Keratocystic odontogenic tumor: A retrospective analysis of genetic, immunohistochemical and therapeutic features. Proposal of a multicenter clinical survey tool

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Objective. In 2005, the World Health Organization reclassified the parakeratinizing odontogenic keratocyst as a neoplasm. This article reviews the research leading to this reclassification, and validates a new survey tool that can be easily used to pool surgical and recurrence data from multiple offices.

Study design. All odontogenic lesions accessioned in the Iowa Surgical Oral Pathology Laboratory between 1949 and 2010 were identified from the database. A survey tool to assess treatment and follow-up was created. A total of 46 surgeons agreed to participate.

Results. A total of 70 keratocystic odontogenic tumors (KOTs) had documented recurrences at follow-up intervals ranging from 6 months to 5 years. Primary tumors that recurred ranged in size as measured by greatest radiographic diameter from 0.7 to 6 cm.

Conclusions. This survey tool is recommended as standard allowing treatment of cases by multiple practitioners to be compared retrospectively or prospectively. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:75-83)

Keratocystic odontogenic tumors (KOTs) were first described by Philipsen in 1956. Over 50 years later the debate continues as to their proper classification, behavior, and recommended therapeutic management.1-4

Until 2005, the lesion was classified as a developmental odontogenic cyst that had an unusually high recurrence rate following simple enucleation when compared to other odontogenic cysts and most odontogenic neoplasms. In 2005, following a series of publications highlighting specific genetic mutations and noting the immunohistochemical similarity of this lesion to a variety of odontogenic neoplasms, the World Health Organization (WHO) reclassified the lesion as a benign cystic neoplasm.5,6

This article reviews the research leading to this reclassification, and introduces a new, standardized survey tool that can be easily used to pool surgical and recurrence data from multiple offices. Because a majority of these tumors are managed in private oral and maxillofacial surgery offices, rather than in large teaching hospitals, prospective multicenter studies regarding therapeutic management and long-term follow-up of large numbers of cases cannot be easily accomplished at single treatment centers. Based on the number of offices that chose to participate in this initial study, this survey tool seems to be an acceptable tool that can be used by researchers wishing to collect treatment and follow-up data over time.

CLINICAL FEATURES

A majority of KOTs occur in males, with many studies suggesting a ratio of about 2:1. Most tumors are first diagnosed in the second, third, and fourth decade of life.7 As with all odontogenic lesions, KOTs can occur in any tooth-bearing site. However, the mandible is the site of occurrence of most KOTs with approximately 75% occurring in the posterior body. Tumors originating in the maxilla can expand superiorly to involve the sinuses, nasal antrum, and even the floor of the orbit.

Similar to other benign tumors, KOTs seldom cause symptoms unless secondarily inflamed. Clinical signs usually include a uniform expansion of the buccal cortical plate of the mandible, or the buccal or palatal alveolus of the maxilla. Crepitus may be evident on palpation.

Statement of Clinical Relevance

Genetic and immunohistochemical features of keratocystic odontogenic tumors have resulted in this lesion being reclassified by the World Health Organization in 2005, and may hold the key to the relatively high recurrence rates following conservative excision when compared to most other odontogenic tumors. This article reviews those features and attempts to retrospectively analyze therapeutic management and follow-up at multiple independent oral surgery offices using a new standardized tool.
If multiple odontogenic cysts are present or develop sequentially the consideration of basal cell nevus (Gorlin, Gorlin—Goltz) syndrome must be considered.

**RADIOGRAPHIC FINDINGS**

KOTs appear radiographically as well-demarcated radiolucencies with thin, well-defined, corticated borders (Figure 1). Despite its reported aggressive nature, the tumor is slowly growing and usually displaces normal anatomy, including teeth. Resorption of tooth structure can happen but does not seem to be more frequent than with other odontogenic tumors or cysts. The radiolucency may appear multilocular depending primarily on the size of the lesion. In a recent study, KOTs larger than 31 mm diameter were more likely to appear as multilocular lesions radiographically.7 Computed tomography (CT) scans are often valuable in assessing these lesions in 3 dimensions prior to surgical management.8

**GROSS FINDINGS**

KOTs at the time of surgery and on the grossing table present as tissue-paper thin strips and sheets of soft tissue. The lining epithelium can separate from the connective tissue and will often appear as a translucent membrane. The fixative will often contain varying amounts of amorphous “cheese-like” material, which if processed can be identified as parakeratin.

**MICROSCOPIC FINDINGS**

Classic microscopic features include a relatively uniform layer of stratified squamous epithelium 6-10 cells in thickness without rete ridge formation (Figures 2 and 3). With hematoxylin-eosin staining, the parakeratinized epithelium will appear pink and is often corrugated or wavy. Focal variations can include lack of corrugation or minimal keratinization (Figure 4). The histopathologic feature that is most important for the diagnosis is the uniform, picket-fence-like basal cell layer with ovoid, hyperchromatic nuclei often polarized away from the basement membrane. The underlying fibrovascular connective tissue can contain varying number of epithelial rests or small microcysts (Figure 5).

Secondary inflammation, which can occur with many odontogenic tumors and cysts, including KOTs, will substantially alter the microscopic features (Figure 5). Acute or chronic inflammation in the connective tissues directly subjacent to the KOT lining will often result in loss of keratinization, increases and decreases in thickness of the epithelium, and loss of the uniform basal cell layer. Additionally, tumor tissue removed following marsupialization can be substantially altered, showing increased thickness and the formation of epithelial tufts and whorls within the stratum spinosum (Figure 6).

**TUMOR SUPPRESSOR GENE STUDIES AND IMMUNOHISTOCHEMISTRY**

Parakeratinizing odontogenic keratocysts were reclassified by the WHO in 2005 as KOTs, in recognition of genetic and downstream immunohistochemical features which were similar to those noted in other benign neoplasms.

In 2004, Agaram et al. examined 10 odontogenic keratocysts for loss of heterozygosity of 10 common tumor suppressor genes as well as the *PTCH* gene.5 Loss of heterozygosity was seen in 7 of 10 cases, with a frequency between 11% and 80% of genes studied. The genes that exhibited the most frequent allelic losses were *p16*, *PTCH*, and *MCC*. The finding of clonal deletion mutations of genomic DNA supported the hypothesis that these cystic lesions were neoplastic.

As early as 1989, Scharffetter et al. documented increased mitotic activity within the epithelial lining of KOTs, the initial building-block for its eventual reclassification by the WHO as a cystic neoplasm.9 During the following 15 years, a variety of immunohistochemical studies suggested that the cystic epithelium of KOTs had many similarities with other benign odontogenic neoplasms and suggested possible reasons for the observation that these tumors exhibit a relatively high recurrence rate.4,10

The epithelial proliferation and apoptotic index of both sporadic and syndromic KOTs in basal cell nevus syndrome have been shown to be similar to ameloblastomas and higher than dentigerous cyst epithelium.12,13 Survivin, an inhibitor of apoptosis, has been shown to be expressed in KOTs but not in...
nonkeratinizing periapical cysts. Keratinocyte growth factor and receptor expression has been identified more often in KOT epithelium compared to dentigerous cysts, and the intensity of growth factor staining is significantly reduced following marsupialization.

Bax and bcl-2 are two important anti-apoptotic and pro-apoptotic factors of the bcl-2 family. Immunoreactivity for bcl-2 is detected in the basal layer of KOTs while orthokeratinizing cysts are negative. KOTs have shown decreased expression of cell adhesion proteins β-catenin and E-cadherin and differences of Wnt-1 and Wnt-10A signaling when compared to dentigerous cysts.

Aberrant sonic hedgehog signaling proteins, which are critical to tissue development, have been identified in KOTs. Epithelial expression of the hedgehog transcriptional effector Gli2 has also been identified in the development of laboratory KOTs from rests of Malassez in transgenic mice.

An evaluation of common tumor suppressor genes has concluded that clonal deletion mutations of genomic DNA suggest a neoplastic rather than cystic origin. Proliferating cell nuclear antigen (PCNA), cell proliferation markers (Ki-67), and tumor suppressor protein (p53) occur more frequently and more intensely in KOTs, compared with the other odontogenic tumors and cysts.

Gadbad et al. have recently suggested that Ki-67 and p53 protein quantitative and qualitative expression can be used as a prognostic marker of aggressive behavior in odontogenic lesions including KOTs. High expression of Ki-67 and expression of p53 have also been identified more often in tumors that recurred.

Vascular endothelial growth factor (VEGF) has been shown to be more prominent in KOTs compared to

Fig. 2. KOT (low power). The specimen consists of a thin fibrovascular connective tissue surrounding a lumen lined by a uniformly thin epithelium with no evidence of rete ridge formation. Keratin partially fills the lumen (hematoxylin-eosin stain, original magnification ×100).

Fig. 3. KOT (high power). The epithelial lining is of uniform thickness with evidence of parakeratin formation, and a prominent, uniform basal cell layer (hematoxylin-eosin stain, original magnification ×400).

Fig. 4. KOT variations. The epithelium within the same tumor can show marked corrugation (upper left) of the parakeratinized surface, minimal corrugation (middle), or little evidence of parakeratin (lower right). All variants exhibit the prominent basal cell layer, which is necessary for the diagnosis (hematoxylin-eosin stain, original magnification ×400).
dentigerous cysts. Use of CD34 to evaluate angiogenesis has shown microvessel density to be significantly higher in KOTs compared to dentigerous cysts, although the densities of both were lower than ameloblastoma. Seif et al. showed a statistically significant difference in microvessel density in ameloblastomas and KOTs when compared to follicular cysts.

Podoplanin, a lymphatic endothelial marker, is highly expressed in ameloblastomas and also in the cytoplasm of most of the basal and parabasal cells and peripheral cells of daughter cysts in the stromal connective tissues of KOTs. Podoplanin expression is absent or weakly positive in orthokeratinizing cysts, suggesting a relationship to proliferative activity and recurrence rates.

Osteopontin, an extracellular protein important in bone remodeling, and often found in malignant epithelial tumors, is important in bone metastasis through a process of osteoclast activation. Cytoplasmic osteopontin was identified in the epithelial cells of 8 of 20 KOTs, whereas no evidence of staining was identified in dentigerous or radicular cysts.

Tsuneki et al. evaluated for the presence of keratins 10 and 17, perlecan, PCNA, and UEA-I lectin in a variety of cystic jaw lesions. Keratin 10 was positive in KOTs and dentigerous cysts. Perlecan was found in unicystic ameloblastomas, KOTs, and lateral periodontal cysts. PCNA+ cells were found frequently in unicystic ameloblastomas and KOTs.

Senguven and Oygur have suggested that the expression rates of cytokines interleukin 1α (IL-1α) and IL-6, which showed a positive correlation with tumor size in ameloblastomas, and wall thickness in KOTs may play a role in the aggressive behavior of these tumors by facilitating bone resorption. By polymerase chain reaction, Wang and Li suggested that increased COX-2 and VEGF expression may be responsible for the increased osteoclastogenic effects of KOT fibroblasts.

Collagen IV, matrix metalloproteinase (MMP) 9, and tissue inhibitor of MMP 2 may be important factors for the establishment of differences in the biologic behavior of dentigerous cysts, radicular cysts, KOTs, and ameloblastomas. Most dentigerous and radicular cysts showed a predominance of continuous staining for collagen IV in the basement membrane of the epithelium, whereas discontinuous staining was observed more frequently in KOTs and ameloblastomas. MMP-9 was identified in epithelial and mesenchymal cells of all the lesions analyzed, but the staining percentage was higher in the epithelium and mesenchyme of KOTs and ameloblastomas.

REVIEW OF TREATMENT FOR KOT’S
Initial attempts to explain the recurrence rates, estimated to be as high as 60% following simple enucleation, included a variety of microscopic and clinic features including the presence of dental lamina rests or satellite cyst formation in the cyst walls, surgical difficulty in identification and removal of the paper-thin epithelial lining, collagenase activity in the fibrovascular connective tissue subjacent to the epithelium, or prostaglandin-induced bone resorption.

In a recent series of 120 cases of KOTs treated with simple enucleation, 28 tumors (26%) recurred. Average follow-up was 86 months with a range of 18-151. There was no correlation between tumor
site, cortical perforation or radiographic features. In another series of 32 KOTs treated with enucleation, 4 recurred during follow-up ranging from 1 to 114 months.27

Because simple enucleation is often deemed inadequate, some reports have claimed success with decompression and subsequent enucleation, whereas others advocate enucleation and excision of overlying mucosa, peripheral osteotomy, and chemical curettage.42-47

Carnoy’s solution is a volatile pharmacologic compound that varies in formulation somewhat from institution to institution. The solution usually consists of 1 g of ferric chloride (FeCl₃) dissolved in 24 mL of absolute ethanol, with 12 mL of chloroform and 4 mL of glacial acetic acid.

Complementary treatment with Carnoy’s solution and peripheral ostectomy has been advocated as an effective treatment for KOTs. An evaluation of 22 cases treated in this manner and followed for 12-78 months reported a recurrence rate of only 4.5%. Complications included wound dehiscence (22.7%), infection (4.5%), and paresthesia (18.2%). These complications were less frequent and less serious than complications associated with cryotherapy.48 Use of Carnoy’s solution on the inferior alveolar vascular—nervous plexus can be utilized as a complementary treatment for the KOTs with low and transient neural morbidity.49

In an analysis of 257 KOTs, 7.4% recurred within 6 years after initial treatment with either enucleation or a combination of enucleation and Carnoy’s solution. The recurrent KOTs were more likely to be multilocular or multifocal than the primary cases and often involved the alveolar bone around remaining teeth.50
In a review of cases published between 1999 and 2010, Johnson et al. found that cases treated with enucleation and enucleation with adjunctive measures other than Carnoy’s solution had recurrence rates of 25.6% and 30.3%, respectively. Marsupialization with adjunctive measures produced a recurrence rate of 15.8%, whereas enucleation with Carnoy’s solution presented a recurrence rate of only 7.9%.51

Marsupialization of large lesions has been shown to decrease tumor size by 47% making follow-up enucleation much simpler.52 Using CT 3-dimensional reconstruction data from 15 patients, the volume of marsupialized KOTs was reduced by half in an average of about 8 months.53 This approach allows for a less invasive approach with excellent results, avoiding extensive disfiguring procedures.53 Treatment of 3 patients with Gorlin–Goltz syndrome, using marsupialization for 10 months, followed by enucleation with peripheral ostectomy resulted in no recurrences during a 5 year follow-up.

In a systematic review of the literature regarding treatment of KOTs from 1999 to 2010, Johnson et al. found only 8 of 206 published manuscripts that met 4 inclusion criteria: (1) tumors were diagnosed by histopathologic evaluation; (2) the patient selection process was described and consisted of consecutive patients; (3) cases had an adequate period of follow-up; (4) treatment was rendered in specific detail to repeat the procedure and each treatment was correlated with recurrence rate.51 This report brings into focus the substantial lack of uniformity in reporting treatment modalities for KOTs and other odontogenic lesions.

The literature has abundant case reports and short series describing both peripheral and central odontogenic tumor recurrences. Due to the fact that many of these lesions are treated in the private clinics of oral and maxillofacial surgeons, or in relatively small, regional hospitals, data regarding treatment and follow-up for odontogenic tumors in general are lacking when compared to prospective, multicenter hospital-based clinical trials for many other tumors, both benign and malignant. Too often, as noted by Johnson et al., a lack of standardization makes meta-analyses difficult.51

**MATERIALS AND METHODS**

Our study was designed to evaluate treatment of KOTs at multiple clinical settings using a standard reporting tool (Figure 7). The clinical trial was approved by the University of Iowa IRB # 201105716. Letters were mailed to 15 oral surgery offices asking for participation. Eleven oral surgery clinics responded and received Internal Review Board approval. The laboratory database was searched for odontogenic tumors and developmental cysts submitted by practitioners in these clinics and individual forms were printed for each accession included in the study.

The individual patient information section, which included the patient name, practitioner, and diagnosis section of the clinical data information form (Figure 7), was auto-filled directly from the database. This made information gathering at each office simple, and provided uniformity of information retrieved. The contributors’ name was necessary because many oral surgery offices had multiple practitioners. A total of 11 offices with 46 oral surgeons participated in the study.

**RESULTS**

The Surgical Oral and Maxillofacial Pathology Laboratory at the University of Iowa has been in continuous operation since 1949 and through December 2010 recorded 100,804 accessions. If odontomas are excluded, the total number of central bony odontogenic neoplasms, as defined by the 2005 WHO classification, system totaled 1133. These are outlined in Table 1.

Central bony odontogenic neoplasms comprised 1.12% of the total accessions. Not included in this number are 483 complex and compound odontomas, which are considered by many to be hamartomas. Also not included in this total were a variety of peripheral odontogenic neoplasms including 165 peripheral odontogenic fibromas, 9 peripheral KOTs, and 3 peripheral ameloblastomas. A total of 439 peripheral giant cell granulomas, and 792 peripheral ossifying fibromas, both of which are considered by most to be reactive lesions, were also identified.

**RETROSPECTIVE ANALYSIS OF TREATMENT FOR KOTS**

KOT comprised 896 central bony tumors, far out-distancing all other odontogenic neoplasms combined. A total of 763 individual KOT case forms were mailed to participating oral and maxillofacial surgery offices. Of these, 446 had surgical management and follow-up information.

The tumors were identified in 474 males and 390 females, a ratio of 1.2:1. In 32 accessions, the sex was

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**Table 1.** Summary of total number of central bony odontogenic neoplasms

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenomatoid odontogenic tumor</td>
<td>21</td>
</tr>
<tr>
<td>Ameloblastic fibroma</td>
<td>18</td>
</tr>
<tr>
<td>Ameloblastic fibro-odontoma</td>
<td>43</td>
</tr>
<tr>
<td>Ameloblastoma</td>
<td>106</td>
</tr>
<tr>
<td>Calcifying epithelial odontogenic tumor</td>
<td>02</td>
</tr>
<tr>
<td>Calcifying cystic odontogenic tumor</td>
<td>15</td>
</tr>
<tr>
<td>Central odontogenic fibroma</td>
<td>23</td>
</tr>
<tr>
<td>KOT (OKC)</td>
<td>806</td>
</tr>
<tr>
<td>Odontogenic myxoma</td>
<td>07</td>
</tr>
<tr>
<td>Squamous odontogenic tumor</td>
<td>02</td>
</tr>
<tr>
<td>Total</td>
<td>1133</td>
</tr>
</tbody>
</table>

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not identified. A total of 539 were from the mandible and 222 from the maxilla, a ratio of 2.4:1. In 135 cases, the location was not stated. A total of 8 tumors were removed from patients with documented Gorlin–Goltz syndrome.

A total of 70 KOTs had documented recurrences at follow-up intervals ranging from 6 months to 5 years. A total of 253 had follow up with no recurrence, and 123 were unknown.

Recurrence by location of the primary tumor, when reported, was posterior mandible 29, posterior maxilla 6, anterior maxilla 5, and anterior mandible 5. Primary tumors that recurred ranged in size as measured by greatest radiographic diameter from 0.7 to 6 cm. Excluding known syndromic KOTs, the average age of the patients for primary tumors that recurred was 41 years with a range from 7 to 74 years.

Most of the documented recurrences (73%) were identified during the first 24 months following initial treatment. The recurrence rate for cases treated with simple curettage was 32%. For tumors treated with excision followed by mechanical or chemical curettage, the recurrence rate was 15%. For tumors that recurred, the primary tumor size, measured by greatest diameter as reported by the clinician, was on average 3.3 cm, whereas overall primary tumor size for all KOTs was 2.9 cm.

**DISCUSSION**

All benign odontogenic neoplasms have been reported to recur, but risk of recurrence alone should not be used as a justification for overaggressive treatment. Recurrences can be related to tumor characteristics at the cellular level, or difficulty in surgical management. The morbidity of the treatment should not exceed the expected morbidity of the tumor. Following initial surgical removal, based on the best scientific evidence available, the most important aspect to assure a successful patient outcome is regular extended follow-up to assure that any recurrent lesions are identified and treated early.

Because tumors that subsequently recurred were larger than those that did not and because larger tumors are more often multiloculated, difficulty accessing the entire tumor may have some effect on recurrence rates.

Regardless of the initial treatment provided, patients treated for KOTs need to be followed radiographically for a minimum of 10 years. Clinical work-up to assess for basal cell nevus syndrome is always prudent when KOTs are diagnosed in patients under 20 years of age, or multiple concurrent or sequential OKCs are identified. In addition, clinicians need to be reminded that a variety of jaw cysts including radicular, dentigerous, lateral periodontal, and glandular odontogenic cysts are clinically and radiographically indistinguishable from cystic neoplasms including KOTs and unicystic ameloblastomas. These can only be diagnosed microscopically, making it imperative that all jaw cysts be submitted for microscopic examination.

The current survey tool is promoted as an attempt to standardize reporting of surgical management for tumors of the jaws so that prospective and retrospective analyses from multiple surgical offices and institutions can be compared, thereby giving rise to more reliable data. Based on responses from 11 offices and 40 practitioners, the form is easy to understand and takes very little office time to complete.

**REFERENCES**


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