

A comparative study of odontogenic keratocyst and orthokeratinized odontogenic cyst using Ki67 and α smooth muscle actin

Vinay Kumar Dandena, Satish Yadava Thimmaiah¹, Mohammad Asif Kiresur², Prahlad Hunsigi, Swathi Roy³, Rashmi M⁴

Department of Oral Pathology and Microbiology, AME's Dental College, Raichur, ¹Department of Oral Pathology and Microbiology, GDCRI, Bengaluru, ⁴Department of Public Health Dentistry, KGF College of Dental Sciences, Kolar, Karnataka, ²Department of Oral Pathology and Microbiology, St. Joseph's Dental College, Eluru, Andhra Pradesh, ³Department of Oral Pathology and Microbiology, Yamuna Institute of Dental Sciences and Research, Yamunanagar, Haryana, India

Abstract

Aim: This study aimed to demonstrate and evaluate the expression of stromal myofibroblasts (MFs) and epithelial cell proliferation using α -smooth muscle actin (α -SMA) and Ki67 markers, respectively, in odontogenic keratocyst (OKC) and orthokeratinized odontogenic cyst (OOC) to correlate their aggressive behavior.

Materials and Methods: Twenty cases of OKC and twenty cases of OOC were stained with α -SMA and Ki67 markers for demonstration of stromal MFs and epithelial cell proliferation, respectively, and ten cases of well-differentiated squamous cell carcinoma were used as positive control. Assessment of the number of α -SMA-positive stromal cells and Ki67-positive epithelial cells determined by MFs and proliferative epithelial cell frequency in 10 high-power fields ($\times 400$) was presented as the mean number of positive cells per field.

Statistical Analysis: Kruskal–Wallis and Mann–Whitney test were used to analyze the difference in the mean number of α -SMA- and Ki67-positive cells per field between OKC and OOC.

Results: The mean number of positively stained cells for α -SMA and Ki67 is significantly higher in OKC compared to OOC.

Conclusion: Impression is that, the different behaviors of these two entities are compatible with their immunohistochemical view. The high value of stromal MFs and proliferative epithelial cells in OKC in comparison to OOC indicates its aggressiveness and potential for recurrence.

Keywords: Odontogenic keratocyst, orthokeratinized odontogenic cyst, squamous cell carcinoma

Address for correspondence: Dr. Satish Yadava Thimmaiah, Department of Oral Pathology and Microbiology, GDCRI, Bengaluru, Karnataka, India.

E-mail: dr.satishyadava@gmail.com

Received: 05.06.2017, Accepted: 31.08.2017

INTRODUCTION

Philipsen presented the term “odontogenic keratocyst (OKC)” in 1956. Shear (2003) termed it as

“keratocystoma.” Other recommendations have been “keratocystic odontogenic tumor” by Riechart and Philipsen (2004). It is a developmental anomaly derived

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How to cite this article: Dandena VK, Thimmaiah SY, Kiresur MA, Hunsigi P, Roy S, Rashmi M. A comparative study of odontogenic keratocyst and orthokeratinized odontogenic cyst using Ki67 and α smooth muscle actin. J Oral Maxillofac Pathol 2017;21:458-9.

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10.4103/jomfp.JOMFP_71_17

from the dental lamina which arises sporadically or in association with Gorlin–Goltz syndrome.^[1]

Philipsen was the first person to describe OKC in 1956.^[2] In 2005 WHO classification, Odontogenic keratocyst was included under tumors as a Keratocystic odontogenic tumor.^[3] In the latest 2017 WHO classification, again they included it under cysts as OKC as the current evidence is lacking to justify the continuation of keratocystic odontogenic tumor as tumor.^[4]

Orthokeratinized odontogenic cyst (OOC), described by Philipsen as an possible type of OKC, is now considered as a separate entity (Wright, 1981).^[5] The OOC described by Schultz in 1927 and Philipsen considered it to be a type of OKC and it was Wright who called it as OKC – orthokeratinized variant – based on its clinical and pathological aspects. In 1998, Li *et al.* suggested the term “orthokeratinized odontogenic cyst.”^[5]

Epithelial lining usually ranges from 8 to 10 cell layers in OKC lesions. The basal layer shows palisaded pattern with uniform nuclei with usually parakeratinized luminal epithelial cells. Cystic wall is usually thin and shows mild-to-moderate infiltration of inflammatory cells if it is secondarily infected.

OOC shows prominent granular layer with orthokeratinized surface.^[6] The recurrence rate in OOC is very less when compared to OKC and it is not associated with nevoid basal cell carcinoma syndrome (NBCCS). The recurrence rate, aggressive behavior, and neoplastic potential of OKC are higher, which suggests the importance of distinguishing between OKC and OOC. A large number of epithelial molecules have been studied to differentiate OKC from OOC such as Ki67, PCNA, and IPO38.

The expression of the human Ki-67 protein is strictly associated with cell proliferation. The fact is that Ki-67 protein is present during all active phases of the cell cycle (G (1), S, G (2), and mitosis), but is absent from resting cells (G (0)), making it an excellent marker for determining the so-called growth fraction of a given cell population. To understand the biological behavior of the lesion, recently tumor stroma has evolved as a particular field of interest. Myofibroblast (MF) is one such stromal factor which has the potential to facilitate progression of neoplastic epithelial lesions that could contribute to their biological behavior.^[7,8]

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded blocks of twenty cases each of OKC and OOC were selected for this

study. Diagnosis was established on the hematoxylin- and eosin-stained slides. Squamous cell carcinomas were taken as positive control which is of ten cases.

Staining procedure

Three micrometer-thick sections were mounted on silane-coated slides. For antigen retrieval, slides were placed in citrate buffer solution, pH = 6, in a microwave oven at 92°C for 10 min. After cooling at room temperature for 20 min, slides were exposed to commercially available primary antibodies (Biogenex Life Science Pvt., Ltd) at dilution of 1:100, for 60 min at room temperature. For antibody detection, universal immune peroxidase polymer anti-mouse rabbit Histofine® (Multi) kit (Nichirei, Tokyo, Japan) was used. Sections were rinsed in phosphate-buffered saline (PBS) for 10 min, reacted with aminoethyl carbazole (AEC) substrate-chromogen kit, rinsed in PBS for 2 min, counterstained in Mayer’s hematoxylin, and mounted with nonaqueous mounting agent.

Evaluating the expression of Ki67 in stained sections

Ten fields were selected in each section, and a grid was placed at basal and suprabasal layers of epithelium. Counts were performed with a BH-2 Olympus microscope ×10 ocular, ×40 objective. Each Ki67-expressed cell was counted, and the total number of positive cells for all the ten examined fields per case was calculated. This allowed the calculation of the mean number of Ki67-positive cells per field [Figures 1 and 2].

Evaluating the expression of α -smooth muscle actin in stained sections

Representative fields were randomly selected in each immunohistochemically stained section. Ten fields were chosen for each section. The grid was placed immediately beneath the cystic epithelial lining. Each α -smooth muscle actin (α -SMA)-positive cell, excluding those surrounding

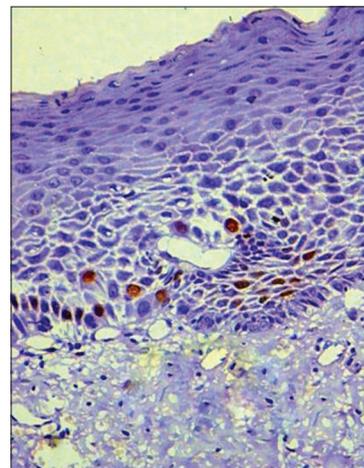


Figure 1: Odontogenic keratocyst showing Ki67 positively stained cells

blood vessels, was counted and the total number of positive cells for all the ten examined fields per case was calculated. This allowed calculation of the mean number of α -SMA-positive cells per field [Figures 3 and 4]. Similar to the evaluation of OKC and OOC, the mean number of α -SMA and Ki67 was calculated in squamous cell carcinoma which was positive control [Figures 5 and 6].

Statistical analysis

Kruskal–Wallis and Mann–Whitney tests were used to determine the mean number of α -SMA-positive cells and Ki67-positive cells per field between OKC and OOC.

RESULTS

Cystic lesions show that spindle cells with α -SMA positivity were located beneath and parallel to the basement membrane of the odontogenic epithelium. Additional small aggregates and short, delicate

bundles of similar cells were found within the fibrous wall. Blood vessel walls which take α -SMA served as positive control for the specificity of the stain. The value of α -SMA-positive cells in OKC (19.42 ± 3.9) was significantly higher than that seen in OOC (9.9 ± 1.8) ($P < 0.05$) [Table 1].

The value of Ki67 positively stained cells in OKC (7.4 ± 0.54) was significantly higher than that seen in OOC (5.06 ± 0.84) ($P < 0.05$) [Table 2].

DISCUSSION

This study aimed to measure the expression of stromal MFs and epithelial cell proliferation using α -SMA and Ki67, respectively, in OKC and OOC to compare their biological behavior. We found that the mean number of stromal MFs and Ki67-positive cells per high-power field was considerably higher in the OKC than OOC.

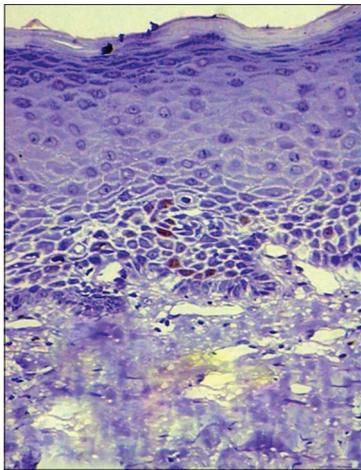


Figure 2: Orthokeratinized odontogenic cyst showing Ki67 positively stained cells

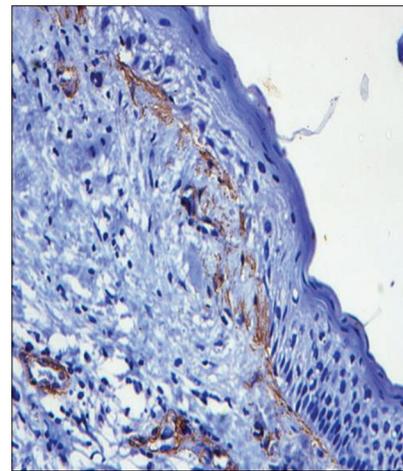


Figure 3: Odontogenic keratocyst showing α -smooth muscle actin positively stained cells

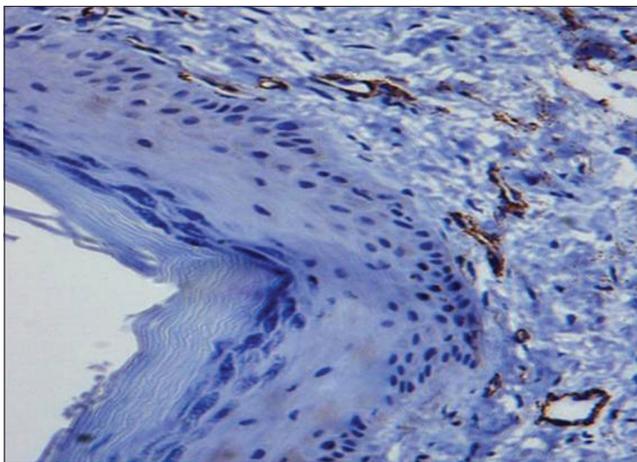


Figure 4: Orthokeratinized odontogenic cyst showing α -smooth muscle actin positively stained cells

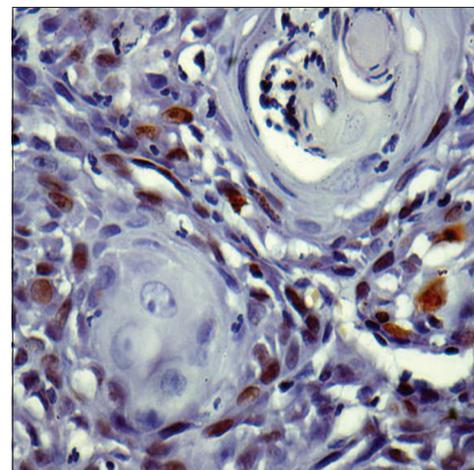


Figure 5: Squamous cell carcinoma showing Ki67 positively stained cells

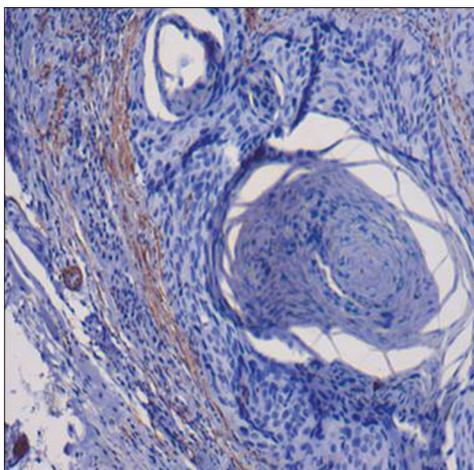


Figure 6: Squamous cell carcinoma showing α -smooth muscle actin positively stained cells

Table 1: Value of α -smooth muscle actin-positive cells

Lesion	Mean \pm SD
OKC	19.42 \pm 3.9
OOC	9.9 \pm 1.8
SCC	25.15 \pm 1.3

SD: Standard deviation, OOC: Orthokeratinized odontogenic cyst, OKC: Odontogenic keratocyst, SCC: Squamous cell carcinoma

Table 2: Value of Ki67-positive cells

Lesion	Mean \pm SD
OKC	7.4 \pm 0.54
OOC	5.06 \pm 0.84
SCC	10.66 \pm 0.52

SD: Standard deviation, OOC: Orthokeratinized odontogenic cyst, OKC: Odontogenic keratocyst, SCC: Squamous cell carcinoma

OKC is highly aggressive and recurs at greater frequency than other types of odontogenic cysts. The recurrence rate ranges from 3% to 60%. Studies show that the OKC recurred in at least 42.6%, compared with only 2.2% for the OOC. Thus, it suggests the importance of distinguishing between OKC and OOC.^[9]

Due to low recurrence rate and less aggressive behavior with different histopathological features, OOC is now considered as a different entity. Several studies proved that OKC and OOC are distinct from each other, both clinically and histologically. The exact occurrence of OOC is not clear either because of the improper identification or classification of this entity in literature. Clinicopathological studies on OKC have reported the orthokeratinized variant as ranging from 3.3% to 12.2%.^[10]

These lesions look similar clinically but are different. OOCs are usually single asymptomatic lesions, occurring in the third to fourth decade with a male predilection. They occur more frequently in the posterior region of the mandible,

not seen in patients with NBCCS. OKC shows similar findings regarding age, sex, and site of occurrence, but they are associated with NBCCS patients and the lesions are usually multiple.^[11,12]

Radiographically, OOCs tend to be unilocular lesions and are more often associated with impacted teeth as compared to OKC. Differences in the staining pattern of numerous immunohistochemical markers suggest the aggressiveness of OKC compared to OOC.^[13]

Decreased expression of Ki67 and p63 in OOCs as compared to OKC indicates the low proliferative activity.^[10]

Bcl-2 was found to be negative in the basal cell layer as against a positive expression in OKC. Immunohistochemical studies on the epithelial lining and the capsule using cytokeratins and extracellular matrix proteins revealed that OOC was a well-formed and more organized cyst as compared to OKC. Many of such studies were evident to say why OKC is now considered a neoplasm.^[1]

CONCLUSION

This study enlightens on the pertinent differences between OOC and OKC and how a thorough pathological examination of a keratinizing cyst is very important as there may be a tendency of clinical misdiagnosis which in turn could affect the prognosis of the patient.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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