Orthokeratinized Odontogenic Cyst

A Clinicopathologic Study of 61 Cases

Qing Dong, MDS; Shuang Pan, MDS; Li-Sha Sun, PhD; Tie-Jun Li, DDS, PhD

• ***Context.*—Orthokeratinized odontogenic cyst (OOC) is a relatively uncommon developmental cyst comprising about 10% of cases that had been previously coded as odontogenic keratocysts. Odontogenic keratocyst was designated as keratocystic odontogenic tumor (KCOT) in the new World Health Organization classification and OOC should be distinguished from KCOT for differences in histologic features and biologic behavior.**

***Objective.*—To analyze the clinicopathologic features of 61 cases of OOC in a Chinese population.**

***Design.*—Clinicopathologic analysis was performed on 61 cases of OOC. Immunohistochemical expression of Ki-67 and p63 was evaluated in 15 OOCs and 15 typical KCOTs.**

***Results.*—The 61 patients with OOC ranged from 13 to 75 years (average, 38.93 years). The lesions developed mainly in the third and fourth decades (57.38%) with a distinct predilection for males (72.13%). Six (9.84%) lesions were found in the maxilla and 55 (90.16%) in the mandible. The most common sites were in the mandibular molar and ramus region. Of the 54 cases with radiographic record, 47 (87.04%) were unilocular and 7 (12.96%) were multilocular radiolucencies. Twenty-seven of the 54 cysts were associated with an impacted tooth. Follow-up of 42 patients revealed no recurrence during an average period of 76.8 months after surgery. Compared with KCOTs, expression level of Ki-67 and p63 was significantly lower in OOCs, suggesting a lower proliferative activity.**

***Conclusion.*—Orthokeratinized odontogenic cyst is clinicopathologically distinct from KCOT and should constitute its own clinical entity.**

(*Arch Pathol Lab Med.* 2010;134:271–275)

Orthokeratinized odontogenic cyst (OOC) is a relatively uncommon developmental cyst comprising about 10% of cases that had been previously coded as odontogenic keratocysts (OKCs).[1–6] In 1981, Wright[2] reported 59 cases of what he then termed ''orthokeratinized variant of OKC,'' which showed little clinical aggressiveness. Subsequently several studies have discussed the clinical and pathologic differences between typical OKC and OOC.[3–5,7,8] The lesion has been termed variously as an ''orthokeratinized variant of OKC''[2–4] or a ''jaw cyst with orthokeratinization.''[5] Li et al[6] suggested a descriptive term ''orthokeratinized odontogenic cyst,'' which also reflected its most plausible histogenic origin. The new World Health Organization classification for head and neck tumors has designated OKC as keratocystic odontogenic tumor (KCOT) and reclassified it as a neoplasm in view of its intrinsic growth potential and propensity to recur.[9] According to this new classification, OOC should not be part of the spectrum of KCOT and should be distinguished from the latter.[9] The aims of this study were to analyze the clinicopathologic features of 61 cases of OOC and to compare the proliferative activity between epithelial linings of OOC and KCOT by immunohistochemical labeling of Ki-67 and p63.

Accepted for publication May 8, 2009.

From the Department of Oral Pathology, School and Hospital of Stomatology, Peking University, Beijing, China.

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Tie-Jun Li, DDS, PhD, Department of Oral Pathology, School and Hospital of Stomatology, Peking University, 22 S Zhongguancun Ave, Haidian District, Beijing 100081, China (e-mail: litiejun22@vip.sina.com).

MATERIALS AND METHODS

A total of 583 cases coded as KCOT or previously as OKC were reviewed from the files of the Department of Oral Pathology, Peking University Hospital and School of Stomatology, during the period from 1985 to 2008. After reviewing the patient details, clinical information, and histology, we identified 61 OOC cases based on the criteria established by Vuhahula et al[5] and Li et al.[6] For inclusion in this series, all or a predominant portion of the lining epithelium exhibited orthokeratinization and the basal cells showed no tendency to palisade. Clinical data, including age, gender, lesion location, radiologic features, surgical procedures, and information on recurrence, were reviewed. The location of the center of lesion in the maxilla or mandible was classified as anterior (between the right and left canines), premolar, or molar regions. The radiographic features of OOC were also compared with that of 85 typical KCOTs. To avoid the distortion caused by an x-ray, the size of lesion was expressed as the ratio of the largest diameter of the lesion and the width of the mandibular first molar in the panoramic radiographic films.

Immunohistochemical expression of Ki-67 and p63 were studied in 15 OOCs together with 15 KCOTs. All selected cases were primary jaw cysts and the tissue specimens had been routinely fixed in 10% neutral formalin, processed and embedded in paraffin. Immunohistochemical studies were performed on 4-μm–thick paraffin sections using avidin-biotin-peroxidase complex method. The antibodies used were as follows: rabbit anti–Ki-67 monoclonal antibody and mouse anti-p63 monoclonal antibody (Zymed Lab, San Francisco, California; working solution, 2 hours at 37°C). To enhance the immunostaining, sections were pretreated by microwave heating in 0.01M citrate buffer (pH 6.0) for 10

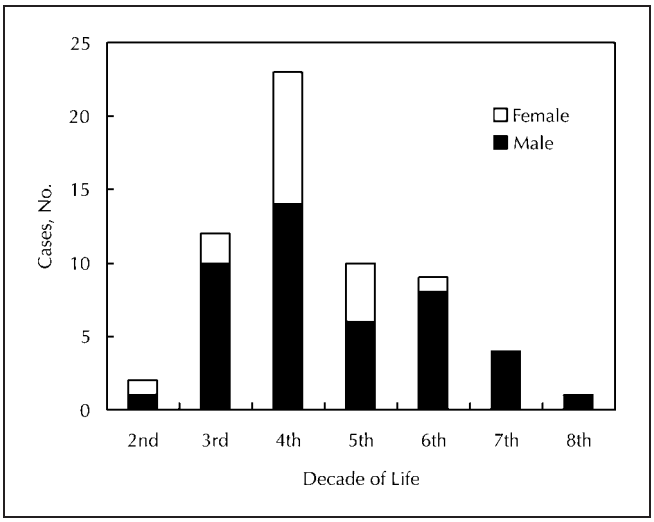

Figure 1. *Distribution of age and gender of the patients.*

minutes. Staining was revealed using 3,3′-diaminobenzidine reagent (Dako, Carpinteria, California).

The percentage of Ki-67– and p63–positive cells within the lining epithelium was calculated using an image analysis–based computer system (Image-Pro Plus 6.0 analysis software [Media Cybernetics Inc, Bethesda, MD]). About 8 to 10 high-power fields (×400, approximately more than 4500 cells) were observed in each case. All quantitative data were analyzed using SPSS 13.0 software (SPSS Inc, Chicago, Illinois). Levene test was used for equality of variances. Independent-sample *t* test and Satterthwaite approximate *t* test were used to determine significant differences between the OOC and KCOT groups.

RESULTS

The 61 patients with OOC included 44 men and 17 women (ratio, 2.59:1). The age at diagnosis ranged from 13 to 75 years (average, 38.9 years), with a predilection for the third and the fourth decades (57.4%). Twenty-four of 44 male patients were diagnosed between the third and the fourth decades, whereas in female patients most (13 of 17) were between the fourth and the fifth decades. There was a second peak incidence in the sixth decade in male patients (Figure 1). The mandible was affected in 55 (90.2%) cases and the maxilla in 6 (9.8%) lesions. The most common sites were in the mandibular molar and ramus region (46 of 61; 75.4%) (Table 1). None of the maxillary lesions affected the sinus. Of the 54 OOC lesions with available radiograghs, 47 (87.0%) were unilocular radiolucencies. Multilocular lesions were found in 7 (13.0%) cases. Twenty-seven (50.0%) cysts were found to be associated with an impacted tooth. Reviewing the radiographic records of 85 typical KCOTs from our own file, 57 (67.1%) were found to be unilocular and 28 (32.9%) multilocular. Only 32 (37.7%) cases were associated with an impacted tooth. The cyst size of OOCs, as measured using radiographic film, ranged from 1.6 to 15.5 (mean, 4.8), which was slightly lower than that of KCOTs (mean, 5.1; $P = .556$). Jaw swelling was the most common presenting symptom (46 cases; 75.4%). Thirteen (21.3%) patients also complained of pain and 2 (3.3%) patients also presented with infection. The duration of symptoms varied from 2 days to 20 years (mean, 16 months), with 29 (47.5%) patients having a duration of less than 3 months.

Table 2. Summary of Follow-up Data

Follow-up Period	Cases, No.	No Sign of Disease	Recurrence
6 mo–2 y	9	9	0
>2–4 y	11	11	0
>4–10 y	15	15	0
>10 y	7	7	0
Lost to follow-up	19	NA	NA

Abbreviation: NA, not applicable.

The OOC lining epithelium was mostly thin and uniform with an average thickness of 4 to 9 cells (Figure 2, a). The orthokeratinized surface layers were relatively thick, and onion-skin–like. There was a prominent granular layer beneath the keratinized layer. A hypocellular spinous cell layer was usually made up of polyhedral to flattened cells with eosinophilic cytoplasm. The basal layer cells exhibited low cuboidal or flat morphology with little tendency of nuclear hyperchromatin and palisading. The epithelial linings of OOCs and KCOTs showed variable reactivity to the 2 antibodies used. Ki-67–positive cells in the OOC lining epithelium were mainly detected in the basal cell layer (Figure 2, c). In KCOT, the distribution of Ki-67–positive cells was mostly confined to the suprabasal layers (Figure 2, d). p63 was expressed in the basal and part of the suprabasal layers of OOC linings (Figure 2, e), whereas its expression in KCOT epithelium was seen in all cell layers except for the surface parakeratinized layer (Figure 2, f). By quantification, the percentages of Ki-67– and p63–positive cells determined by an image analyzer were significantly higher in KCOTs than in OOCs ($P < .001$) (Figure 3).

Enucleation, with or without curettage, was performed in 52 cases. Two cases required a combination of marsupialization followed by enucleation. The other 7 cases were treated with peripheral ostectomy due to the radiologically multilocular features and relative larger size. Related follow-up data were available on 42 patients with respect

Table 1. Anatomic Location of Orthokeratinized Odontogenic Cysts (OOCs) and Comparison With Keratocystic Odontogenic Tumors (KCOTs) Reported Previously by Our Group

Lesion	Cases, No.	Maxilla (%)				Mandible (%)				Maxilla-Mandible Ratio
		Anterior	Premolar	Molar	Total	Anterior	Premolar	Molar and Ramus	Total	
OOC	61	3 (4.9)	2 (3.3)	1 (1.6)	6 (9.8)[b]	2 (3.3)	7 (11.5)	46 (75.4)	55 (90.2)[b]	1:9.17[b]
KCOT[a]	461	34 (7.4)	23 (5.0)	60 (13.0)	117 (25.4)	38 (8.2)	30 (6.5)	276 (59.9)	344 (74.6)	1:2.94

[a] Data previously reported by our group.[11]
[b] The difference is significant ($P < .001$) by binomial test.

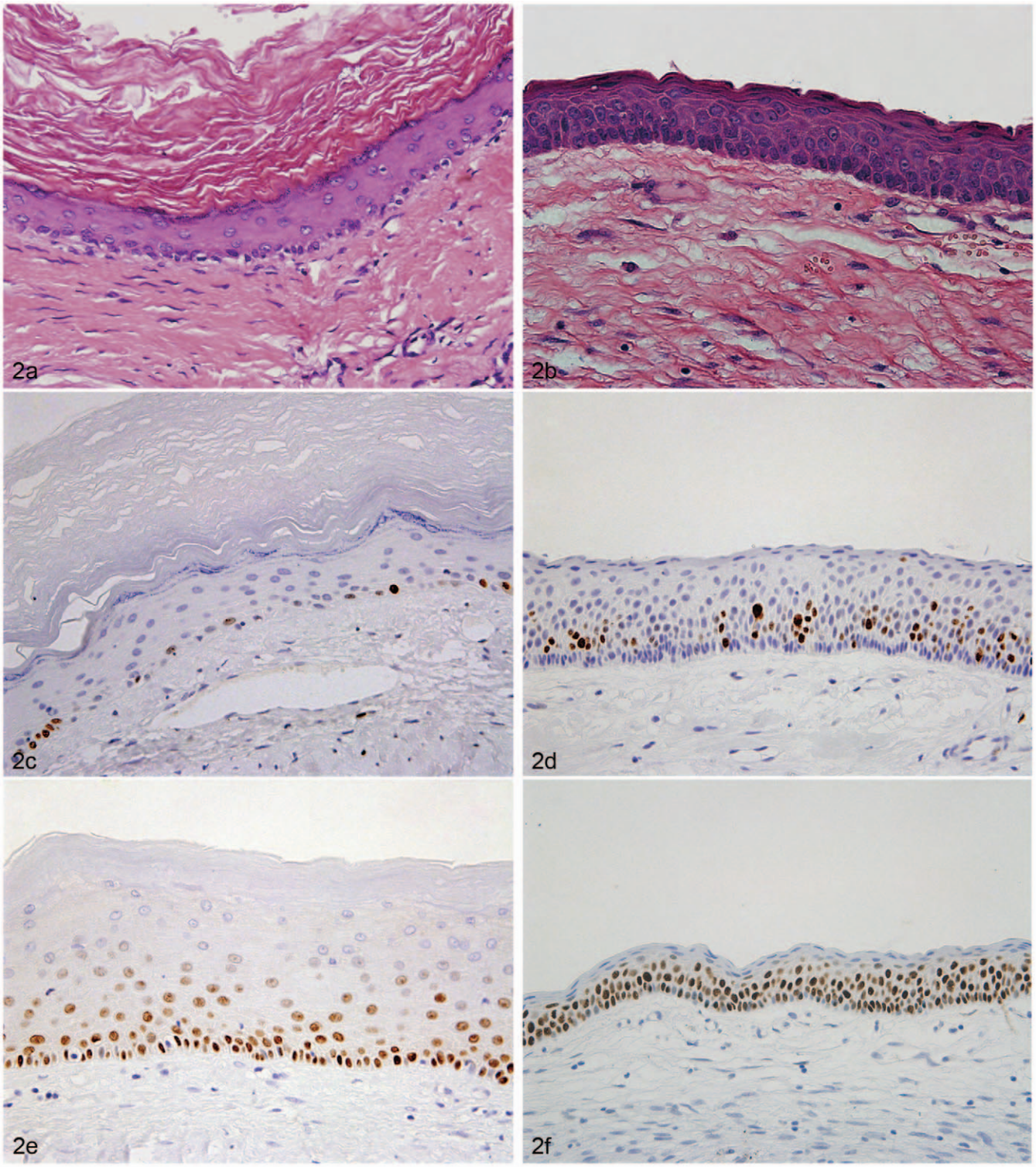

Figure 2. *a, The epithelial lining of orthokeratinized odontogenic cyst shows onion-skin–like surface orthokeratinization, prominent granular cell layer, and flattened basal cells. b, The keratocystic odontogenic tumor lining shows typical features of surface corrugations and a polarized layer of basal cells (hematoxylin-eosin, original magnifications ×400 [a and b]). c and d, Immunoreactivity for Ki-67 in orthokeratinized odontogenic cyst and keratocystic odontogenic tumor (original magnifications ×400 [c and d]). e and f, Immunoreactivity for p63 in orthokeratinized odontogenic cyst and keratocystic odontogenic tumor (original magnifications ×400 [e and f]).*

to recurrence (Table 2), and the remaining 19 patients were lost to follow-up. The follow-up period ranged from 6 to 282 months with an average of 76.8 months. None of the patients showed any sign of recurrence.

COMMENT

In the present study, we presented the largest series of OOC cases, which appeared to represent 10.5% of cases

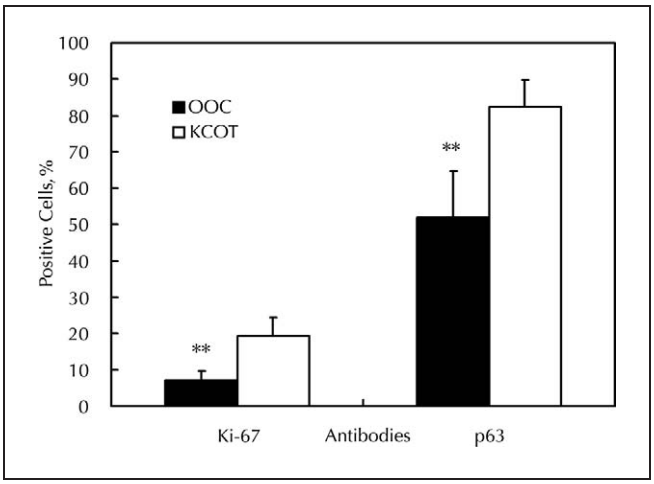

Figure 3. *Distribution of the percentage of positive cells for the Ki-67 and p63 antibodies. ** P < .001. Abbreviations: KCOT, keratocystic odontogenic tumor; OOC, orthokeratinized odontogenic cyst.*

previously coded as OKC or KCOT from our files. The reported incidence of OOC varies in different series, ranging from 5.2% to 16.8% among cases that had been previously coded as KCOT.[1–6,10] The average age at diagnosis of patients with OOCs was 38.9 years. Orthokeratinized odontogenic cyst had a male predominance with a male to female ratio of 2.59:1, which was consistent with the pooled data of all OOC cases reported in English literature (2.26:1)[2–6,11] but higher than that reported for KCOT (ranging from 1.42:1 to 1.76:1).[12–15] Namely, OOCs occurred more frequently in male patients compared with typical KCOTs. The mandible was far more commonly involved than the maxilla (90.6% versus 9.4%) and the most common location was in the mandibular molar and ramus region. The mandible-maxilla ratio of the present series was 9.17:1, higher than that reported for KCOTs (ranging from 2.08:1 to 4.4:1).[13,15–17] Radiographically, OOCs more frequently presented as unilocular radiolucencies (87.0%) in comparison with KCOTs (67.1% from our own file and ranging from 69.4% to 73.3% by other reports[18–20]).

It is interesting to note that half of our cases were found to be associated with an impacted tooth. This had been reported with various frequency by several authors, averaging about 60.8% in the literature.[2–6,21] It has been reported that about 7% to 47.8% of typical KCOTs are associated with an impacted tooth.[4,16,18–20,22–24] This finding aroused the interest of several authors. Vuhahula and colleagues[5] found that reduced enamel epithelium that had completed its tooth-forming function had the capability to keratinize under appropriate stimuli, thus forming a true dentigerous cyst with keratinization. As to KCOTs, most authors believed that they originated from dental lamina[25–27] or surface epithelium or hamartomatous proliferation of odontogenic epithelium.[28] The histogenesis of KCOTs and OOCs may vary and needs further investigation. The possibility should be considered that a cyst in a pseudodentigerous relation, in which the crown of an unerupted tooth was not inside the cyst, might be clinically and radiologically misinterpreted as a dentigerous cyst. Thus, radiographic diagnostic imaging in 3 dimensions[29] was advocated to visualize the lesions and assist in diagnosis and analysis of the possible histogenesis of OOCs.

Histologic examination demonstrated several striking differences between the epithelial lining of orthokeratinized and parakeratinized cysts. Although the typical KCOT exhibits a highly cellular parakeratinized epithelial lining with surface corrugations and a palisaded layer of basal cells (Figure 2, b), the OOC lacks these features. Instead, the thin, uniform, orthokeratinized lining epithelium was characterized by onion-skin–like luminal surface keratinization, prominent stratum granulosum, and low cuboidal or flattened basal cell layer with little tendency of nuclear palisading. Our immunocytochemical results demonstrated that the epithelial linings of OOC differed from KCOT by containing significantly fewer Ki-67–positive proliferating cells, which were mostly confined to the basal cell layer. The high, predominantly suprabasal proliferative activity of the KCOT lining, as demonstrated here and previously,[30,31] was not shared by OOC. *p63*, a member of the *p53* tumor suppressor gene family, plays a major role in the maintenance of epithelial stem cells, as well as in their terminal differentiation.[32] In the absence of p63, stem cells and their progenies die by apoptosis, and the crippled stem cells are unable to bolster cell proliferation and self-renewal.[33] The present study demonstrated that p63 expression in OOCs was significantly less intensive in comparison with KCOTs, indicating epithelial cells in OOCs may possess a lower proliferative and self-renewal potential. Interestingly, p63 expression has been reported to be more intensive and diffuse in malignant odontogenic tumors and benign odontogenic tumors exhibiting local aggressiveness compared with other odontogenic tumors.[34] These findings thus appear to reflect the variations in epithelial cell maturation and proliferation between the 2 types of lining epithelia; namely, those of OOC seem to assume a different cell differentiation and exhibit a lower cellular activity than those of KCOT.

The KCOT is of particular interest because it is clinically more aggressive than other forms of odontogenic cyst and tends to recur after surgery. Figures for the incidence of recurrence in reported series have varied from 12% to 60%.[13,19,22,35] The notion for separation of OOCs from KCOTs was mainly supported by a number of studies that indicated a significantly lower recurrence rate of OOCs following surgery.[2–6] The present study confirmed that OOC had little tendency to recur. None of the 42 patients who had been followed for 6 to 282 months after surgery showed any sign of recurrence. Furthermore, such features as multiplicity and association with nevoid basal cell carcinoma syndrome, which commonly occur in KCOTs, were not observed in the present series or in other reports.[1–6] Therefore, OOC exhibits a number of distinctive clinical, pathologic, and behavioral features that varied substantially from KCOTs. It appears to represent an uncommon but consistent group of odontogenic developmental cysts that cannot be classified as other established types and should therefore constitute its own clinical entity.

We gratefully acknowledge the patients for their cooperation. This work was supported by grants 30625044, 30572048, and 30872900 from the National Nature Science Foundation of China and grant 20050001110 from the Specialized Research Fund for the Doctoral Program of Higher Education in China.

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