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ORIGINAL ARTICLE

A immunohistochemical study of the peripheral ameloblastoma

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AIM: Peripheral ameloblastoma (PA) is a rare variant of ameloblastoma occurring in the extraosseous region. With regard to the histogenesis of the tumor, two major sources of origin are considered: odontogenic epithelial remnants and the gingival epithelium. In this study, we examined the immunohistochemical profiles of cytokeratins (CKs) and Ki-67 labeling index (LI) of PAs, and discuss the histogenesis and the biologic behavior of the PA.

MATERIALS AND METHODS: Eight cases of PA were retrieved from the pathology files of 212 cases of ameloblastoma that had been registered at our hospital. Immunohistochemical staining was performed in seven cases using monoclonal antibodies of six CKs (7, 8, 13, 14, 18, and 19) and Ki-67.

RESULTS: All cases of PA expressed CK13, 14, and 19. CK18 was positive staining in six cases, and CK8 in five cases. This staining pattern was similar to that in intraosseous ameloblastomas (IAs). The mean of Ki-67 LI of PAs (1.91%) was significantly lower than that of IAs (4.82%) ($P = 0.002$).

CONCLUSION: We consider that the PA originates from odontogenic epithelial remnants rather than from the gingival epithelium, and the Ki-67 LI of the tumor is a good prognostic indicator.
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Keywords: peripheral ameloblastoma; cytokeratin; Ki-67; intraosseous ameloblastoma; odontogenic

Introduction

Ameloblastoma is the most common odontogenic tumor, generally occurring in the intraosseous region of the jaws. However, it rarely appears in the gingiva or oral mucosa, known as the peripheral ameloblastoma (PA) (Sciubba *et al*, 2001), which comprises 1.3–10% of all ameloblastomas (Gardner *et al*, 2005). In addition to 160 cases of PA in a review of Philipsen *et al* (2001), some cases have been reported up to now (Orsini *et al*, 2000; Tajima *et al*, 2001; Wettan *et al*, 2001; Marucci *et al*, 2004; Lopez-Jornet and Bermojo-Fenoll, 2005; Martelli-Junior *et al*, 2005).

The PA is usually a painless sessile or pedunculated gingival lesion with smooth, granular, or warty surface. Most lesions are clinically considered as a fibroma or granuloma (Neville *et al*, 2002). Histologically, the PA consists of proliferating ameloblastic epithelium with follicular pattern under the mucosal epithelium. Frequently, the continuity between tumor nests and the mucosal epithelium is presented (Neville *et al*, 2002).

With regard to the histogenesis of PA, two major sources of origin are considered: odontogenic epithelial remnants and the basal cell layer of the gingival epithelium (Philipsen *et al*, 2001; Gardner *et al*, 2005). Tajima *et al* (2001) suggested that the PA originates from the odontogenic epithelium, as the tumor cells are positive for CK19, which is usually expressed in intraosseous ameloblastoma (IA) immunohistochemically. On the other hand, Vigneswaran *et al* (1993) described that the PAs are tumors analogous to cutaneous basal cell carcinomas (BCCs), based on the evidence of the presence of a peritumorous band-like peanut agglutinin staining, which is characteristic of BCCs and is not seen in IA. Gardner (1997) also considered that the PA and the BCC of the gingiva share the same histology.

The recurrence rate of the PA is much lower (19%) than that of the IA (33%) (Buchner and Sciubba, 1987). The PAs do not require extensive surgical treatment and usually show a better prognosis than the IA, but some cases of malignant transformation of PA (Edmondson *et al*, 1982; Lin *et al*, 1987; McClatchey *et al*, 1989; Baden *et al*, 1993; Califano *et al*, 1996), recurrent PA with dysplasia (Wettan *et al*, 2001), or PA with significant bone involvement (Tajima *et al*, 2001; Ide *et al*, 2002) have been reported.

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In this study, we examined the immunohistochemical profiles of cytokeratins (CKs) and Ki-67 labeling index (LI) of PAs compared to those of IAs, and discuss the histogenesis and biologic behavior of the PA.

Patients and methods

The pathology files of 212 cases of ameloblastoma that had been registered at our hospital for a 33-year period were re-evaluated. All tumor patients were Japanese. Eight cases of PA were retrieved from the files and were reviewed clinically and histopathologically.

Tissues obtained by surgery (eight cases of PA and ten cases of IA) were fixed in 10% buffered formalin followed by paraffin embedding. One case of PA containing bone tissue in its specimen was decalcified in 10% formic acid solution for a week at room temperature before paraffin embedding. Histologic sections from paraffin-embedded blocks were stained with hematoxylin and eosin (HE). Ten cases of IA, which showed typical histologic appearances (five follicular ameloblastomas and five plexiform ameloblastomas), were selected for comparison with the PAs immunohistochemically. Immunohistochemical stain was performed on the serially prepared sections except one decalcified case by avidin–biotin–peroxidase complex technique using following monoclonal antibodies: CK7 (LP5K 1:20; Novocastra, Newcastle, UK), CK8 (C-51, 1:60; Novocastra), CK13 (KS-1A3, 1:200; Novocastra), CK14 (LL002, 1:40; Novocastra), CK18 (DC-10, 1:40; Novocastra), CK19 (RCK108, 1:100; Dako, Glostrup, Denmark) and Ki-67 (MM1, 1:200; Novocastra). To enhance immunostaining, antigen retrieval was carried out in citrate buffer (0.01 mol l^{-1}, pH 7.0) at 121°C for 10 min in an autoclave.

Two investigators (M.K. and M.Y.) confirmed the diagnosis of the specimens and evaluated the intensity of staining for CKs [classified – (negative), + (weak), + + (moderate), or + + + (strong)]. To investigate the Ki-67 LI, three fields of each specimen were evaluated using a 20× lens. The mean Ki-67 LI was defined as the percentage of total tumor cells that were Ki-67 positive. The results were based on at least 1200 randomly counted tumor nuclei within each case.

Databases were created using all the recorded clinical and pathologic outcomes. For comparing a Ki-67 LI on PA and IA, the Mann–Whitney *U*-test was used. $P < 0.05$ was considered to be significant.

Results

We retrieved eight cases of PA from the files, amounting to approximately 3.8% of all ameloblastomas in our registry. Clinical and histopathologic findings of these cases are summarized in Table 1. The average age at the time of the diagnosis was 57.5 years. Three maxillary tumors were located in the molar region. Five mandibular tumors were located from the anterior to premolar regions. Radiographically, six cases showed no evidence of bone involvement while two cases presented superficial bone resorption with a cup-like appearance (Figure 1). In seven cases, the tumor was excised with the

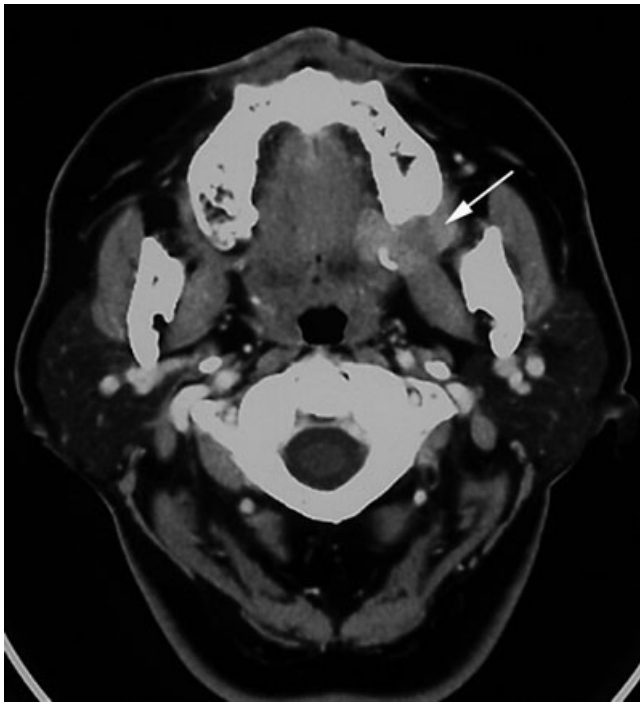

Figure 1 Computerized tomography image shows left maxillary bone resorption of cupping appearance caused by the retromolar tumor (arrow) (case 8)

Table 1 Details of eight cases of peripheral ameloblastoma

No.	*Age/Sex*	*Location*	*Clinical diagnosis*	*Bone resorption*	*Histologic feature*	*Continuity*	*Treatment*	*Recurrence*
Case 1	61/F	L, I	Ameloblastoma	+	Follicular(A), plexiform	+	Excision	–
Case 2	60/F	L, I	Epulis	–	Follicular(A)	–	Excision	–
Case 3	70/M	U, P	Fibroma	–	Follicular(A)	–	Excision	–
Case 4	36/M	L, P	Epulis	–	Follicular(A)	+	Excision	–
Case 5	60/M	L, P	Gingival tumor	–	Follicular	–	Excision	–
Case 6	67/M	L, P	Verrucous carcinoma	–	Follicular(A)	+	Excision	–
Case 7	45/F	U, P	Epulis	–	Follicular(A)	+	Excision	–
Case 8[a]	61/F	U, M	Ameloblastoma[b]	+	Follicular, plexiform	+	Partial maxillectomy	–

L, lower gingiva; U, upper gingiva; I, incisor region; P, premolar region; M, molar region; A, acanthomatous change. Continuity: between the tumor and the surface epithelium.
[a]Decalcified case.
[b]Diagnosed by biopsy specimen at another hospital.

surrounding soft tissue. Partial resection of the maxilla was performed in one case. This case was treated as IA in our hospital, because it was diagnosed as 'ameloblastoma' by biopsy specimen at another hospital (case 8). The average follow-up duration of the PAs was 56.1 months (21–93 months). The recurrent tumor has not been detected in any treated cases from clinical and radiologic examinations.

Histologically, these tumors consisted of proliferating ameloblastic epithelium with follicular pattern under the gingival epithelium (Figure 2a). In two cases, the plexiform component was presented (Figure 2b). Acanthomatous change was seen in the center of the tumor nests in six cases (Figure 2c). The continuity between the tumor nests and the gingival epithelium was observed in five cases (Figure 2d). In case 8, neoplastic islands were not found within the bone.

Immunohistochemical profiles of CKs and Ki-67 of the tumors are summarized in Table 2. With regard to stratified squamous CKs, all cases of PA and IA were stained for CK13 and CK14 (Figure 3a,c). As regards simple epithelial CKs, all cases were positive for CK19 (Figure 3d), but negative for CK7. Six cases of PA showed positive staining for CK18 (Figure 3b) that was observed in all cases of IA. CK8 was weakly positive in five cases of PA (Figure 3e) and nine cases of IA. For CK8, CK13, and CK18, the positive cells were noticed mainly in the central area of the tumor nests in all positive cases of PA. This immunostaining pattern was similar to that in IAs.

The mean of Ki-67 LI of PAs was 1.91% (0.98–2.97%) while its mean of IAs was 4.82% (2.12–11.25%). The mean of Ki-67 LI of PAs was significantly lower than that of IAs ($P = 0.002$).

Discussion

In a review by Philipsen *et al* (2001), the PAs tend to develop in the premolar region of the mandible while the IAs show marked predilection for the molar region of the mandible. Macroscopically, the PA is a painless gingival lesion, the surface of which is usually smooth, and sometimes granular, papillary, or warty (Philipsen *et al*, 2001; Gardner *et al*, 2005). Radiologically, superficial bone resorption may be rarely noticed as cupping or saucerization (El-Mofty *et al*, 1991; Gardner, 1997). As mentioned above, the PAs do not show typical clinical and radiologic features of ameloblastoma. Our cases of PA and IA showed different anatomic distribution as reported previously (Table 3). Radiologically, there were only two cases of PA that showed superficial bone resorption. Six cases of PA were clinically considered as a epulis, fibroma, gingival tumor, or verrucous carcinoma. However, histologic diagnosis of the PA is not difficult because the lesions consist of proliferating epithelium with the same histologic cell types and

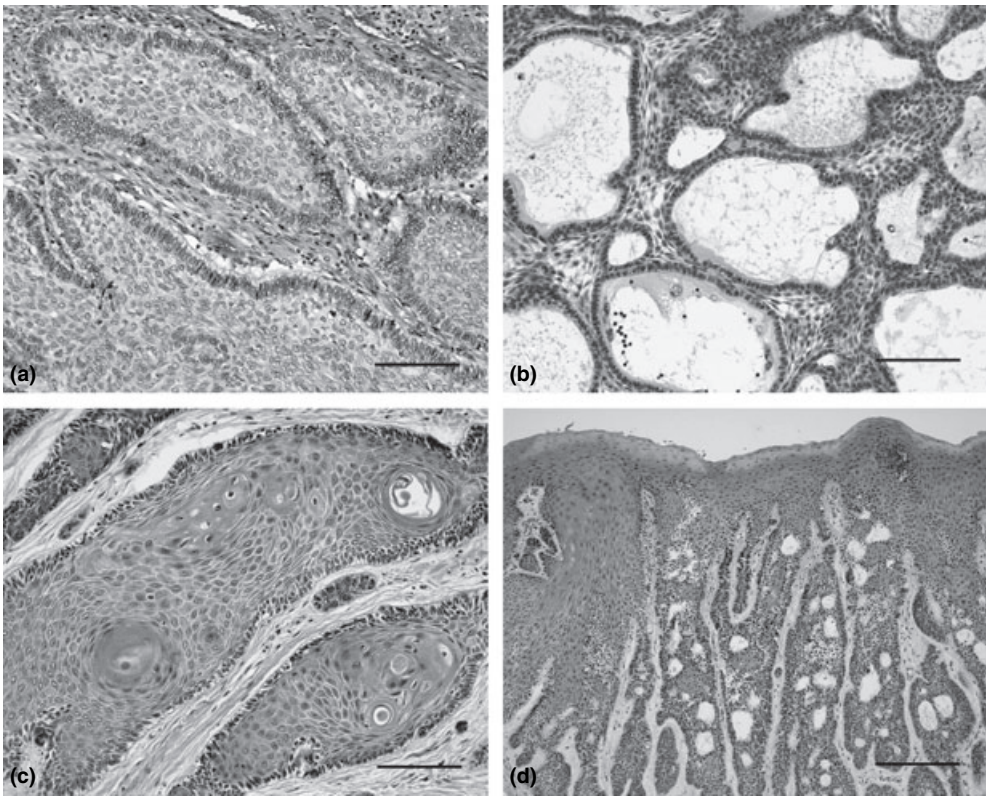

Figure 2 Histologic findings of the peripheral ameloblastoma (PA). All cases of PA show the proliferating epithelial nests of follicular pattern (**a**), mixed with plexiform pattern in two cases (**b**). Acanthomatous change in the central area of the follicular tumor nests (**c**). The continuity between the tumor and the gingival epithelium is presented (**d**) (HE, **a–c**: bar 100 μm, **d**: bar 200 μm)

Table 2 Summary of the immunohistochemical staining in PAs and IAs

	CK7	*CK8*	*CK13*	*CK14*	*CK18*	*CK19*	*Ki67 LI (%)*
PA (case 1)	−	+	+ +	+ + +	+ +	+ +	0.98
PA (case 2)	−	+	+	+ + +	+	+	2.06
PA (case 3)	−	+	+ +	+ + +	+ +	+	1.89
PA (case 4)	−	−	+	+ + +	+	+ +	1.80
PA (case 5)	−	−	+	+ + +	−	+	1.85
PA (case 6)	−	+	+ +	+ + +	+ +	+	1.84
PA (case 7)	−	+	+ +	+ + +	+ +	+ + +	2.97
PA (case 8)	NS	NS	NS	NS	NS	NS	NS
IA 1(F)	−	+	+ +	+ + +	+ +	+ + +	7.69
IA 2(P)	−	−	+	+ + +	+	+ +	3.30
IA 3(F)	−	+	+ +	+ + +	+ +	+ +	3.30
IA 4(P)	−	+	+ +	+ + +	+	+ + +	2.05
IA 5(P)	−	+	+ +	+ + +	+ +	+ + +	11.25
IA 6(F)	−	+	+ +	+ + +	+ +	+ +	2.82
IA 7(F)	−	+	+ +	+ + +	+ +	+ + +	3.89
IA 8(P)	−	+	+	+ + +	+ +	+ + +	7.81
IA 9(F)	−	+	+ +	+ + +	+	+ +	3.96
IA 10(P)	−	+	+ +	+ + +	+ +	+ + +	2.12

PA, peripheral ameloblastoma; IA, intraosseous ameloblastoma; F, follicular type; P, plexiform type; CK, cytokeratin; −, negative; +, weakly positive; + +, moderately positive; + + +, strongly positive; NS, not stained (case 8 was decalcified in 10% formic acid, so it was unsuitable for immunohistochemistry). The PAs had significantly lower percentage of Ki67 labeling index (LI) than the IAs ($P = 0.002$).

follicular patterns as seen in ameloblastoma (Philipsen *et al*, 2001). The continuity between the tumor nests and the gingival epithelium was reported in about 50% of cases (Neville *et al*, 2002). This finding was seen in our five cases. The PAs showing the continuity with the surface epithelium should be differentiated from other epithelial neoplasms – squamous cell carcinoma or verrucous carcinoma.

Intraosseous ameloblastomas are tumors of odontogenic epithelial origin (Neville *et al*, 2002). It is considered that the PA is derived from odontogenic epithelial remnants or basal cells of the gingival epithelium (Philipsen *et al*, 2001; Gardner *et al*, 2005). Immunohistochemically, previous reports show that the IAs express various kind of CKs – CK7, 8, 13, 14, 18, and 19 (Ong'uti *et al*, 1999; Tateyama *et al*, 2001; Crivelini *et al*, 2003). In particular, tumor cells are positive for CK13, 14, and 19 in almost all reported cases, while only a few cases are stained for CK7. On the other hand, Vigneswaran *et al* (1993) described that the PAs are tumors analogous to cutaneous BCCs, based on the evidence of the presence of a peritumorous band-like peanut agglutinin staining, which is characteristic of BCCs and is not seen in IA. Previous studies of the BCC show that the cutaneous BCCs also express various kind of CKs – CK7, 8, 14, 8/18, and 19 (Yoshikawa *et al*, 1998; Kruger *et al*, 1999; Yamamoto and Asahi, 1999). Yamamoto and Asahi (1999) showed that all BCCs (29 cases) did not stain for CK13 and only one case stained for CK19. In our results, all cases of PA expressed CK13, CK14, and CK19. CK18 was positive in six cases, and CK8 in five cases. CK profiles of PAs were similar to those of IAs, but somewhat different from those of cutaneous BCCs. From these findings, it is suggested that PA originates from odontogenic epithelial remnants rather than from basal cells of the gingival epithelium.

As regards immunohistochemistry for Ki-67, Sandra *et al* (2001) showed that the mean Ki-67 LI of the follicular ameloblastomas is 5.05% and that of the plexiform ameloblastoma is 3.07%. Rosenstein *et al* (2001) showed that the mean Ki-67 LI of the cystic ameloblastoma and solid ameloblastoma are 4.3% and 2.8%, respectively. Buchner and Sciubba (1987) showed that the recurrence rate of the PA is much lower (19%) than that of the IA (33%). In our cases, the mean of Ki-67 LI of the PAs (1.91%) was significantly lower than that of the IAs (4.82%) ($P = 0.002$), and recurrence has not been found in any cases until now. However, some cases of malignant transformation of PA (Edmondson *et al*, 1982; Lin *et al*, 1987; McClatchey *et al*, 1989; Baden *et al*, 1993; Califano *et al*, 1996), recurrent PA with dysplasia (Wettan *et al*, 2001), or PA with significant bone involvement (Tajima *et al*, 2001; Ide *et al*, 2002) have been reported. It seems that Ki-67 LI is useful in differentiating a malignant transformation and in predicting the prognosis of the PA.

In conclusion, the immunohistochemical similarity of CK profiles between PAs and IAs suggests that they are tumors of the same origin, and that the PA is derived from odontogenic epithelial remnants rather than from the gingival epithelium. From the finding of lower Ki-67 LI of the PAs than that of the IAs, we consider that the Ki-67 LI is a factor related to the recurrence and the malignancy of the PA, and will be a good prognostic indicator of the PA.

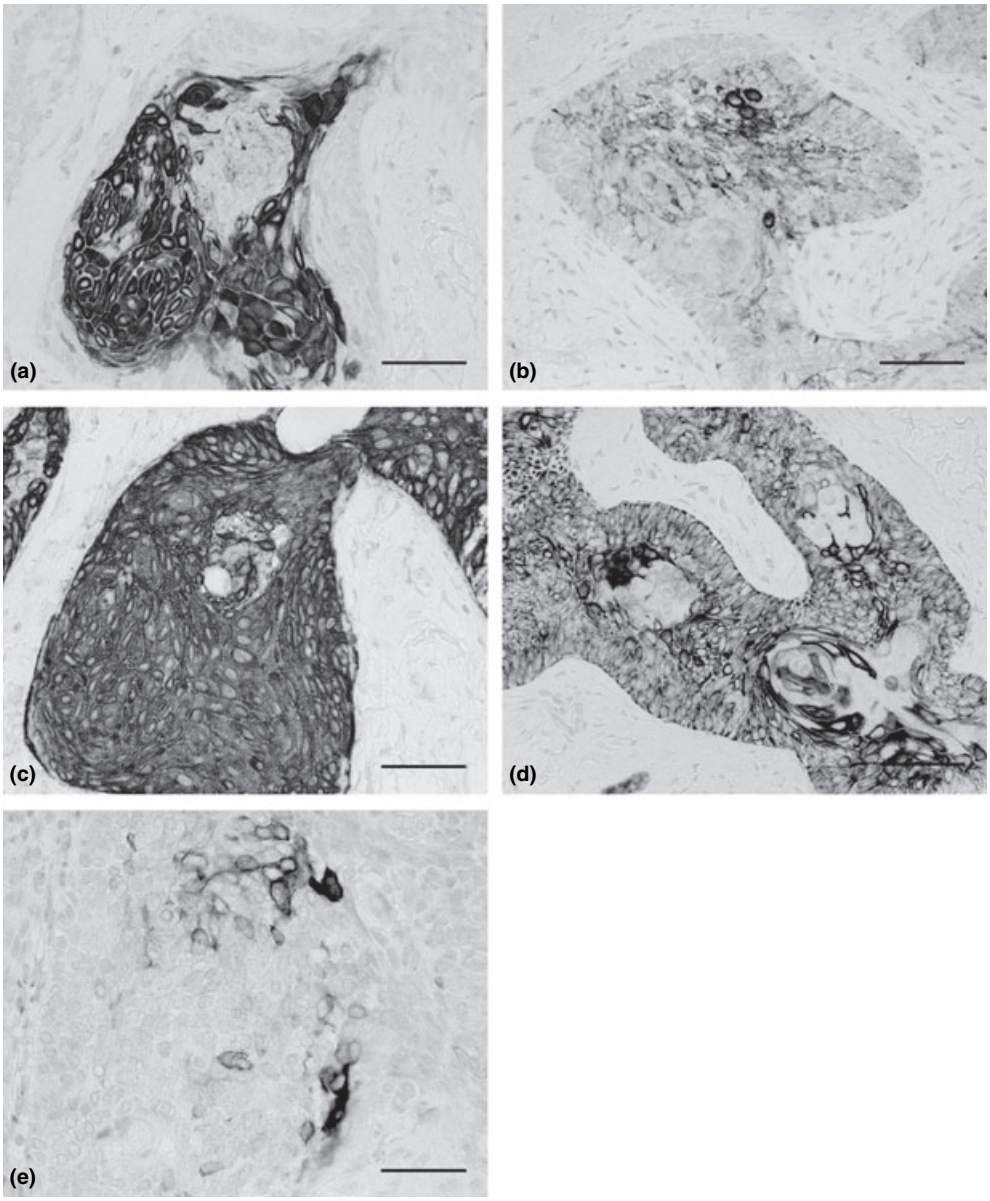

Figure 3 Immunohistochemical findings of the peripheral ameloblastoma (bar: 50 μm). The positive cells are mainly in the central area of the tumor nests for CK13 (**a**) and CK18 (**b**). All the tumor cells are strongly positive for CK14 (**c**). The tumor cells are diffusely positive for CK19 (**d**). Some tumor cells in the central area of the tumor nests are positive for CK8 (**e**)

Table 3 Anatomic distribution of PAs compared with IAs

	Maxilla			*Mandible*		
	Anterior	*Premolar*	*Molar*	*Anterior*	*Premolar*	*Molar*
PA ($n = 135$), Philipsen *et al* (2001)	6	8	24	28	44	25
PA ($n = 8$), our cases	0	2	1	2	3	0
IA ($n = 204$), our cases	5	1	13	14	22	149

PA, peripheral ameloblastoma; IA, intraosseous ameloblastoma.

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