Clear Cell Variant of Calcifying Epithelial Odontogenic Tumor: Is It Locally Aggressive?

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The calcifying epithelial odontogenic tumor (CEOT) is a rare benign odontogenic neoplasm of the jaws, accounting for approximately 1% of all intraosseous odontogenic tumors.¹⁻³ Some histologic variants have been described, including CEOT with Langerhans cells,^{4,5} with cementum-like and bone-like material, combined epithelial odontogenic tumor and adenomatoid odontogenic tumor, myoepithelial cells, and the clear cell variant of calcifying epithelial odontogenic tumor (CCCEOT).⁶ The diagnosis of CCCEOT is very difficult and other clear cell lesions that affect the oral cavity should be excluded. According to some authors it is more aggressive when compared with other CEOT variants showing a recurrence rate of $10.52\%^7$ and should be considered as a low-grade odontogenic

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© 2009 American Association of Oral and Maxillofacial Surgeons 0278-2391/09/6701-0031\$34.00/0 doi:10.1016/j.joms.2007.11.029 carcinoma.^{1,3,6} To our knowledge, there are only 19 cases of CCCEOT in the oral cavity published in the English language literature. We report an additional case of CCCEOT affecting the mandible of a 65-year-old male and discuss its clinicopathologic and immunohistochemical findings.

Report of a Case

Our biopsy service (Oral Pathology, Piracicaba Dental School-UNICAMP, São Paulo, Brazil) received a specimen from a 65-year-old male of a painless swelling located between the mandibular right lateral incisor and canine that had been growing slowly for about 3 years. The clinical information sent by the private dentist was of a fibrous, firm, sessile, and painless lesion covered by a smooth and regular mucosal surface in the anterior mandibular gingiva between the right lateral incisor and canine, which measured nearly 1.5 cm in its maximum dimension. The periapical radiograph showed a well-defined unilocular radiolucent lesion with radiopacities dispersed throughout the lesion. In addition, there was resorption of the bone crest between the right mandibular lateral incisor and canine; however, the roots of the adjacent teeth were not displaced, and there was no sign of the root resorption (Fig 1). The lesion was removed by complete surgical excision.

Hematoxylin and eosin-stained sections showed multiple mucosal fragments represented by a dense connective tissue that was replaced by irregular strands, cords, and nests of epithelial cells. The polyhedral epithelial cells disclosed an abundant and eosinophilic cytoplasm; roundto-oval relatively large nuclei with dense chromatin, and evident intercellular bridges. These cells showed mild pleomorphic and hyperchromatic nuclei. In significant portions, the epithelial cells had a clear, foamy, vacuolated cytoplasm (Figs 2-4). The calcifications areas and Liesegang's rings were observed, and amyloid-like deposits were not visualized. However, these amyloid-like deposits could be observed in Congo red staining under polarized light (Fig 5). The periodic acid Schiff (PAS) showed numerous globules of positive material, which was removed by prior



FIGURE 1. Periapical radiograph showing a well-defined unilocular radiolucent lesion with radiopacities dispersed throughout the radiolucency, with resorption of the bone crest between the right mandibular lateral incisor and canine.

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diastase digestion. Immunohistochemical reactions carried out using avidin-biotin complex technique showed positivity for cytokeratin cocktail (clone: AE1/AE3, Dako, Carpinteria, CA, dilution 1:500) (Fig 6), ck7 (clone: OV-TL12/30, Dako, dilution 1:400) and ck14 (clone: NCL-LL002, Novocastra Laboratories, Newcastle, England, dilution



FIGURE 2. Microscopic features of the CCCEOT showing nests of polyhedral epithelial cells with clear cells and calcification (hematoxylin-eosin stain, original magnification $\times 100$).

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FIGURE 3. High-power view exhibiting mild pleomorphism on polyhedral and clear cells (hematoxylin-eosin stain, original magnification ×400).

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1:200) in the epithelial neoplastic cells whereas the stromal component was positive for vimentin (clone: Vim 3B4, Dako, dilution 1:400) (Fig 7). The other immunomarkers (1, S-100 protein, Dako, dilution 1:10,000; 2, muscle specific actin, clone: HHF-35, Dako, dilution 1:800; 3, anti-human melano-some, clone: HMB-45, Dako, dilution 1:200; 4, Desmin, clone: D33, Dako, dilution 1:1,000) were negative in the present case. Analyzing all features described above the final diagnosis of central CCCEOT was established. The patient has been followed for about 24 months with no sign of recurrence observed.

Discussion

The first case of CCCEOT was reported by Abrams and Howell⁸ in 1967. There are currently 20 reported



FIGURE 4. Microscopic features of the CCCEOT showing nests of polyhedral epithelial cells compounded by large and dark nucleus and clear cells with foamy and clear cytoplasm (hematoxylin-eosin stain, original magnification ×200).

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FIGURE 5. Amyloid-like material stained by Congo red (A) and shown by polarized light showing the characteristic green birefringence in a darkened background (B) (Congo red, original magnification \times 200).

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cases (including the present one) in the English-language literature (Table 1). Of these 20 cases, 12 were central and 8 peripheral lesions. The male:female ratio was 1:1.2 for the central and 1.66:1 for the peripheral lesions. Overall ages ranged from 14 to 68 years with a mean age of 40.84 years. The mean age for the intraosseous CCCEOT is, however, considerably higher (46.36 years) than for the extraosseous variant (33.25 years). The surgical management modalities in the 20 cases were as follows: complete or partial resection in 6 patients (30%), excision in 10 (50%), enucleation in 3 (15%), and curettage in 1 (5%). The follow-up ranged from 4 months to 13 years (mean, 3.6 years), and it was not available in 1 case. Recurrence was reported in 2 cases of central CCCEOT (10.52%) that occurred 4 months after curettage in 1



FIGURE 6. Epithelial, polyhedral, and clear cells displaying immunopositivity for cytokeratin cocktail (AE1/AE3) (immunohistochemical stain, original magnification $\times 100$).

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FIGURE 7. Stromal cells showing immunoreactivity for vimentin (immunohistochemical stain, original magnification ×100).

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case, and 13 years after partial resection in the other. None of the peripheral lesions recurred.

The diagnosis of CCCEOT is usually based on the finding of some areas of typical epithelial clear cells within the tumor. The nuclei show considerable variation in size and shape, with rare mitotic figures. In addition, the CCCEOT can present irregular strands, cords, and nests of the polyhedral epithelial cells with abundant, eosinophilic cytoplasm, round to oval relatively large nuclei with dense chromatin, and evident intercellular bridging in association with clear epithelial cells.⁹ Deposition of extracellular amyloid-like material and calcifications are also typical.¹⁰

Tumors with a conspicuous clear cell component in the head and neck region can impose serious problems with respect to differential diagnoses. Tumors constituted by clear cells are most often of epithelial origin but can also be present in melanocytic and mesenchymal neoplasms.² Clear cells are characteristic cellular components of the epithelial lining of most lateral periodontal cyst and gingival cysts in adults, or they may be found as clear cell rests of the dental lamina within the connective tissue of these cysts.¹ With considerably less frequency clear cells may occur in certain odontogenic epithelial tumor entities such as ameloblastoma, calcifying odontogenic cyst and calcifying odontogenic tumor.¹¹⁻¹³ Clear cells have also been described in other lesions of the jaw, including salivary gland tumors such as mucoepidermoid carcinoma and acinic cell carcinoma, and metastatic disease originating from kidney, thyroid, and lung carcinomas.^{6,14,15} Carcinoma of salivary gland origin is ruled out by the absence of actin and S-100 expression, and clear cell odontogenic carcinoma is excluded by the minor degree of atypia, good circumscription of the lesion, and presence of calcified and

Authors	Age (yr)	Gender	Site	Size (cm)	Treatment	Follow-Up
Abrams and Howell ⁸	16	F	Gingiva	0.5	Excision	FOD 3 yr
	50	Μ	Intraosseous	1.2	Enucleation	FOD 3 yr
Anderson et al ⁶	68	F	Intraosseous	3.0	Curettage	Recurrence at 4 mo
Wallace and MacDonald ¹³	65	Μ	Intraosseous	NR	Excision	FOD 22 mo
Greer and Richardson ⁵	37	F	Intraosseous	0.5	Enucleation	FOD 13 mo
Oikarinen et al ³	36	F	Intraosseous	10.0	Enucleation	FOD 2 yr
Wertheimer et al ²²	20	Μ	Gingiva	1.5	Excision	NR
Yamaguchi et al ²³	36	Μ	Intraosseous	2.5	Partial resection	FOD 2 yr
Ai-Ru et al ²⁴	32	F	Gingiva	NR	Partial resection	FOD 10 yr
	42	F	Intraosseous	NR	Resection	FOD 2 yr
Schmidt-Westhausen et al ¹⁴	38	Μ	Intraosseous	2.5	Resection	FOD 2 yr
Hicks et al ²¹	59	F	Intraosseous	3.8	Resection	FOD 3 yr
Houston and Fowler ²⁵	64	Μ	Gingiva	1.5	Excision	FOD 4 yr
	27	Μ	Gingiva	1.4	Excision	FOD 4 yr
Kumamoto et al ⁹	14	F	Intraosseous	NR	Partial resection	Recurrence at 13 yr
Orsini et al ¹²	32	Μ	Gingiva	NR	Excision	FOD 4 yr
Mesquita et al ¹⁶	48	F	Gingiva	2.0	Excision	FOD 30 mo
Anavi et al^7	27	Μ	Gingiva	1.0	Excision	FOD 1 yr
Germanier et al ¹¹	44	F	Intraosseous	3.0	Enucleation	FOD 1 yr
Rangel et al†	65	Μ	Intraosseous	1.0	Excision	FOD 1 yr

Table 1. WELL-DOCUMENTED REPORTED CASES OF CCCEOT

Abbreviations: CCCEOT, clear-cell calcifying epithelial odontogenic tumor; F, female; FOD, free of disease; M, male; NA, not available; NR, not reported.

*Eighteen cases were reported in 16 articles.

†Present report.

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amyloid-like material.¹⁰ Moreover, most clear cell salivary gland tumors are positive for PAS reaction with diastase digestion and most of them are malignant. Occasionally, melanocytic tumors may show a predominant clear cell component, but most of these tumors arise in the soft tissue and they have been described only rarely in the head and neck region. In addition, they show positivity for S-100 protein and melanoma associated antigen (HMB-45). Although metastatic disease originating from thyroid, kidney, and lungs may present clear cells, the absence of PAS positivity excluded metastatic renal cell carcinoma.¹⁰ The lack of mitotic figures and the generally good circumscription of the lesion are not typical of metastatic disease of any origin. The diagnosis of CCCEOT in our case was supported by microscopically biphasic pattern, presence of apple-green birefringent/ Congo red-positive material between tumor islands, small calcifications, good circumscription of the lesion, and lack of mitotic figures.

There are few reports about immunohistochemical findings in CCCEOT.^{3,9,14} Kumamoto et al⁹ found immunoreactivity of the tumor cells for all wide-spectrum cytokeratins (CKs), CK 8, 13, and 19 but were negative for CK 10 and 20. For Anavi et al⁷ the tumor cells were positive for cytokeratin cocktail only. For Mesquita et al¹⁶ all epithelial cells (polyhedral and clear) strongly expressed CK AE1/AE3 and CK14. In the present case, the immunohistochemical findings

were similar to those other cases published in the literature.^{3,9,14} Our analysis showed that all cells (polyhedral and clear) strongly expressed CK AE1/ AE3, CK7, and CK14 but were immunonegatives for S-100, muscle specific actin, desmin, and anti-human melanosome. The stromal component, however, was positive for vimentin.

Although considered benign in nature, CCCEOT is designed as locally aggressive for some investigators because its moderate recurrence rate. Some authors report that the presence of clear cells may indicate increased tumor aggressiveness.^{7,13,17-19} Anavi et al⁷ described the clinical and radiographic features of CCCEOT and compared them with those reported for CEOT without clear cell component, and concluded that there is evidence to support the classification of CCCEOT as a distinct, more aggressive variant of CEOT but not as a separate entity. This assumption may have been influenced by described cases of clear cell odontogenic tumor with local bone invasion and metastatic spread. In addition, Veness et al²⁰ described initially a case of CEOT that recurred twice after excision, with a progressive increase in the cellularity, loss of differentiation, and presence of vascular and lymph node metastasis, suggesting a malignant transformation process. Kumar et al¹⁵ reported a case with initial diagnosis of CEOT that showed posterior widespread infiltration, necrosis, and metastatic lesion in vertebrae and hip. Final diagnosis was metastasizing clear cell odontogenic carcinoma. Therefore, it is unknown whether this is attributable to sampling error, or to clear cell change predominating during the natural history of the tumor.

Treatment of CCCEOT involves surgery; enucleation of involved tooth, or "in bloc resection" in some cases including any associated soft tissue mass. Tumor-free surgical margins should be obtained to reduce the risk of local recurrences. Analyzing the literature, we observed that the recurrent cases of CCCEOT occurred probably because of inadequate treatment.^{9,13} On the other hand, the follow-up of some CCCEOT cases is short, not permitting concise conclusions of biological behavior of these tumors. The clinical course of the present case did not show recurrence after 24 months. Based on our findings and the literature review, we can state that CCCEOT is not locally aggressive as mentioned previously because the cases reported have generally shown little evidence of aggressiveness even years after relatively conservative treatment. Moreover, Hicks et al²¹ reported recurrence rates of 14% for CEOT and our literature review (n = 19) has detected 10.5% for CCCEOT. Further CCCEOT cases should be reported to increase our knowledge about this rare lesion and to confirm our findings.

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