Calcifying Odontogenic Cyst: A Clinicopathologic Study of 57 Cases With Immunohistochemical Evaluation for Cytokeratin

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Purpose: A clinicopathologic study of all cases accessioned as calcifying odontogenic cyst (COC) from 1971 to 1996 from the files of the Oral Pathology Laboratory at Temple University School of Medicine was undertaken.

Materials and Methods: Microscopic slides and clinical histories of cases diagnosed as calcifying odontogenic cyst were reviewed and analyzed. Ten cases were processed for cytokeratin immunohistochemical staining.

Results: Fifty-seven cases were reviewed, 28 males and 29 females. Patients' ages ranged from 7 to 83 years, with a mean age of 49.8 years. Thirty-four cases involved the mandible and 23 were from the maxilla. Seventeen were found both centrally and peripherally. The most common clinical complaint for peripheral lesions was a nodular growth on the gingiva. Although lining epithelial cells were strongly positive for cytokeratin, full-blown ghost cells and disintegrating ghost cells were nonreactive.

Conclusion: Calcifying odontogenic cyst can occur in any age-group, intraosseously or extraosseously, and as a solid lesion. No recurrences were found after surgical removal in the current series.

The calcifying odontogenic cyst (COC), described separately by Gorlin et al in 1962 and by Gold in 1963, is derived from odontogenic epithelial remnants within the mandible or maxilla, or from the gingiva. Gold originally termed the lesion keratinizing and calcifying odontogenic cyst (KCOC), which accurately describes the histologic features of most cases, but the World Health Organization and most contemporary publications prefer use of COC as the accepted term. Most COCs occur centrally within the jawbones, but peripheral lesions of the gingiva or edentulous alveolar ridge are reported in 17% to 20% of cases. The unique histopathologic features include a fibrous capsule and a lining of odontogenic epithelium. The basal cells of this lining epithelium vary from cuboidal to columnar and resemble ameloblasts. A layer of loosely arranged epithelium, which suggests stellate reticulum, overlies the more basal epithelial cells. The defining microscopic feature of this lesion is the presence of variable numbers of altered epithelial cells without nuclei. They tend to be lightly eosinophilic and retain the basic cell outline, and have been termed "ghost cells." Gorlin et al suggested that this feature was a possible analog to the cutaneous...
calcifying epithelioma of Malherbe. Individual ghost cells may undergo calcification, or lose cell outline and form sheetlike areas of calcified keratin.\textsuperscript{1,2} Hyalinized areas that suggest immature or dysplastic dentin have been reported adjacent to the cystic epithelium.\textsuperscript{5} The cystic epithelium reacts positively to monoclonal and polyclonal antikeratin antibodies, but the ghost cells have been reported as nonreactive.\textsuperscript{8,9}

The purpose of this study was to do a clinicopathologic evaluation of a series of COCs and to perform an immunohistochemical evaluation for cytokeratin in a selected number of cases using monoclonal antibodies and the standard avidin-biotin peroxidase method.

**Materials and Methods**

Cases diagnosed as calcifying odontogenic cyst were retrieved from the files of Temple University's School of Medicine Oral Pathology Laboratory. The period encompassed January 1971 to May 1996. Clinical histories were reviewed, and data including age, sex, anatomic site, radiographic description, and recurrent status were recorded.

Microscopic slides of all cases were reviewed to confirm the diagnosis. Ten cases with sufficient tissue in the paraffin blocks were selected for immunohistochemical staining of cytokeratin using a monoclonal anti-epithelial keratin mix, AE1/AE3 (Immu-Mark Anti-Cytokeratin Universal Kit, ICN Pharmaceuticals Inc, Costa Mesa, CA). Representative tissue samples were deparaffinized and processed following the manufacturer's instructions.

**Results**

Fifty-seven cases were identified and retrieved. Analysis showed 28 were from males and 29 from females. The ages of the patients ranged from 7 to 83 years, with a mean age of 49.8 years. Thirty-four cases involved the mandible, and 23 cases were from the maxilla. Seventeen cases were reported in peripheral locations, whereas 38 occurred centrally within the jawbones. Two cases were found both centrally and peripherally. Thirty cases occurred in the anterior segments of the jaws, and 27 were reported in posterior (premolar, molar) segments.

The radiographic appearance, as reported by the contributing surgeons, was primarily that of a discrete radiolucency (29 cases). In six cases, the surgeon described the lesion as radiopaque, and in three cases, the lesions were described as mixed. In 19 cases, no radiographic information was provided. An association with an unerupted tooth was reported in five cases, one case was associated with a supernumerary tooth, two cases occurred at the apex of a nonvital tooth, and one case was located at the apex of a vital tooth. Root resorption was reported in one case.

The most common clinical sign reported was a raised or expansive process (27 cases); in 14 cases, pain was reported as a symptom, in 43 cases, lesions were reported as asymptomatic.

No recurrences after surgical removal were reported.

**Histology**

All cases of COC were lined by odontogenic epithelium. The basal cells were cuboidal or columnar, and in many cases resembled ameloblasts (Figs 1, 2). Cells above the basal layer were loosely arranged and resembled the stellate reticulum of an enamel organ. Toward the lumen, epithelial cells appeared circular, enlarged, and lightly eosinophilic. The nuclei had disintegrated and disappeared. These large ballooning cells, so-called ghost cells, occluded the cyst lumen and spilled into the connective tissue wall in many cases. A hyalinized, eosinophilic, and partially mineralized material was occasionally seen in the connective tissue adjacent to the epithelium. Ghost cells showed varying degrees of dystrophic calcification. In six cases that were reported as radiopaque lesions massive calcification was observed.

**Immunohistochemistry**

Immunohistochemical staining with AE1/AE3 mix showed strong staining for lining epithelial cells and cells that resembled stellate reticulum (Figs 3, 4). The ballooning, degenerating ghost cells were only faintly stained or not stained at all. The hyalinized material showed no reaction product to the epithelial antibodies.

**Discussion**

The COC is an odontogenic lesion that has been characterized as a discrete entity since 1962.\textsuperscript{1,2} It shares features with other entities, including the calcifying epithelioma of Malherbe, calcifying epithelial odontogenic tumor, craniopharyngioma, adenomatoid odontogenic tumor, and ameloblastoma.\textsuperscript{1,10} Our results confirm the data reported in the literature that the COC occurs equally in men and women. Most reported COCs occur in the maxilla, and 15% to 20% are reported to be extraosseous. Our data showed a predilection for the mandible (60%); 30% occurred in peripheral locations. Radiographically, COC is described as a well-circumscribed radiolucency, although variations do occur.\textsuperscript{4,6,7,11} Calcified material may be present, ranging from tiny flecks to large radiopaque masses.\textsuperscript{4,6,7,11} In about half of the cases, patients experience alveolar bone expansion.\textsuperscript{4,6,7,11} Extraosseous lesions are typically sessile or pedunculated.\textsuperscript{5}
About 65% of COCs are reported to occur in the incisor/canine region, and approximately 20% are associated with an odontoma.4,6,7,11 About 53% of COC in our series occurred in the anterior segments of the jaws. The mean age of patients with COC is 33 years, with most cases occurring between the second and third decades.4,6,7,11 However, the mean age of patients in the current study was 50 years. When associated with an odontoma, the mean age of occurrence is 17 years.4,6 Although there are reported differences in occurrence when comparing cases arising earlier rather than later in life, there did not appear to be any differences in distribution of lesions in terms of sex or location relative to age in this study. The treatment of choice is surgical excision.4,6,7,11 Recurrences, although reported, are rare.4,6,11 Our 57 cases experienced no recurrence.

Pan-cytokeratin (AE1/AE3) immunostaining showed strong reactivity in the cyst lining epithelium, but only faint or no reactivity in ghost cells and hyalinized areas. Similar findings that ghost cells were slightly or nonreactive with monoclonal and polyclonal antibodies against keratins have been reported.4,8,9 However, with the conventional Rhodamine B method, ghost cells and orthokeratinized cells of oral epithelium are strongly stained for keratin.8 Apparently keratin filaments in ghost cells have lost their reactivity for anti-keratin antibodies. This phenomenon is similar to what occurs in the hornified cells in oral stratified squamous epithelium.8,9 In the hornified cells of oral epithelium,
there is a dense packing of keratin filaments and cross-linking of sulfhydryl groups into disulfide bonds. Ultrastructural study of COCs also showed gradual dense packing of keratin filaments in ghost cells and hyalinized areas. The antigenicity of keratin filaments is probably lost in this packing and cross-linking process.

Ghost cells have been considered to be the product of aberrant keratinization of the lining epithelium of COC. Ultrastructural study shows large bundles of keratin filaments and gradual packing of filaments into a homogeneous mass. This is similar to the keratinization process in oral epithelium, in which there also is formation of a thickened cell envelope in hornified cells. However, most ghost cells do not show a thickened cell membrane. Thinning of the cell membrane in hornified cells results from accumulation of involucrin proteins on the inner aspect of the cell membrane. Immunostains for involucrin in COC have shown that only a limited number of ghost cells are positive. Therefore, the aberrant keratinization of ghost cells occurs in the absence of a thickened cell membrane produced by involucrin. The thickened cell membrane in hornified cells of stratified epithelia acts as a permeability barrier. Lack of such a barrier in degenerating keratinizing cells would allow an influx of calcium ions and water to cause cellular swelling. The ghost cells in COC are prominently swollen and often become large, spherical bodies.

Ultrastructural study has shown mitochondrial and endoplasmic reticulum residues scattered among keratin filaments in ghost cells. Degeneration of these membranous structures liberates free fatty acids that attract calcium ions. It seems likely that ghost cells become calcified by this mechanism, truly a dystrophic calcification. A similar calcification process occurs in hyalinized areas of COC, where ghost cells with their cell membranes broken are packed and fused together. Although collagen fibrils have been observed in areas adjacent to hyalinized keratin masses and sometimes among keratin filaments, dentin or dentinlike material could not be confirmed ultrastructurally.

Several authors have devised classifications based on the cystic or solid histomorphology of COC, and have suggested that the solid variant may be neoplastic. In some of our cases, a cystic lumen was evident, whereas in others the lumen was occluded by confluent masses of ghost cells and hyalinized material. The "solid" variant of COC seems to represent the ultimate phase of evolution of the COC and not necessarily a separate entity. Ghost cell masses also can be seen in the connective tissue wall of the COC. This happens when the lining epithelium has been disrupted by large numbers of swollen ghost cells. In time, fibroblasts adjacent to ghost cells proliferate and produce collagen fibrils that mingle with the keratin filaments to form hyaline masses. Because recurrence is uncommon, there seems to be no clinical justification for subclassifying these lesions.

It seems unwarranted to name the solid lesions as "dentinogenic ghost cell tumors." First, there is no ultrastructural evidence that the hyalinized material is dentin. The so-called dentinoid material has been reported associated with multinucleated giant cells, indicating the hyalinized material can induce a foreign body reaction. Second, ghost cells are dead or dying cells, not neoplastic cells. It has been shown that ghost cells may occur in odontomas, ameloblastomas, and craniopharyngiomas. In lesions that are indisputably neoplastic, it seems more logical to term them as ameloblastomas with COC (or a ghost cell component). Hong et al have used the term ameloblastoma ex COC. References