were included in this study. The patients were diagnosed histopathologically as having a KCOT (orthokeratinized KCOTs were excluded) based on the clinical and histopathologic criteria given by the WHO in 1992. The mean age of the patients was 34.2, years with a range of 14-82 years. Additionally, 40 cases (28 inflamed and 12 non-inflamed KCOT) of the total 186 patients (22 males and 18 females, 1.2:1 ratio) were selected from the archives of the Department of Oral Pathology, Swami Devi Dyal Dental College, Panchkula, India [Table 1]. A retrospective radiographic analysis was performed on 186 cases to gain insight into the radiographic characteristics The radiographs consisted of panoramic radiographs, lateral and posteroanterior skull conventional radiographs and posteroanterior chest radiographs.

Table 1: Radiographic appearance and histopathologic characteristic of 186 patients with keratocystic odontogenic tumors

Radiographic appearance	Number of cases (%)	Non-inflamed KCOT (%)	Inflamed KCOT (%)
Unilocular	128 (68.8)	92 (71.87)	36 (28.13)
Multilocular	38 (20.43)	16 (42.10)	22 (57.90)
Multiple KCOT (non syndromic)	14 (7.5)	11 (78.57)	3 (21.43)
NBCCSS	06 (3.22)	05 (83.33)	01 (1.67)

KCOT: Keratocystic odontogenic tumors

Histopathologic features

Immunohistochemical evaluation

Forty OKCs (28 non-inflamed and 12 inflamed) were selected randomly and two sets of 4-μm sections were prepared. One set of sections was stained by Harris hematoxylin and eosin for histologic diagnosis while the other set was stained with PCNA for immunohistochemical analysis.

The inflammatory density score was determined by counting the inflammatory cells adjacent to the basement membrane, up to a depth corresponding with one histopathological field (HPF) under 40× magnification. It was evaluated in 10 separate fields and the inflammatory density score for each case was calculated as the average of all HPFs examined. Inflammatory density was graded as follows. Grade 0: No inflammation, Grade 1: <15 cells/field, Grade 2: 15-50 cells/field and Grade 3: >50 cells/field. Data from each individual field were tabulated and then grouped for analysis. This approach was preferred because, often, there are focal variations in the epithelial lining as well as in the inflammatory infiltrate density within each cyst, and proliferation may not be equally distributed along the lining. Therefore, analysis of data, from each individual field, is potentially more sensitive in the detection of relationships between inflammation, epithelial morphology and expression of proliferation markers. The positively stained PCNA areas showed uptake of brown color. PCNA-positive cells were counted in 1000 cells of each sample: 50 cells in the basal layer and 50 cells in the suprabasal layer. Ten representative fields at the 400× magnification we selected and cells were counted in each of the mentioned layers. Thus, by selecting 10 random areas that were not in continuum with each other, a possible error in recounting the same cell was minimized.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) and Epi-Info 6.04d software. Difference between the mean of two independent groups was observed by the *T* test if data was normally (evenly) distributed. Differences between the

different variables were analyzed using the ANOVA test. A P value < 0.05 was considered significant.

RESULTS

The radiographic presentation of KCOT can be broadly divided into four types:

a. Unilocular KCOT: In this lesion, a radiograph shows a well-circumscribed radiolucent cystic lesion surrounded by a thin smooth or scalloped radiopaque border that is often associated with impacted teeth [Figure 1].
b. Multilocular KCOT: Here, a radiograph reveals at least two round or ovoid radiolucent cystic lesions that are uniform or differently sized and overlap each other. The cysts are partially divided by fibrous or bony septa.
c. Multiple KCOT (excluding nevoid basal cell carcinoma syndrome [NBCCS]): Radiographs of this entity show multiple discrete cysts distributed in the jaws. This entity is not associated with any anomaly and neoplasia [Figure 2].
d. NBCCS or Gorlin–Goltz syndrome is characterized by five main components: Multiple nevoid basal cell carcinomas, jaw cysts (also known as multiple KCOTs), congenital skeletal anomalies, ectopic calcifications and palmar and plantar pits [Figure 3].

Relationship between proliferating cellular nuclear antigen uptake and radiographic features

Of the 40 cases analyzed with PCNA, 16 were unilocular cases, 14 were multilocular cases, eight were multiple cases (excluding NBCCS) and two were NBCCS cases. Histologically, 28 were confirmed as non-inflamed KCOT cases and 12 were confirmed as inflamed KCOT cases. The results of immunohistochemical evaluations are shown in Tables 2 and 3 and Figures 4-11.

On comparing the four types of KCOTs, the expression of PCNA in the multilocular group was significantly higher than that in the unilocular type ($P < 0.05$). Syndromic KCOT had a significantly different PCNA expression than the other groups, whereas there was a non-significant difference between multiple and multilocular KCOTs. In the presence of inflammation, there was a statistically significant increase in the proliferative activity of multilocular and multiple cysts, whereas there was no significant increase in the

Table 2: Radiographic appearance and histopathologic characteristic of 40 patients with keratocystic odontogenic tumors

Radiographic appearance	Total number of cases	Number of cases		Total mean PCNA expression (Mean ± SE)
		Non-inflamed KCOT	Inflamed KCOT	
Unilocular	16	14	02	262.5^{a}±1.25
Multilocular	14	05	09	283^{b}±0.99
Multiple KCOT (Non syndromic)	08	05	03	282^{b}±1.41
NBCCSS	02	01	01	298.25^{c}±1.89
F value				214.68*

*Significant at 1%, Means followed by different superscript letters (a, b, c) within a column are significantly different; Tukey's test: 0.05; KCOT: Keratocystic odontogenic tumors; PCNA: Proliferating cellular nuclear antigen; NBCCSS: Nevoid basal cell carcinoma syndrome

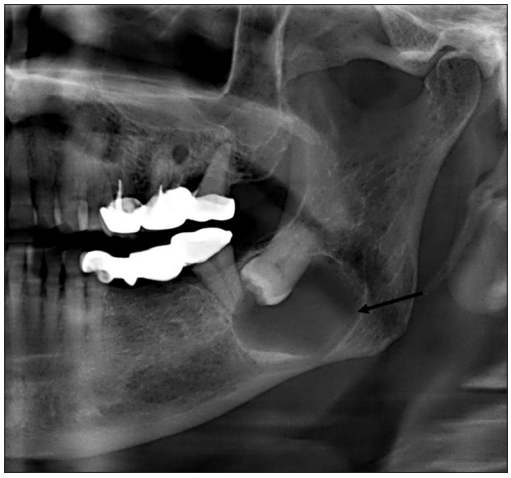

Figure 1: Panoramic radiograph showing well-defined unilocular radiolucency with sclerotic margins in the mandibular left posterior region (large arrow).

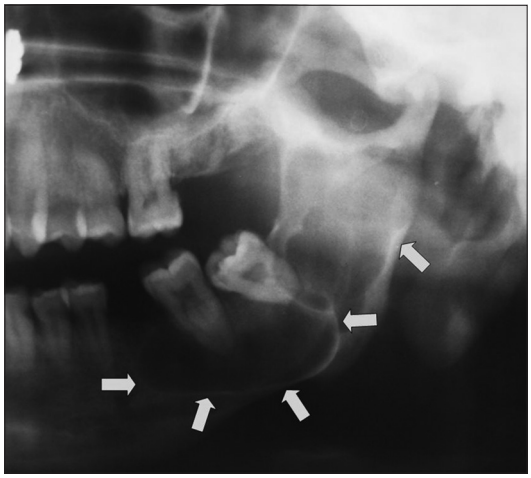

Figure 2: Section of the panoramic radiograph shows multiple radiolucencies in the mandibular left posterior region (arrows).

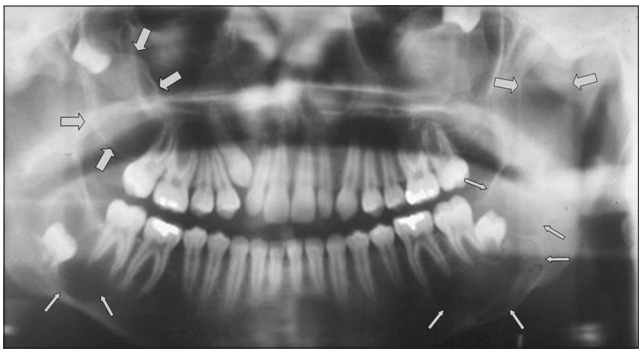

Figure 3: Radiograph reveals well-defined multiple unilocular radiolucencies in the right and left posterior regions of the maxilla (large arrow) and left and right mandibular posterior regions (small arrow) in syndromic keratocystic odontogenic tumors.

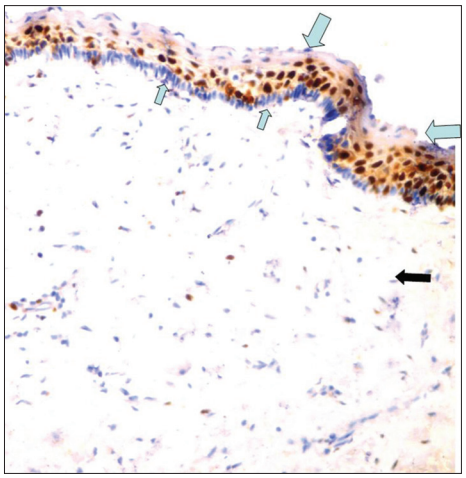

Figure 4: Proliferating cellular nuclear antigen immunostaining of unilocular keratocystic odontogenic tumors shows parakeratinized corrugated epithelium (large arrow) with palisading pattern of basal cells (small arrow) and mild amountof infl ammation in connective tissue stroma (black arrow) (40×).

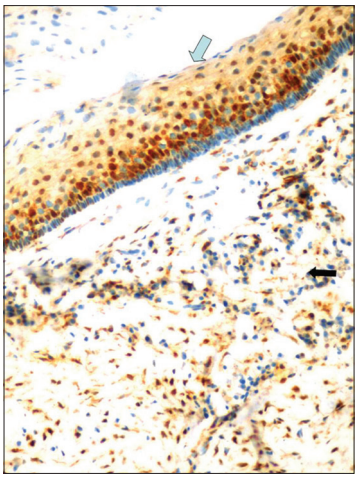

Figure 5: Proliferating cellular nuclear antigen immunostaining of unilocular inflamed keratocystic odontogenic tumor sample shows non-keratinized epithelium (large arrow) and dense amount of infl ammatory cells in connective tissue stroma (black arrow) (40×).

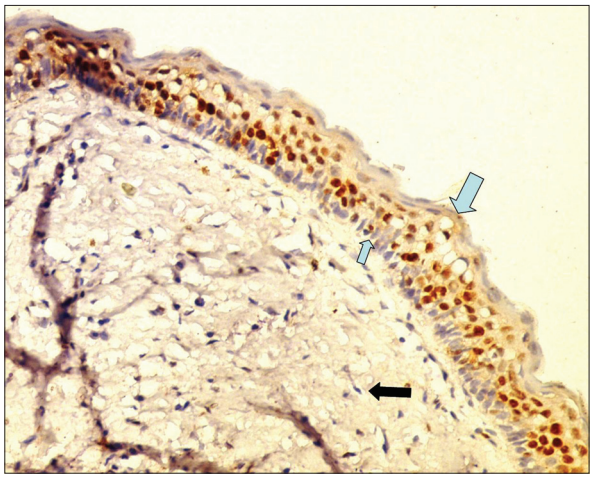

Figure 6: Proliferating cellular nuclear antigen immunostaining of multilocular keratocystic odontogenic tumors shows corrugated six to seven-cell layered thick parakeratinized epithelium (large arrow) with palisading pattern of basal cells (small arrow) and minimal amount of inflammation in connective tissue stroma (black arrow) (40×).

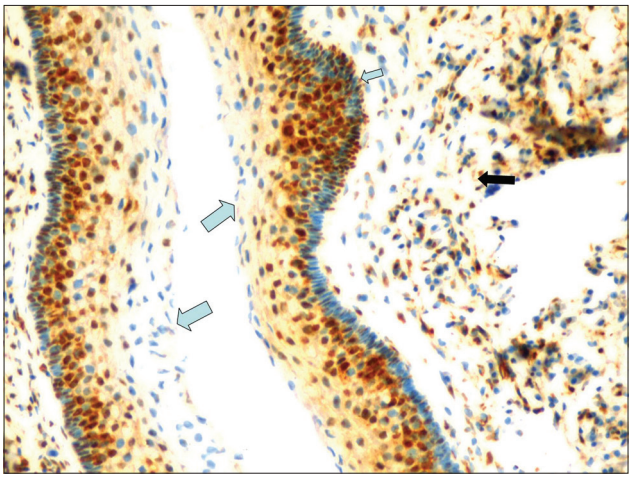

Figure 7: Proliferating cellular nuclear antigen immunostaining in multilocular inflamed keratocystic odontogenic tumor sample reveals non-keratinized (large arrow) proliferating epithelium with rete ridge formation (small arrow) and dense amount of infl ammatory cells in connective tissue stroma (black arrow) (40×).

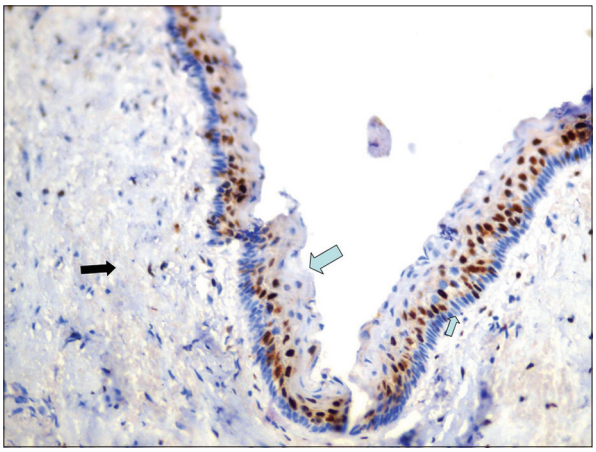

Figure 8: Proliferating cellular nuclear antigen immunostaining of multiple keratocystic odontogenic tumors reveals corrugated parakeratinized epithelium with palisading pattern of basal cells and mild amount of inflammation in connective tissue stroma (40×).

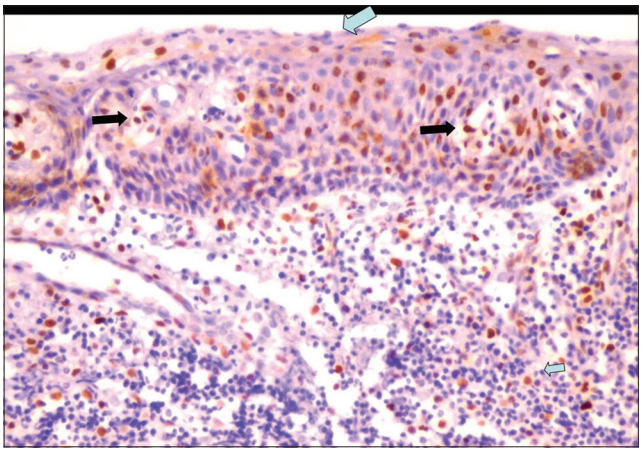

Figure 9: Proliferating cellular nuclear antigen immunostaining of multiple inflamed keratocystic odontogenic tumors reveals non-keratinized (large arrow) proliferating epithelium with arcading (black arrow) and dense amount of inflammatory cells in.

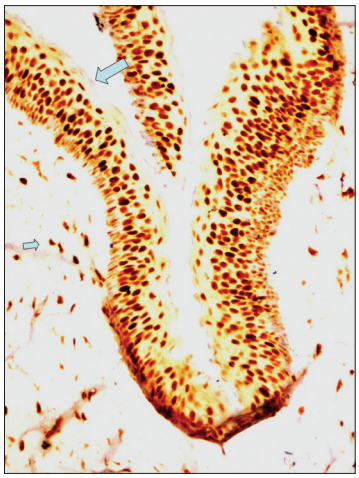

Figure 10: Proliferating cellular nuclear antigen immunostaining of syndromic keratocystic odontogenic tumors reveals parakeratinized epithelium (large arrow) and mild infl ammation (small arrow) in connective tissue stroma (40×).

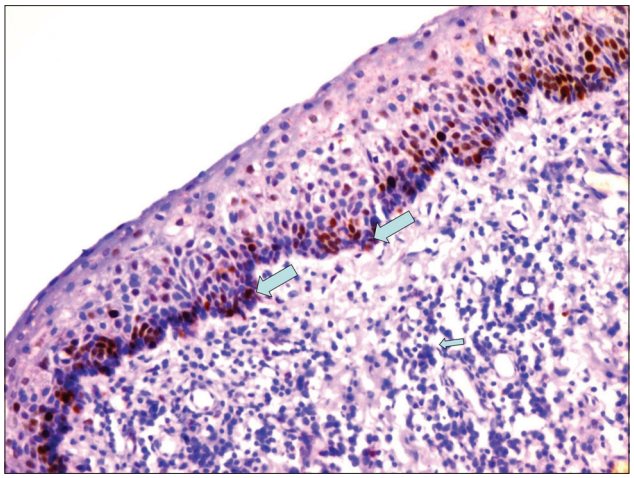

Figure 11: Proliferating cellular nuclear antigen immunostaining of syndromic inflamed keratocystic odontogenic tumors shows proliferating epithelium with serrated ridges and dense amount of infl ammation in the underlying connective tissue stroma (40×).

Table 3: Comparative evaluation of proliferating cellular nuclear antigen expression between inflamed and non-inflamed Keratocystic odontogenic tumors

Radiographic appearance	Type of cyst	Mean PCNA expression (Mean±SE)	*F* value
Unilocular	Non inflamed	262^{a}±1.25	0.500 (*P*=0.05)
	Inflamed	263^{a}±1.41	
Multilocular	Non inflamed	278^{a}±1.85	50.00** (*P*=0.0193)
	Inflamed	288^{b}±2.25	
Multiple	Non inflamed	276^{a}±2.65	64.08** (*P*=0.013)
	Inflamed	288^{b}±1.85	
Syndromic	Non inflamed	298^{a}±1.95	0.125 (*P*=0.050)
	Inflamed	298.5^{a}±2.50	

**Significant at 1%, Means followed by different superscript letters (a, b, c) within a column are significantly different; Tukey's test: 0.05; PCNA: Proliferating cellular nuclear antigen

proliferative activity in unilocular and syndromic KCOTs [Figures 5, 7, 9 and 11].

DISCUSSION

Although the WHO in 2005 has described OKC as a tumor,[3] there is no universal agreement regarding its biological behavior and mode of treatment. The typical radiographic features of KCOT are unilocular, multilocular or multiple well-circumscribed radiolucent lesions surrounded by a thin radiopaque border with a smooth or loculated periphery.[8] The present study has evaluated the clinicodemographic characteristics of 186 patients, and has found 68.88% unilocular, 20.43% multilocular, 7.5% multiple (non-syndromic) and 3.22% syndromic KCOTs.

Epithelial cell proliferation has been extensively discussed in several studies,[5-7] but few have investigated the relation of proliferation with radiographic features. Using PCNA as a proliferative marker, the present study shows increased expression in the suprabasal layer than in the basal layer. This increase was found to be statistically significant in both inflamed and non-inflamed KCOTs. Similar findings were reported in previous studies done by Li et al.,[9] in 1994, Murtadi et al.,[10] in 1996 and Piatelli et al.,[11] in 1998 using PCNA as a proliferative marker. This suggests that the highest proliferative activity is in the suprabasal cell layers. Olivera et al.,[7] believe that OKC exhibits proliferation and maturation patterns different from other lesions. Li et al.,[9] in 1994 hypothesized that a unique epithelial differentiation process exists, in which the basal cells assume some characteristics of preameloblasts, indicating that it might have entered, to some extent, toward ameloblast differentiation. Browne et al.,[12] supported this hypothesis by stating that for a cell to enter a differentiation pathway, it must first leave the cell cycle. The presence of differentiated cell in the basal layer probably accounts for the fact that the major proliferation compartment is suprabasal. This peculiar distribution of PCNA-positive cells in a suprabasal location in OKC could be due perhaps to inductive influences of the underlying connective tissue as suggested by Piattelli et al.,[11] in 1998. When the influence of inflammation on the proliferative activity of the epithelium was studied, it was found that there was a statistically significant increase in the proliferative activity of multilocular and multiple cysts, whereas there was a non-significant increase in the unilocular and syndromic KCOTs. Kaplan and Hirshberg[13] and De paula et al.,[6] have obtained contradictory results as they have observed significant and non-significant increases in PCNA expression in OKC with inflammation. Therefore, authors suggest that inflammation increases the neoplastic behavior of

multilocular and multiple KCOTs and does not affect the unilocular and syndromic ones.

The varying amount of inflammation present in the connective tissue wall causes irregular and increased proliferation of the epithelium, resulting in increased proliferative potential of KCOT and hence its aggressive behavior.[6] In our present study, majority of the inflamed KCOTs (22/38) were multilocular. Thoma advocated that multilocular cyst develops from an epithelial sprout that branches and forms, at each end of the branch, a small cyst. These become larger and, as they crowd together and fuse the intercystic tissue, are resorbed such that a single space results with a lobular outline and partial septa that appears to subdivide the cavity.[14] Hence, we can hereby suggest that multilocular KCOTs are more aggressive than the unilocular ones. Shear also supported the same by suggesting that size of the cyst did not seem to influence its prognosis after surgery, but those whose radiographic images showed a multilocular appearance had a higher recurrence rate than those with a unilocular appearance.[15]

CONCLUSION

In our study, majority of the unilocular cysts have a lesser proliferative potential than multilocular cysts and, hence, are less biologically active and should not be treated as a tumor. When there is inflammation, there is a statistically significant increase in the proliferative activity of multilocular and multiple cysts, whereas there is no significant increase in proliferation seen in unilocular and syndromic KCOTs. We suggest that inflammation increases the neoplastic behavior of multilocular and multiple KCOTs and does not affect the unilocular and syndromic ones. Therefore, we conclude that aggressive treatment should be reserved for selective cases, in contrary to other authors, who believe that all OKCs behave as a tumor and should be treated aggressively.

REFERENCES

1. Payne TF. An analysis of the clinical and histopathologic parameters of the odontogenic keratocyst. Oral Surg Oral Med Oral Pathol 1972;33:538-46.
2. Philipsen HP. Om keratocystedr (Kolesteratomer) and kaeberne. Tandlaegebladet 1956;60:963-71.
3. Philipsen HP. Keratocystic odontogenic tumour. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. Head and Neck Tumours. Pathology and Genetics. WHO Classification of Tumours. Lyon: IARC Press; 2005. p. 306-7.
4. Ba K, Li X, Wang H, Liu Y, Zheng G, Yang Z, et al. Correlation between imaging features and epithelial cell proliferation in keratocystic odontogenic tumour. Dentomaxillofac Radiol 2010;39:368-74.
5. Sudiono J, Zain RB. PCNA expression in epithelial linings of odontogenic cysts. Ann Dent Univ Malaya 2003;10:1-5.
6. De Paula AM, Carvalhais JN, Domingues MG, Barreto DC, Mesquita RA. Cell proliferation markers in the odontogenic keratocyst: Effect of inflammation. J Oral Pathol Med 2000;29:477-82.
7. De Oliveira MG, Ida SL, Chaves AC, Rados PV, Filho MS. Immunohistochemical analysis of the patterns of p53 and PCNA expression in odontogenic cystic lesions. Med Oral Patol Oral Cir Bucal 2008;13:E275-80.
8. Blanchard SB. Odontogenic keratocysts: Review of the literature and report of a case. J Periodontol 1997;68:306-11.
9. Li TJ, Browne RM, Matthews JB. Quantification of PCNA+cells within odontogenic jaw cyst epithelium. J Oral Pathol Med 1994;23:184-9.
10. El Murtadi A, Grehan D, Toner M, McCartan BE. Proliferating cell nuclear antigen staining in syndrome and nonsyndrome odontogenic keratocysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;81:217-20.
11. Piattelli A, Fioroni M, Santinelli A, Rubini C. Expression of proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. Oral Oncol 1998;34:408-12.
12. Browne RM. Per[cyst] ent growth: The odontogenic keratocyst 40 years on. Ann R Coll Surg Engl 1996;78:426-33.
13. Kaplan I, Hirshberg A. The correlation between epithelial cell proliferation and inflammation in odontogenic keratocyst. Oral Oncol 2004;40:985-91.
14. Thoma KH. Oral Surgery. Treatment of cysts of oral region. 5th ed. Saint Louis: The CV Mosby Company; 1969. p. 1002-15.
15. Shear M, Speight PM. Cysts of the oral and maxillofacial regions. 4th ed. Singapore: Blackwell Munksgaard; 2007. p. 14.

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