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Glandular odontogenic cyst in China: report of 12 cases and immunohistochemical study

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OBJECTIVE: The purpose of this study was to present 12 additional cases of glandular odontogenic cyst (GOC) in the Department of Oral Pathology, School of Stomatology, Wuhan University, People's Republic of China, and to investigate their immunohistochemical cytokeratins (CKs) expression in the epithelial components.

METHODS: A total of 12 GOCs were reviewed clinically and radiographically, and immunohistologic CKs AE1, 7, 8/18, 10/13, 14, 16, 19 and 20 were performed by using a standard biotin-streptavidin immunoperoxidase technique on paraffin sections.

RESULTS: The present series showed that eight occurred in males and four in females. The mean age was 37.6 years with a peak incidence occurring in the third decades (six of 12). Mandibles were more affected than maxillas (7:5), especially anterior mandible (four of seven). Radiographically, ratio multilocular to unilocular radiolucencies was 5:7 usually with well-defined borders. Histologically, cystic spaces were lined by non-keratinized stratified epithelia containing focal plaque-like or whirlpool-like thickenings; surface epithelial layer-containing eosinophilic cuboidal cells; mucous cells; and mucin pools of microcystic areas in the epithelium. Immunohistochemistry showed that epithelium of GOCs stained for CKs AE1, 7, 8/18, 10/13, 14 and 19 with slight changes in their patterns, and no reaction to CKs 16 and 20.

CONCLUSIONS: Most clinical and histologic features in this study were analogous to those reported west population, although with slight difference between them. Histologically, the morphology of the epithelium strongly suggested an odontogenic origin, and CKs expression of GOC was similar to that of odontogenic epithelium, suggesting histochemically that GOC might be derived from odontogenic epithelium.

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Keywords: clinicopathologic; cytokeratin; glandular odontogenic cyst; immunohistochemistry

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Introduction

Glandular odontogenic cyst (GOC) is a uncommon jawbone cyst of odontogenic origin, first described in 1988 by Gardner et al. (1) as a distinct clinicopathologic entity. In 1987, Padayachee and Van Wyk (2) reported two cases with multilocular mandible cystic lesions that were similar to botryoid odontogenic cysts (BOC) but with a glandular element, and proposed the term 'sialo-odontogenic cyst'. Because the possible salivary gland histogenesis has not been established and the histologic features were highly indicative of an odontogenic origin, World Health Organization (WHO; 3) in 1992 named GOC (or sialo-odontogenic cyst) as an independent histopathologic entity, and listed the lesion as a developmental odontogenic epithelial cyst, and defined this lesion as 'a cyst arising in the tooth-bearing areas of the jaws and characterized by an epithelial lining with cuboidal or columnar cells both at the surface and lining or cyst-like spaces within the thickness of the epithelium'.

A total of 20 different CKs have so far been identified in human tissues. In epithelial cells CK expression patterns differ according to cell type, developmental stage, differentiation status, anatomical site and degree of complexity, and has been regarded as an useful tool in identifying different epithelial types and origins (4). In the present study, we analyzed the clinical and histopathologic features of 12 new cases of GOC and immunohistochemical findings concerning the expression of CKs AE1, 7, 8/18, 10/13, 16, 19 and 20, seven of which have been reported previously by Wang et al. (5) in Chinese, and compared our findings with those reported in the literature.

Materials and methods

From March 1965 to July 2002, a total of 12 GOCs were reviewed from files of the Department of Oral Pathology, School of Stomatology, Wuhan University, Hubei Province, People's Republic of China. In this period approximate 7023 represented jaw cysts. Clinical information including age, sex, size, anatomical site, duration of symptom, radiographic description, treatment, follow up, and recurrence status was recorded. The GOC cases

Table 1 Characterization of the applied monoclonal antibodies

Antibody	*Clone*	*Source*	*Dilution*	*Retrieval of antigen*
AE1	AE1	Zhongshan, Beijing, China	1:100	Pepsin
CK7	OV-TL12/30	Zhongshan, Beijing, China	1:100	Pepsin
CK8/18	Zym5.2	Zhongshan, Beijing, China	1:100	Pepsin
CK10/13	DE-K13	Zhongshan, Beijing, China	1:50	Trypsin
CK14	LL002	Maixin, Fuzhou, China	1:100	Heating
CK16	LLO25	Maixin, Fuzhou, China	1:50	Heating
CK19	KS19.1	Maixin, Fuzhou, China	1:50	Heating
CK20	KS20.8	Maixin, Fuzhou, China	1:100	Trypsin

were diagnosed based on the classic criteria described by Gardner et al. (1) and WHO (3) regarding histologic typing of odontogenic cysts and tumors.

All tissue specimens were fixed in 10% neutral-buffered formalin (18–48 h) and routinely processed and embedded in paraffin, including decalcification in 25% formic acid for 48–72 h if necessary. Histopathologic diagnosis was confirmed by two experiential pathologists on hematoxylin and eosin-stained section for each case, and periodic-acid Schiff (PAS) and Alcian Blue-stained slides were also used.

Immunostaining for CKs AE1, 7, 8/18, 10/13, 14, 16, 19 and 20 was performed using a standard biotin-streptavidin immunoperoxidase technique on paraffin sections. Details of antibodies used were listed in Table 1. Tissue sections of oral mucosal epithelium and salivary gland were stained as positive controls. For negative control, phosphate-buffered saline (PBS) was applied to substitute for the primary antibody. Immunohistochemical reactivity for CKs was detected in the cytoplasm of the epithelial cells of GOC. Cytokeratin (CK) immunostaining was semiquantitatively analyzed in the epithelial lining of the cysts according to description by Semba et al. (6) as follows: $--$, negative; $-+$, heterogeneously and weakly positive; $+$, heterogeneously and markedly positive; $+-$, homogeneously and weakly positive; $++$, homogeneously and markedly positive.

Results

Clinical data

The mean age of the 12 patients at diagnosis was 37.6 years, with a range of 21–64 years. The peak incidence was in the third decades (six of 12, 50%). There was a male predominance with a male-to-female ratio of 2:1. Five lesions were situated in the maxilla and seven in the mandible, and four cases were in anterior mandible regions. The mean size of the cysts was 4.2×3.5 cm with maximum diameter ranging from 2 to 8 cm. Duration of symptom before the initial medical consultation averaged 10.8 months (range: 2–28). The most common presenting symptom was painless swelling of jaw, and found in 11 cases, three of which accompanied pain. One case was discovered during routine dental treatment. In no case paresthesia, dysphagia, nodal enlargement were noted. The lesions tended to exhibit buccal or lingual expansion. Radiographically, The lesions appeared as either a multilocular (five of 12) or unilocular (seven of 12) radiolucencies, usually with well-circumscribed borders, although they were scalloped in two cases. Two of 12 cases were found to be associated with an impacted tooth, and four and two of them appeared to be root resorption and tooth displacement, respectively (Figs 1 and 2). Provisional diagnosis as ameloblastoma/odontogenic keratocyst (AB/OKC) or OKC was in three

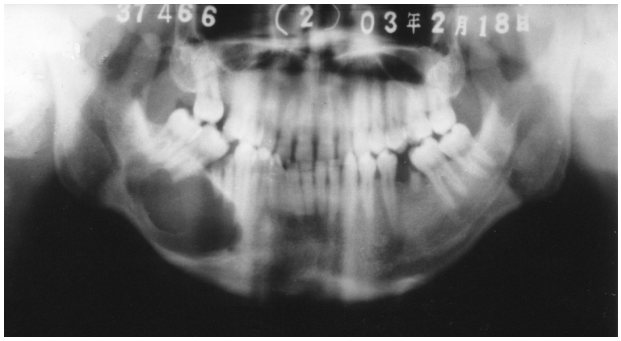

Figure 1 Panoramic radiograph showing unilocular well-circumscribed radiolucency extending from tooth 43 to 47, with resorption of root apices of 44 to 47 and displacement of 46.

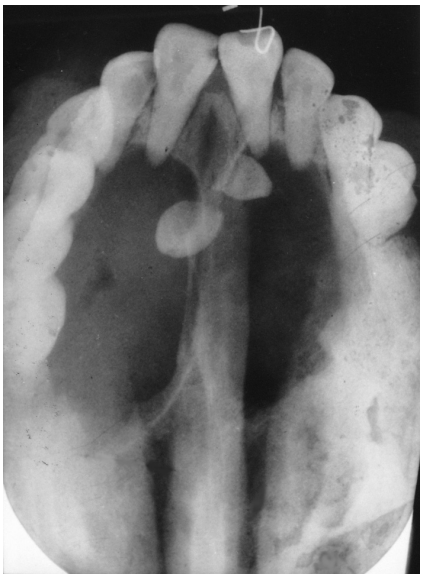

Figure 2 Occlusal radiograph showing a large, unilocular well-defined radiolucency in the maxilla with two impacted teeth.

cases. Clinical impression of dentigerous (DC) and radicular (RC) cyst was suggested in two and one patients prior to surgery, respectively. The remaining six cases were coded with a non-specific term 'jaw cyst'. Four cases were treated by enucleation and five by curettage. Extirpation with segmental ostectomy of maxilla/partial mandibulectomy was in three cases. Ten of 12 cases were adequately followed with respect to recurrence. The follow-up period ranged from 2 to 40 years, with a mean period of 13.3 years. Only one cyst recurred in the third years post-operatively.

Histopathology

Histopathologic examination revealed that these lesions were either multicystic (five of 12) with thin fibrous septa or unicystic (seven of 12; Fig. 3a). All specimens consisted of luminal epithelium and surrounding connective tissue. The cystic spaces were lined by non-keratinized stratified squamous epithelium that varied in thickness from 2 to approximately 14 cells. It exhibited a flat interface with the subjacent stroma but sometimes undulating course. The surface layer of the epithelium was composed of eosinophilic cuboidal or low-columnar cells that were sometimes ciliated (Fig. 3b). Plaque- and whirlpool-like epithelial thickenings were noted, with occasionally local irregular papillary projections into the lumen (Fig. 3c). In many areas, numerous intraepithelial microcysts or glandular-like structures were lined by eosinophilic cuboidal cells similar to those seen in the surface of the epithelium, and filled with mucous material which stained positively with diastase-resistant PAS and Alcian Blue. Some of the glandular structures communicated directly with main cystic cavity. The basal cells without polarity of the nuclei and spinous cells were occasionally vacuolated. No mitotic figures were noted. Small satellite cysts and epithelial islands entrapped in the connective tissue were found in the wall of two cysts. Small irregularly shaped calcifications were scantily present in the fibrous stroma in two cases. In all cases but two, the underlying connective tissue consisted of densely fibrous tissue free of chronic inflammatory cells. Infiltration moderately by chronic inflammatory cells were noted in underlying connective tissue in remaining two cases, one of which the cystic epithelium showed proliferating rete processes, similar to those in inflammatory RC. Erosion of the surrounding bone was seen occasionally. None of our cases showed areas of hyalinization. In addition, mucoepidermoid carcinoma

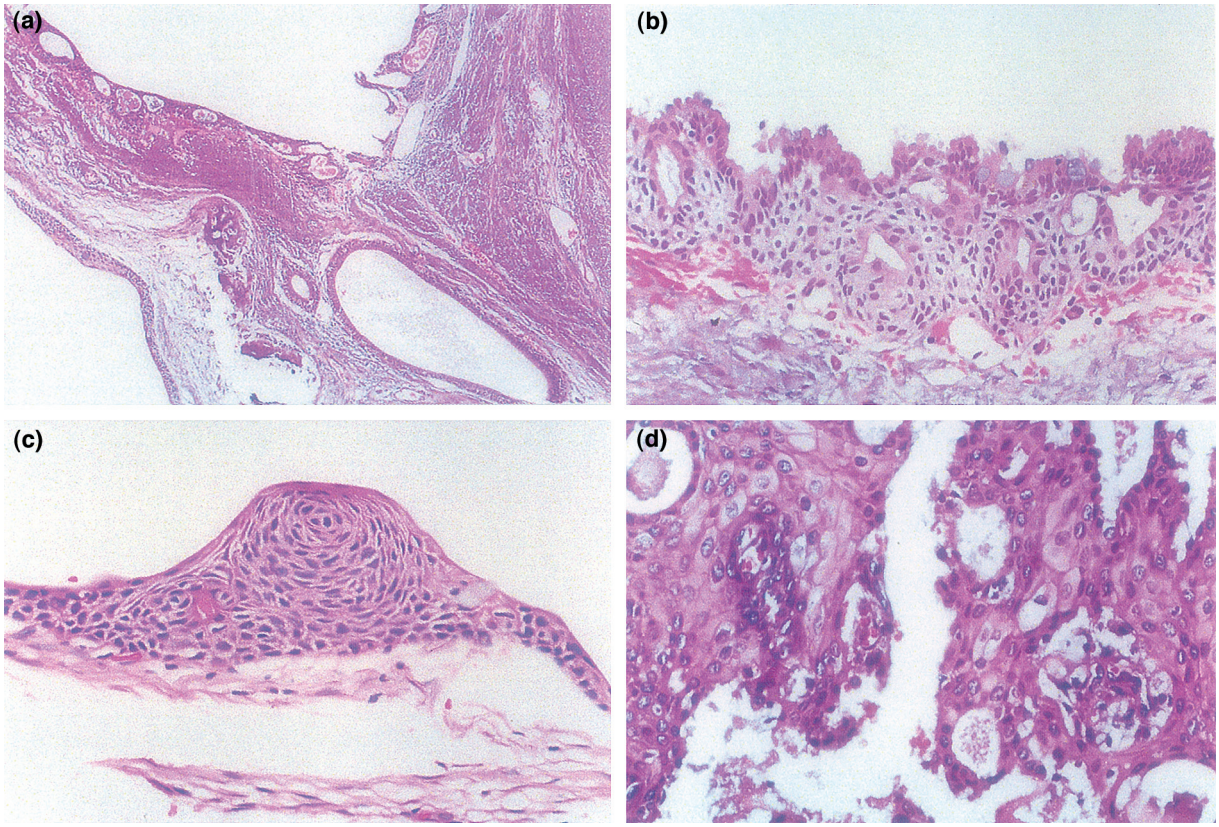

Figure 3 Histopathologic findings. (a) Low-power photomicrograph showing multiple cystic spaces surrounding residual bone trabeculum centrally. Cysts are lined by stratified epithelium, which in upper cyst contains numerous mucous-secreting goblet cell and intraepithelial gland-like structures [hematoxylin and eosin (H & E), original magnification ×10]. (b) Cyst wall consisting of non-keratinized epithelial layer and connective wall. Superficial layer of epithelium consists of eosinophilic cuboidal or low-columnar cells with cilia forming irregular papillary projections. Glandular-like structures filled by mucinous materials can be seen within epithelial lining (H & E, original magnification ×40). (c) Epithelial lining showing whirlpool-like thickenings bulging into the cystic cavity (H & E, original magnification ×40). (d) Part of lesion presenting mucoepidermoid carcinoma-like proliferation (H & E, original magnification ×40).

(MEC)-like proliferation in the wall were present in one cyst in which clear cell change was noted focally (Fig. 3d).

Immunohistochemistry

The results of immunohistochemical staining for GOC are summarized in Table 2. The results revealed positive reactivity for CKs AE1, 7, 8/18, 10/13, 14 and 19 with slight changes in their patterns whereas reactivity of CKs 16 and 20 was negative in epithelium of all GOCs. Expression of CK14 was detected homogeneously and intensely in basal cell layer and plaque-like areas, and heterogeneously and less intensely in suprabasal layer of cystic epithelium (Fig. 4a). CK19 were expressed homogeneously and strongly in all cell layers of GOC except for two cases strongly positive in basal layer of the cyst lining epithelium (Fig. 4b). Epithelium of the GOC stained with AE1 was analogous to CK19 but weaker when compared with the latter antibody. CK7 and CK8/18 was positive mostly in the surface and suprabasal layer of the cystic epithelium, and absent or sporadically weak positive cells in the basal layer of the lining epithelium(Fig. 4c), but CK7 staining patterns were more homogeneously and intensely positive in the surface and plaque-like areas than CK8/18, respectively. CK10/13 was only heterogeneously and markedly

Table 2 Cytokeratin expression in the lining epithelium of glandular odontogenic cyst (GOCs)

	Cytokeratin							
Epithelial layer	*AE1*	*CK7*	*CK8/18*	*CK10/13*	*CK14*	*CK16*	*CK19*[b]	*CK20*
Surface	+−	++	+	−−	−−	−−	++	−−
Suprabasal[a]	+−	−+	−+	+	−+	−−	++	−−
Basal	+−	−−	−−	−−	++	−−	++	−−
Mucous cells	−−	−−	−−	−−	−−	−−	−−	−−
Plaque	+−	++	+−	+−	++	−−	++	−−

[a]Suprabasal layer is identified only in the thickened area of lining epithelium.
[b]Two cases strongly positive only in basal layer of the cyst epithelium.

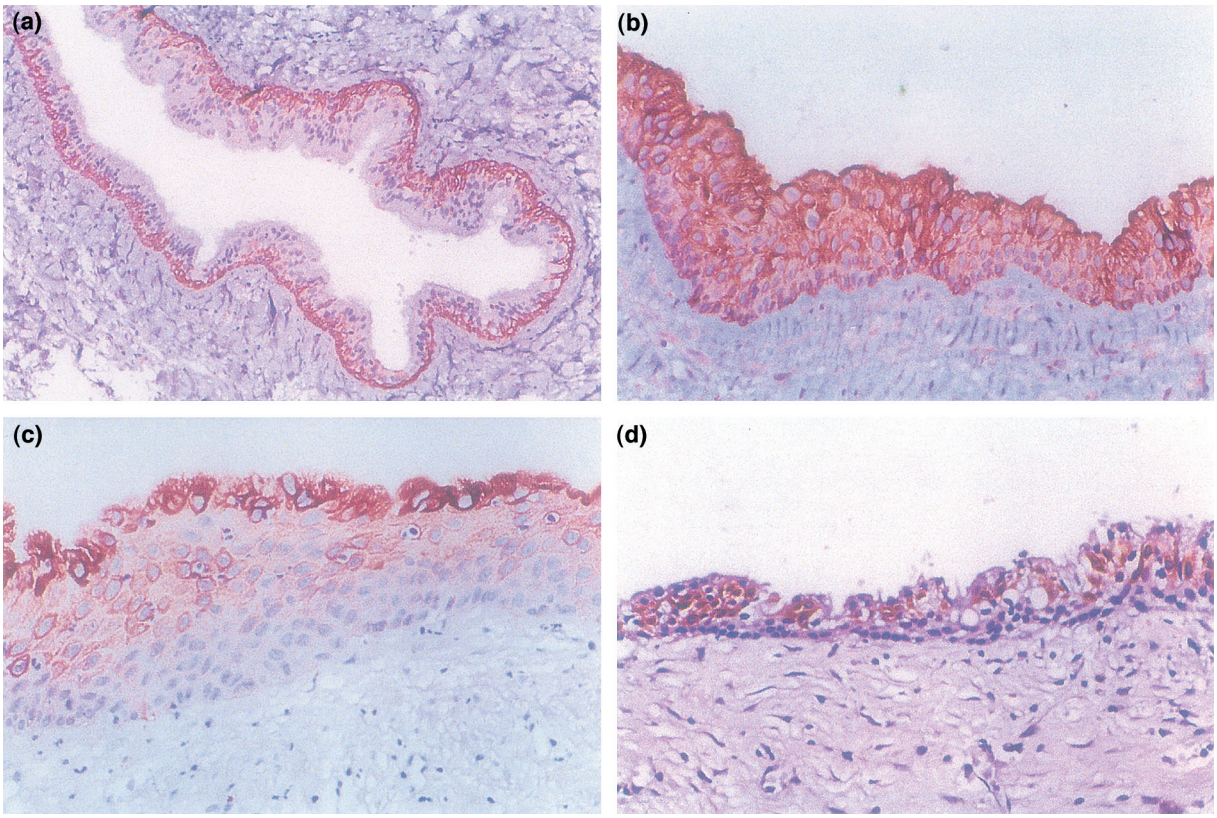

Figure 4 Immunohistochemical findings for cytokeratins. (a) CK14 is positive homogeneously in the basal cells and heterogeneously in suprabasal cells (SP; original magnification ×10). (b) CK19 is positive homogeneously in all of the cell layers of glandular odontogenic cyst (GOC) (SP, original magnification ×40). (c) CK8/18 is strongly positive in the surface cells, heterogeneously and weakly in suprabasal cells (SP, original magnification ×40). (d) CK10/13 is positive heterogeneously and strongly in the suprabasal cells (SP, original magnification ×40).

positive in the suprabasal layers (Fig. 4d), and homogeneously and weakly positive in plaque-like areas of the lining epithelium. Mucous cells of all GOCs gave no reaction with all of CK antibodies used.

Discussion

The GOC is a rare lesion with a frequency rate of only 0.012% (7) to 1.3% (8) of all jaw cysts, in accordance with present study, giving a prevalence of 0.17%. Our Medline search revealed 73 cases of GOC in the English literature and data were summarized in Table 3. The present study showed that there was a slight preponderance for male and the third decades. In accordance with previous reports, the mandible was affected more often than the maxilla, especially anterior mandible (7, 9–12). A slight predilection in this study for unilocular lesion, was in concurrent with Manor et al. (13), but in contrast to earlier reports (9, 10, 14). The most common symptom was an asymptomatic slow-growing swelling. Accompanied pain was relatively unusual probably due to stretching of, or pression on neurovascular bundles or inflammation of the cyst. Radiographically the GOC is usually localized intraosseously and may appear as unilocular or multilocular radiolucent lesion with well-defined borders, sometimes presenting as peripheral osteosclerosis and scalloping. Root resorption and displacement of tooth are also noted. But clinical and radiographic finding of GOC varied and was often not pathognomonic (15, 16). An incisional biopsy, fine-needle aspiration, electrophoresis, and exfoliative cytologic examination of cyst contents as adjuventive tests are essential to differentiate between GOC and other similarly presenting lesions (17). But their true nature can only be determined by pathologic examination.

The present 12 lesions completely fulfilled the histodiagnostic criteria of GOC proposed by Gardner et al. (1) and WHO (3). However, they exhibited some unusually histologic features. Solid proliferation of the epithelial elements into underlying connective tissue, epithelial island, and satellite microcysts interspersed within the fibrous stroma, and erosion of the surrounding bone were observed, and corresponding to Toida et al. and Ficarra et al. (10, 18). There were also reported aggressive and neoplastic features in GOC including MEC-like, AB-like, and squamous odonto-

Table 3 Clinical features at presentation of previous glandular odontogenic cyst (GOCs)

Year	*Author*	*Male/female*	*Mandible/maxilla*	*Multilocular/unilocular*	*References*
1987	Padayachee and Van Wyk	1/1	2/0	2/0	(2)
1988	Gardner et al.	3/5	6/2	1/7	(1)
1990	Lindh and Larsson	0/1	1/0	1/0	(35)
	Ficarra et al.	1/0	1/0	1/0	(18)
1991	Sadeghi et al.	1/0	1/0	1/0	(36)
	Patron et al.	3/0	3/0	1/2	(11)
1992	Van Heerden et al.	1/1	2/0	0/2	(8)
1993	Gardner and Morency	1/0	1/0	1/0	(12)
1994	Takeda	1/0	1/0	0/1	(37)
	Jean et al.	1/0	0/1	0/1	(38)
	de Carvalho et al.	0/1	1/0	1/0	(39)
	Toida et al.	0/1	1/0	1/0	(10)
	Semba et al.	0/1	1/0	1/0	(6)
1995	Hussain et al.	1/3	4/0	4/0	(17)
	Econompopoulou and Patrikiou	1/0	0/1	0/1	(15)
1996	Savage et al.	0/1	1/0	0/1	(40)
	Ide et al.	0/1	1/0	0/1	(24)
	High et al.	1/1	2/0	2/0	(20)
1997	Manojlovic et al.	0/1	1/0	1/0	(41)
	Ramer et al.	0/1	1/0	1/0	(9)
	Baliga et al.	1/0	0/1	0/1	(42)
	de Sousa et al.	1/0	1/0	1/0	(30)
	Magnusson et al.	4/3	5/2	2/5	(7)
1998	Koppang et al.	2/0	1/1	1/1	(14)
1999	Chavez and Richter	1/0	1/0	1/0	(43)
2000	Hisatomi et al.	0/1	1/0	0/1	(19)
	Tosios et al.	2/1	2/1	NR	(34)
	Damm and Fantasia	0/1	1/0	0/1	(44)
	Lin et al.	1/0	1/0	1/0	(45)
2001	Barreto et al.	0/1	1/0	1/0	(46)
	Bhatt et al.	0/1	1/0	1/0	(47)
2002	Farman et al.	0/1	1/0	0/1	(48)
	Noffke and Raubenheimer	4/5	6/3	2/7	(16)
2003	Manor et al.	4/1	2/3	1/4	(13)
	Ertas et al.	1/0	1/0	1/0	(49)
2004	Tran et al.	1/0	1/0	0/1	(50)
	Osny et al.	1/0	1/0	1/0	(51)
Total	–	39/34	58/15	32/38	

NR: no records.

genic tumor-like epithelial proliferation in the cyst wall (1, 10, 11, 19), and showing a wide histopathologic spectrum of GOC. In addition, one case with infiltration greatly by chronic inflammatory cells showed proliferating rete processes similar to those seen in inflammatory RC.

The most interesting histologic feature in current series was MEC-like proliferation in one case, concurrent with Toida et al. (10). But in most areas of this cyst, classic histologic picture of GOC were visualized. Thus, the lesion was diagnosed as MEC-like GOC, but not CMEC.

While the histogenesis of GOCs remains uncertain, most authors believed that they originate from odontogenic epithelium (20–22). Important evidence in support of such a concept is the morphology of the epithelium. GOC arising in tooth-bearing areas led to suggestion of an odontogenic epithelial origin, whilst histopathologically salivary components were noted (20). The thin, cuboidal or columnar epithelium is reminiscent of reduced enamel epithelium (21). The plaque-like or spherical structures sometimes are also seen commonly in odontogenic lesions such as lateral periodontal cyst, gingival cyst of adults and rarely in DC, adenomatoid odontogenic tumor (AOT) and AB (1). Intraepithelial duct and mucous-producing cells with or without cilia have been ubiquitously reported in odontogenic lesions (22, 23). Histologic variations including squamous odontogenic tumor-like, AB-like proliferation and other features observed in odontogenic lesion were also visualized (1, 9–11, 14, 19, 24). Additionally in present study, small irregularly shaped calcifications were seen in the fibrous stroma in two cases, similar to those observed by Ramer et al. (9).

Our immunohistochemistry revealed that CK expression of GOCs showed a high degree of tissue specificity. Epithelium of GOCs stained for CKs AE1, 7, 8/18, 10/13, 14 and 19 with slight changes in their patterns, but no staining for CKs 16 and 20 could be visualized, which shared an similar distribution of CKs with odontogenic epithelium. Previous report showed that CK14 and 19 were expressed in all kinds of odontogenic epithelial cells of developing tooth germs and in epithelial cells of some odontogenic tumors (4, 25–29). We also found that the two CKs were strongly expressed in epithelium of GOC in spite of small differences in different specimens and different cell layers, and indicating histochemically that epithelium of GOC may be odontogenic origin in nature.

In this study, CK8/18 was positive mostly in surface, suprabasal layers and plaque of GOC, and similar to those described by Mackenzie and co-workers (28) in RC and DC, but in contrast to other study (14, 26, 30–33) in odontogenic lesion. Differences in reactivities for CK8 and 18 may be due to differing availability of the epitopes, which monoclonal antibodies recognize, or possibly to the expression of different subfamilies of CK in different tissues (29). Again, it is possible that technical or genuine biologic differences are responsible for the divergent results (14).

In addition, our results showed that CK10/13 were positive sporadically in suprabasal layer of GOC, corresponding to previous report in GOC and other odontogenic lesion (4, 14, 29, 31). As for CK7 and AE1, reacted with GOC in surface, suprabasal layer, plaque of GOC and moderately with all cell layers of GOC, respectively, in agreement with previous findings (9, 14, 31).

It should be noted that CK16 was negative in GOCs, suggesting that high-recurrent rate of GOC is not due to cell proliferation in epithelium of the cyst. Immunohistochemistry of bcl-2, Ki-67 and p53 in epithelium of GOC also suggested that biologic behavior of GOC was not associated with cell proliferation, and incomplete removal of the cyst because of its multicystic configuration, tendency of the epithelium to separate from the connective tissue, or growth through the cancellous spaces of bone may account for its high-recurrence rate (34). In general, our findings of a series of CKs in GOCs appeared to reflect the variations in epithelial cell type, maturation and differentiation of GOC to some degree, and small difference in CKs expression between GOC and the other odontogenic epithelia may be related to developmental differences or otherwise.

Various treatment modality for GOC ranging from curettage, enucleation to *en bloc*, partial ostectomy have been recommended (1, 2, 5–20, 24, 30). In the present series, nine lesions were first treated by conservative treatment, Extirpation with segmental ostectomy of maxilla/partial mandibulectomy were in remaining three cases. The post-operative course has been uneventful except for one case treated by curettage recurring in the third years post-operatively. In view of the high-recurrence rate associated with conservative treatment of these cysts and their invasion potential, we suggest that a more aggressive removal, rather than simple conservative methods, be considered so as to minimize rate of recurrence.

It should be emphasized that the relative rarity of GOC make evaluation of the behavior and prognosis difficult. Furthermore, more than half of the published cases had been followed for periods <2 years or were very recent cases, and the recurrences developed after the third years post-operatively (1, 2, 6–20, 24, 29, 30). So we speculate that the incidence of recurrence could be higher than those described previously, and suggesting that carefully long follow up be essential, in agreement with Koppang et al. (14).

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