Analysis of the Neoplastic Nature of Odontogenic Keratocyst and its Comparison with other Selected Benign Odontogenic Tumours: An Immunohistochemical Analysis
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ABSTRACT
Odontogenic keratocyst (OKC) first described by Philipsen in 1956 constitutes approximately 11% of all cysts of the jaws. Adenomatoid odontogenic tumour (AOT) is an uncommon, benign epithelial lesion of odontogenic origin. The aim of this study was to analyse the expression of Bcl-2 in OKC and its comparison with other selected benign odontogenic tumours (OTs). Ten formalin fixed paraffin embedded blocks of OKCs, five each of AOT and unicystic ameloblastoma Bcl-2 protein is characterized by its ability to inhibit apoptosis. OKC were characterized by higher expression of Bcl-2 in basal cell epithelium. AOT and unicystic ameloblastoma differed from OKC in a wide spectrum of apoptosis and/or cell cycle-related protein expressions, higher proliferation in the basal cell layer, and vice versa, lower proliferation in the suprabasal cell layer. The solitary OKC seems to be less biologically aggressive and should be classified as a cyst rather than a tumour, means that at least few of OKCs manifests as ordinary cysts. Some of the present study findings could support the theory that OKCs are with high proliferative, probably that these lesions are developmental cysts with some neoplastic properties because of the high intrinsic growth potential. WHO recommends the term KCOT as it better reflects the neoplastic nature of the lesion; however, this reclassification has not yet been universally accepted.

Keywords: Adenomatoid odontogenic tumour, Apoptosis, Bcl-2 immunohistochemical analysis, Neoplastic, Odontogenic keratocyst, Unicystic ameloblastoma

INTRODUCTION
First described by Philipsen in 1956, the OKC constitutes approximately 11% of all cysts of the jaws. It is an aggressive cystic lesion with a predilection for recurrence and the recurrence rate is higher than that of other odontogenic cysts. In 1967, Toller suggested that the OKC should best be regarded as a benign cystic neoplasm rather than simply as an odontogenic cyst. In the years since, numerous reports influenced the World Health Organization (WHO) to reclassify this lesion in 2005 as a keratocystic odontogenic tumour (KCOT), and define it as a benign unilocular or multilocular intraosseous tumour of odontogenic origin. KCOT is an old entity with a new name[1]. WHO recommends the term KCOT as it better reflects the neoplastic nature of the lesion; however, this reclassification has not yet been universally accepted. This OT is characterized by proliferation of odontogenic epithelium with a typical corrugated parakeratin layer and palisading of the basal cells. KCOT exhibits a ‘propensity to grow along the internal aspect of the jaws, causing minimal expansion’. Additionally, KCOTs associated with neviod basal cell carcinoma syndrome (NBCCS) occur earlier and exhibit a greater tendency to recur than non-syndromic KCOTs[2].

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The reported frequency of recurrence in various studies ranges from 5 to 60%. Some mechanisms have been suggested to explain its behaviour and high tendency for recurrence, such as the difficulty to remove it in one piece due to the thin, friable nature of the cyst wall, satellite small daughter cysts, production of bone resorptive factors in the cyst wall and increased proliferation of the epithelial lining of the cyst[3].

Orthokeratinized odontogenic cyst (OOC) is a relatively uncommon developmental cyst comprising about 10% of cases that had been previously coded as OKCs. The lesion has been termed variously as an ‘orthokeratinized variant of OKC’ or a ‘jaw cyst with orthokeratinization’. Suggested a descriptive term ‘Orthokeratinized Odontogenic cyst’, which also reflected its most plausible histogenic origin, OOC should not be part of the spectrum of KCOT and should be distinguished from the latter[4].

Unicystic ameloblastoma, a variant of ameloblastoma first described by Robinson and Martinez in 1977, refers to those cystic lesions that show clinical and radiologic characteristics of an odontogenic cyst but in histologic examination show a typical ameloblastomatous epithelium lining part of the cyst cavity, with or without luminal and/or mural tumour proliferation[5,6].

AOT is an uncommon, benign epithelial lesion of odontogenic origin. Described for the first time by Dreiblat in 1907 as an adenoameloblastoma, and among others has also been named ameloblastic adenomatoid tumour. In 1969 Philipsen and Birn proposed the term AOT, indicating that it did not constitute a variety of ameloblastoma, and was accepted as such in the first WHO classification of OTs established in 1971. The term AOT is without doubt the most appropriate, in that these tumours are clearly benign and, in contrast to the ameloblastoma, present a very low recurrence[6].

Bcl-2, among all proto-oncogenes, located at chromosome 18q21, is characteristically able to stop programmed cell death (apoptosis) without promoting cell proliferation. Its gene product, the Bcl-2 protein, acts as a cell death suppressor that facilitates cell survival by regulating apoptosis. Investigations on the immunoreactivities of Bcl-2 protein have been demonstrated in tooth germs, ameloblastomas, KCOTs and dentigerous cysts. TUNEL-positive cells have been detected exclusively in the surface layer of KCOTs, indicating marked levels of apoptosis. Thus, Bcl-2 inhibits apoptosis to facilitate cellular proliferation in the basal and suprabasal layers, whereas apoptosis maintains the homeostasis of the thickness of the lining epithelium and allows the synthesis of large amounts of keratin in the surface layer of KCOTs[7].

The Bcl-2 family is recognized as a cell division regulator, which can inhibit apoptosis and produce extended cell survival[8].

The aim of the present study is to determine the neoplastic nature of OKC and compare its expression with other selected OTs.

**MATERIALS AND METHODS**

Ten formalin fixed paraffin embedded blocks of OKCs, five each of AOT and unicystic ameloblastoma were retrieved from the archive of the Department of Oral Pathology, Coorg Institute of Dental Sciences, Virajpet by random sampling. Serial sections of tissue were cut at 4 μm thickness were used for haematoxylin and eosin and immunohistochemical staining. OKC cases associated with NBCCS and orthokeratotic OKCs were excluded from the study.

**Immunohistochemistry**

Paraffin sections were subjected to immunohistochemical staining for Bcl-2, obtained from BioGenex Life Sciences Pvt Ltd, Hyderabad by using the HRP system. For Bcl-2 antigen retrieval, deparaffinised sections were micro waved in citric acid buffer (pH 6.0), at 120°C for 10 minutes. After microwave treatments, the sections were rinsed in working Tris buffer saline TBS, (1MTris pH 7.4–20 ml, sodium chloride-3.4 gms, make up to 400 ml with distilled water) and treated with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature to block endogenous peroxidase activities, and then treated with the primary antibodies for 60 minutes. After incubation, the sections were rinsed in TBS and incubated with the secondary antibodies.
(antimouse immunoglobulins) which were conjugated with peroxidase-labeled dextran polymers for 1 hour at room temperature. After rinsing with TBS, they were treated with 0.02% 3, 3-diaminobenzimine in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.005% hydrogen peroxide to visualize the reaction products finally, these sections was counterstained with hematoxylin.

For control studies on antibodies, the primary antibodies were replaced with preimmune mouse IgG subclasses.

RESULTS
All stained areas demonstrating positivity for Bcl-2 were identified at a magnification of 40X and the number of positively stained cells was counted on 10 representative areas of the epithelium using a 100X objective with a minimum of 80 cells in the epithelium. The intensity of Bcl-2 positivity was graded as described: Grade I: (-) fewer than 5% positive cells or no staining, Grade II: (+) 5% to 9% positive, Grade III: (+) 10% to 24% positive, Grade IV: (++) 25% to 50% positive and Grade V: (+++) more than 50% positive.

The immunoreactivity for Bcl-2 protein was present in most of the epithelial cells. Staining intensity for Bcl-2 in the studied OTs are shown in Table 1. Difference was observed in staining intensity among KCOT, unicystic ameloblastoma and AOT. A strong positivity for Bcl-2 was observed in 8 of 10 OKC, moderate positivity within 2 of 10 OKC and moderate positivity with all unicystic ameloblastoma and three of the five AOTs two had mild one was poorly stained for IHC with AOT.

In this study, the role of the apoptosis-related factors in lining epithelium of OKCs, unicystic ameloblastoma and AOT cells were examined.

Collected data were analyzed using the SPSS software for Windows. Data analysis was performed using Chi Square test, with the level of significance set at p < 0.05.

DISCUSSION
Different types of odontogenic cysts and tumours arise from derivates of embryologic dental lamina. The potential for further proliferation of these epithelial remnants during formation of a cyst is different and thus lead to variations in their molecular expression and biological behaviour, due to an underlying mechanism that remains highly speculative. It is presumed that epithelia of OKCs originates from the odontogenic epithelium of the dental lamina or its remnants, prior to tooth formation. However, it has also been suggested that OKCs could be derived from basal cells of the oral mucosa[1].

The KCOT exhibits locally destructive and highly recurrent behaviour; the histopathology of the KCOT reveals budding of the basal layer into the connective tissue and frequent mitotic figures; and finally, the KCOTs are associated with an inactivation of PTCH, the tumour suppressor gene. The presence of a genetic component suggests that the patient’s ethnic origin, which is ‘family history’, may have a role to play[2]. The significant differences observed between the global groups in their systematic review suggest that ethnic origin of the KCOT patient is important. It would be valuable to determine whether there are any clinical and radiological features that could suggest an increased risk of recurrence. The likelihood of recurrences and the ability to study these outcomes statistically is affected by numerous variables including subtle discrepancies in treatments, differences in the locations of the cysts, presence or absence of infection, associated teeth, involvement of mucosa, size of the lesion, and association with the Gorlin Syndrome. Because of these numerous factors, the treatment and prognosis of KCOTs must be managed in a case by case manner[9]. According to these factors may led to the recharacterization of the keratocyst as KCOT.

<table>
<thead>
<tr>
<th>Odontogenic tumour</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade IV</th>
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<tr>
<td>Adenomatoid odontogenic tumour</td>
<td>01</td>
<td>0</td>
<td>02</td>
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<td>Unicystic ameloblastoma</td>
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While the cyst is a benign neoplasm, its recurrence rate may be as high as 17–56% with simple enucleation. If an adjunctive treatment is added, such as the application of Carnoy’s solution or decompression before enucleation, the recurrence rate is reported to be between 1 and 8.7%. Finally, resection is uniformly reported to result in essentially no recurrences but may be unacceptably extensive given the benign nature of the disease[10].

The redefinition and the reclassification of KCOT modified the prevalence and frequency of distribution of OT. KCOT has a lightly male predominance, it is most frequent in the third decade of life and the mandible is the site of occurrence. This epidemiological profile of KCOT could modify the epidemiological profile of OTs, there was increase in prevalence OT in males in the period 2005-2008. If this increase is related to KCOT should be established. A research protocol designed ex-profeso will be necessary to clarify this important issue[11]. In a Chinese series, the amount of OT without KCOT was 1054 while they increased to 1642 when KCOT was included. These data mean a 55.7% increase and obtained an increase of 92% when KCOT was included as OT. These findings should be taken cautiously because it is result of a reclassification and not associated with a real increment in the cases of OT[12]. The report of an increase of 92% in a period of time of 4 years in very specific and uncommon neoplasias could be misunderstood by health carriers, specifically the managers of the preventive programmes of oral public health and to influence strategies of prevention[11].

The family of Bcl-2 proteins biologically constitutes one of the most relevant classes of apoptosis-regulatory gene products. Bcl-2 and bax are widely regarded as the most important apoptotic regulators, and their relative levels determine the fate of cells. Bcl-2 protein expression in the mitochondrial outer membrane inhibits cytochrome translocation into the cytosol, which is a critical step in the apoptotic process. On the contrary, bax is a pro-apoptotic antagonist of Bcl-2 and has been characterized as a Bcl-2 binding protein that shares significant sequence homology with Bcl-2. The excess of Bcl-2 homodimers favours cell survival, while the excess of bax homodimers favours cell death. Over expressed bax also counters the death repressor activity of Bel-2[13–15].

Vered et al. concluded that distinction between OKCs and ameloblastoma can be made from all the other cystic lesions based on the immunoprofile of SHH related factors and SHH induced Bcl-2, could provide more evidence that an OKC belongs to neoplastic group of lesions rather than to the cystic type of lesions. The activation of at least two cell cycle regulatory systems is switched on in OKCs and ameloblastomas as the lesions evolve. According to literature, other molecules with function that cause these lesions to have built in aggressive biological behaviour have been identified, such as IPO38, Cyclin D1. This may also serve as the explanation for the observation that not all OKCs demonstrate a similar pattern of clinical behaviour, and that there is a large spectrum of variations among them. Therefore, the type and the number of disturbed cell cycle molecules would more appropriately define the profile of the lesion and ultimately dictate its phenotype[16].

In the present study, a strong positivity for Bcl-2 was observed in 8 of OKC, moderate positivity with all unicystic ameloblastoma and moderate positivity with three of the AOTs, and one AOT was weakly positive,
one AOT showed negative expression. An interesting finding that has been found in our study is the varied expression of Bcl-2 in AOT. Strong positive staining in the basal and suprabasal layer of all OKC was observed. The grade V staining with OKC’s clearly suggest that they are more aggressive than AOT.

8 of 10 OKC expressing a strong positivity in Bcl-2 expression, this could support the theory that OKCs are more aggressive, but other two OKC stained with grade III intensity further means that at least few of OKC manifest as ordinary cysts/less aggressive, 8 of 10 OKC expressing a strong positivity in Bcl-2 expression may suggests that these lesions are developmental cysts with some neoplastic properties because of the high intrinsic growth potential.

High expressions of Bcl-2 in the lining epithelium of OKCs accord with their aggressive clinical behaviour. Although these results support that OKC is a neoplasm, there are not enough genetic studies. Further studies on genomic changes may help for better understanding of the pathogenesis of OKCs.

Considering that there is a regulated balance between cell proliferation, cell differentiation and cell death in this type of lesion, this may explain why KCOTs, though portraying a neoplastic behaviour, with an increase potential to proliferate, do not tend to form tumour masses.

Simple classification of OKC may be as follows:

- **Group 1:** Cyst
  - OKC
  - OOC
- **Group 2:** Syndromic OKCs
- **Group 3** Recurrent/Potential neoplastic/KCOT

OKC which recurred after the treatment could probably grouped as recurrent OKC/ KCOT or Group 3, as the term reflects the neoplastic nature of the lesion.

However, the distribution changes radically from 2005 onwards. When the KCOT was included into OT, they displaced to Odontoma to the second place and now the most frequent OT is the KCOT, the redefinition of OKC as a tumour produced an increase in the frequency and prevalence of OT[11].

In conclusion, although the present investigation is a pilot study, it is generally agreed that some features of OKCs are those of a neoplasia, notably the relatively high proliferative rate of epithelial cells, controversies over the behaviour and management of OKCs still exist. The likelihood of recurrences and the ability to study theses outcomes statistically is affected by numerous variables including: subtle discrepancies in treatments, differences in the locations of the cysts, presence or absence of infection, associated teeth, involvement of mucosa, size of the lesion, and association with the Gorlin Syndrome, WHO’s reclassification of KCOT from cyst to tumour can motivate clinicians to manage the disease in a correspondingly aggressive manner and decrease unnecessary recurrences but because of a reclassification there may lead to pseudo increment in the cases of OTs, this study has found that the few solitary OKC seems to be less biologically aggressive and should be classified as a cyst rather than a tumour few OKC may have neoplastic properties because of the high intrinsic growth potential.

Further prospective studies may provide greater insight into the prognostic implications of this intriguing entity.

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**REFERENCES**


