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# Adenomatoid odontogenic tumor - hamartoma/cyst or true neoplasm; a Bcl-2 immunohistochemical analysis

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## ABSTRACT:

**Objectives:** Adenomatoid odontogenic tumor (AOT) is an uncommon tumor of odontogenic origin, characterized histologically by the formation of ductlike structures with amyloid-like deposits. Histogenesis of AOT is still uncertain and it is often considered as a hamartomatous lesion rather than a true neoplasm. Aim of this study was to analyse the usefulness of detecting important apoptosis in assessing the biological potential of lesion and analyze the expression of Bcl-2 in AOT.

**Methods and Material:** Five formalin fixed paraffin embedded blocks of adenomatoid odontogenic tumor were included in the study and were analysed for Bcl-2 protein is characterized by its ability to inhibit apoptosis.

**Results:**The immunoreactivity for Bcl-2 protein was present in most of the epithelial cells of AOTs. Four of the five AOTs had moderate positivity; one was poorly stained for IHC.

**Conclusions:** adenomatoid odontogenic tumor behaves more aggressively than in most cases that rules out AOT is a cyst. The same activating mutation that causes adenomatoid odontogenic tumor also causes hybrid odontogenic tumors, which is a neoplasm. Thus, based on currently available evidence with the literature and the findings in the present study, it can be considered adenomatoid odontogenic tumor to be a true neoplasm rather than a hamartoma/cyst.

**Key words:** Adenomatoid odontogenic tumor, jaw swelling, Bcl-2

## Introduction

Adenomatoid odontogenic tumour was first described by Dreiblادت, in 1907, as a pseudoadenameloblastoma. In 1948 Stafne considered it a distinct entity, but it was classified by others as a variant of ameloblastoma. As a result, the lesion is known by many names, including adenoameloblastoma, adenoameloblastic odontoma, epithelial tumour associated with developmental cysts, ameloblastic adenomatoid

tumour and adenomatoid or pseudoadenomatous ameloblastoma. Philipsen and Birn proposed the name adenomatoid odontogenic tumour in 1969 and suggested that it not be regarded as a variant of ameloblastoma because of its different behaviour.<sup>1</sup>

The term adenomatoid odontogenic tumour [AOT] was adopted in the initial edition of the World Health Organization's (WHO) Histological Typing of Odontogenic Tumors, Jaw Cysts and Allied Lesions in 1971 and was retained in the second edition in 1992.<sup>2</sup>

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There are 3 variants of adenomatoid odontogenic tumour, the follicular type (accounting for 73% of cases), which has a central lesion associated with an embedded tooth; the extrafollicular type (24% of case), which has a central lesion and no connection with the tooth; and the peripheral variety (3% of cases). Both types of central intraosseous tumours produce a corticate radiolucency, sometimes with radiopaque specks. The follicular type is usually initially diagnosed as a dentigerous or follicular cyst. The extrafollicular type usually presents as a unilocular, well-defined radiolucency found between, above or superimposed on the roots of erupted teeth and often resembling a residual, radicular, globulomaxillary or lateral periodontal cyst. The peripheral type usually presents as a gingival swelling, located palatally or lingually relative to the involved tooth. In two-thirds of cases of the types with a central lesion the radiolucency shows discrete foci and a flocculent pattern of scattered radiopacities. If the tumour has minimum quantities of calcified deposits, intraoral periapical radiography is superior to panoramic radiography in detecting the characteristic radiopacities.<sup>1</sup>

Courtney and Kerr believed AOT was a hamartoma (developmental abnormality) of remnant odontogenic epithelium. Carmo and Silva tried to correlate its clinical behavior with the cell proliferation rate assessed by AgNOR histochemistry.<sup>3</sup> Philipsen and Reichart considered AOT a non-invasive slow-growing benign lesion (hamartomatous) transferring detection by immunohistochemistry in part of the tumoral cells indicated a neoplastic nature.<sup>3, 4</sup>

Bcl-2, a proto oncogene, 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.

The purpose of this immunohistochemical study was to investigate Bcl-2 protein expression in adenomatoid odontogenic tumor, as they are known to play important role as a cell death suppressor that facilitates cell survival by regulating apoptosis and to correlate this expression with the biological nature of AOT, whether it is a cyst/hamartoma or a true neoplasm.

### Materials and methods

Five formalin fixed paraffin embedded blocks of adenomatoid odontogenic tumor 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 and were used for haematoxylin and eosin and immunohistochemical staining.

### 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 min. After microwave treatments, the sections were rinsed in working Tris buffer saline TBS, (1M Tris pH 7.4 - 20ml, sodium chloride - 3.4gms, make up to 400ml with distilled water) and treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature to block endogenous peroxidase activities, and then treated with the primary antibodies for 60 mins. 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 h at room temperature. After rinsing with TBS, they were treated with 0.02% 3, 3 - diaminobenzidine in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.005% hydrogen peroxide to visualize the reaction products finally, these sections were counterstained with hematoxylin.

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

### Results:

The immunoreactivity for Bcl-2 protein was present in most of the epithelial cells of AOTs. Four of the five AOTs had moderate positivity; one was poorly stained for IHC. 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: Grade I: (-) fewer than 5% positive cells or no staining, Grade II: ( $\pm$ ) 5% to 9% positive [Fig 2], Grade III: (+) 10% to 24% positive, Grade IV: (++) 25% to 50% positive and Grade V: (+++) more than 50% positive [Fig 1].

Statistical analysis - 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  $p < 0.05$ .

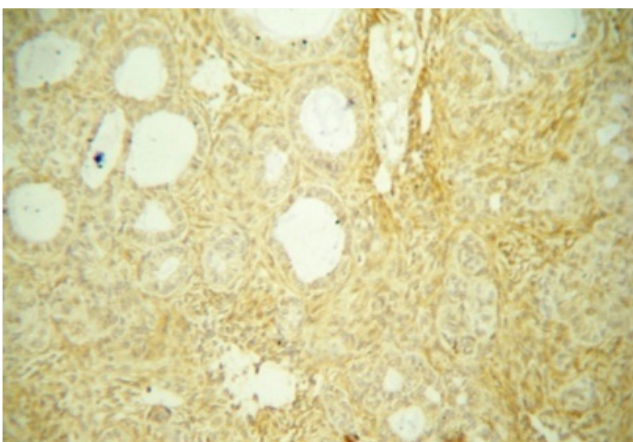


Fig 1. AOT expressing strong positivity for bcl-2

### Discussion;

Knowledge of the biologic behavior of pathologic entities affecting the oral cavity, including the odontogenic tumors, is essential for rendering the most appropriate therapeutic approach and establishing a prognosis for each case. This has led several oral pathologists to investigate different aspects related to the molecular biology of cell populations in tumors, in an attempt to elucidate many points that still remain unclear, resorting to a variety of methodologies.

The origin of adenomatoid odontogenic tumours is controversial, there has long been a debate as to whether it represents anomalous hamartomatous growth, or a true benign neoplasm.

Some believe they originate from the odontogenic epithelium of a dentigerous cyst. Some, but not all of the follicular types of AOT may derive from the odontogenic epithelium of a dentigerous cyst. As far as the extrafollicular variant, concerning the possibility of a tooth erupting through the AOT. The hamartomatous lesion would start at the REE and occasionally would not hinder dental eruption and emphasized that the AOT could remain intraosseous or peripheral in the jaw bones, depending on the spatial position of the tumor and associated tooth. In deeper impacted teeth, there would be more possibility of AOT to be intraosseous follicular or extrafollicular lesion. In tooth impactions next to the alveolar ridge, the tumor could occasionally involve the gingiva during or after the eruption process, which would justify the peripheral variant.<sup>7</sup> Dental lamina remnants likely represent progenitor cells for the peripheral type of this benign odontogenic tumor. Following entrapment, these epithelial remnants proliferate in response to an unknown stimulus, giving rise to the lesion. Furthermore, Malassez remnants found in the periodontal ligament may possibly give origin to an extrafollicular AOT.<sup>1</sup>

In addition to the anterior maxilla, the tumour has been reported in other areas of the jaw, such as the angle of the mandible. Therefore, dental lamina remnants likely represent the progenitor cells for this benign odontogenic tumour. According to this hypothesis, the lesion grows (sometimes while forming a cystic space) next to or into a nearby dental follicle, leading to the "envelopmental theory." Recent studies indicate that the cells of an adenomatoid odontogenic tumour usually differentiate toward an apparent ameloblastic phenotype but fail to achieve further functional maturation.<sup>11</sup> There is no doubt about the AOT origin from the odontogenic epithelium; however the cell directly involved in the pathogenesis is still under discussion, undifferentiated odontogenic epithelium or stratum intermedium cells were also suggested.

The AOT is an uncommon cause of jaw swelling. There is a slightly female over male incidence, an almost 2:1, and appears most often in the second decade of life although larger lesions reported in the literature, the tumors are usually in the dimensions of 1.5 to 3 cm.<sup>5</sup>

Three-quarters of the tumours involved the anterior aspect of the jaws, particularly the incisor-canine premolar region, of which the canine region was the most common site, the size of the lesion usually varied from 15 to 30 mm in diameter. Several larger tumours have been noted, the largest was more than 120 mm.<sup>6</sup>

Kramer (1974) has defined a cyst as 'a pathological cavity having fluid, semifluid or gaseous contents and which is not created by the accumulation of pus.<sup>8</sup> The term hamartoma refers to an excessive but focal overgrowth of cells and tissues native to the organ in which it occurs. Although the cellular elements are mature and identical to those found in the remainder of the organ, they do not reproduce the normal architecture of the surrounding tissue. Examples of hamartoma includes

odontoma, ameloblastic fibroodontomas, and squamous odontogenic tumors.<sup>8,9</sup>

The radiographic findings of AOT frequently resemble other odontogenic lesions such as dentigerous cysts, calcifying odontogenic cysts, calcifying odontogenic tumors, globule-maxillary cysts, ameloblastomas, odontogenic keratocysts and periapical disease. Whereas the follicular variant shows a well-circumscribed unilocular radiolucency associated with the crown and often part of the root of an unerupted tooth, the radiolucency of the extrafollicular type is located between, above or superimposed upon the roots of erupted permanent teeth. Displacement of neighbouring teeth due to tumor expansion is much more common than root resorptions. The peripheral lesions may show some erosions of the adjacent cortical bone. Comparing diagnostic accuracy between intraoral periapical and panoramic radiographs found that intraoral periapical radiographs allow perception of the radiopacities in AOT as discrete foci having a flocculent pattern within radiolucency even with minimal calcified deposits while panoramic often do not. Those calcified deposits are seen in approximately 78% of AOT.<sup>11</sup>

Studies using the AgNOR technique have found no differences in the cellular activity of the ameloblastoma and the adenomatoid odontogenic tumor. Few studies show significant higher incidence of PCNA labelling in the cases of Ameloblastoma than in the cases of AOT. Higher PCNA labelling index may indicate higher cellular proliferation rate, which would explain the more aggressive biologic behaviour of the ameloblastoma compared to the adenomatoid odontogenic tumor. Though the other studies show the reduced expression of AGNOR/PCNA in AOT than Ameloblastoma, it could not be regarded as hamartoma/cyst as still the expression is seen in AOT in reduced level. AOT is not a hamartoma/cyst as there

is no malformation of the tissue or the cystic fluid in the lumen.<sup>2</sup>

It is also stated that as we recognize that the adenomatoid odontogenic tumor is not a tumor but rather a cyst that has a hamartomatous intraluminal proliferation of epithelial cells derived from Hertwig epithelial root sheath. While at times this proliferation may fill the lumen to give the impression of a solid tumor, a close inspection will reveal its emergence from an epithelial lining. The calcifications seen in these cysts, which represent attempts of the root sheath epithelium to induce root dentin, have been identified as dentinoid material. Therefore, the more appropriate term is adenomatoid odontogenic cyst or AOC "the catchy abbreviation AOT prevailed, which unfortunately is also incorrect" and an "outdated term" and "therefore, the more appropriate term is adenomatoid odontogenic cyst or AOC." For a lesion that generally is not a fluid-filled pathologic cavity and often has a predominantly solid component, it is difficult to understand how this change can be considered an improvement or how it will contribute to better clinical management of patients who have this bland tumor.<sup>13</sup>

The long-term debate as to whether AOT is an anomalous developmental hamartomatous growth or a true benign neoplasm has not been settled and it likely never will be. This is due, in part, to difficulties with precise definitions of what seem to be, at least superficially, simple terms and concepts. Investigators who prefer to consider AOT to be a hamartoma point to the limited size of most cases (attributed to its minimal growth potential) and to the lack of recurrence (even following definitely incomplete removal) to support their belief. Those who prefer to consider AOT to be a nonaggressive non-invasive benign neoplasm presumably believe that the limited size of most cases stems from the fact that most are detected early (often on a routine dental radiograph) and removed before the slow-growing

tumor reaches a clinically noticeable size. They also point to the considerable size of some reported cases that had gone undetected or untreated for many years and resulted in facial asymmetry and distortion that rival many ameloblastomas. Additional support comes from the microscopic features of the lesional tissue that show greater departure from the arrangement of the normal odontogenic apparatus than should be expected in a developmental anomaly. Based on currently available evidence, that AOT is "most appropriately considered a benign embryonal neoplasm."<sup>2,4</sup>

### Treatment and prognosis

Conservative surgical enucleation or curettage has proven to be the treatment of choice for all types of AOTs. All forms show identical benign biological behaviour, and in only three out of 750 cases, recurrence of this tumor was verified.<sup>10</sup>

Conservative surgical enucleation is the treatment modality of choice. For periodontal intrabony defects caused by AOT guided tissue regeneration with membrane technique is suggested after complete removal of the tumor. Recurrence of AOT is exceptionally rare. Only three cases in Japanese patients are reported in which the recurrence of this tumor occurred. Therefore, the prognosis is excellent.<sup>11</sup>

**Conclusions;** Histologically the AOT shows a greater departure from the arrangement of normal dental apparatus that would be expected in developmental anomaly that rules out AOT is hamartoma. However, it is well known that, in a few instances, lesions in adenomatoid odontogenic tumor behave more aggressively than in most cases that rules out AOT is a cyst. The same activating mutation that causes adenomatoid odontogenic tumor also causes hybrid odontogenic tumors, which is a neoplasm. Thus, based on currently available evidence with the literature and the findings in the

present study, it can be considered adenomatoid odontogenic tumor to be a true neoplasm rather than a hamartoma/cyst.

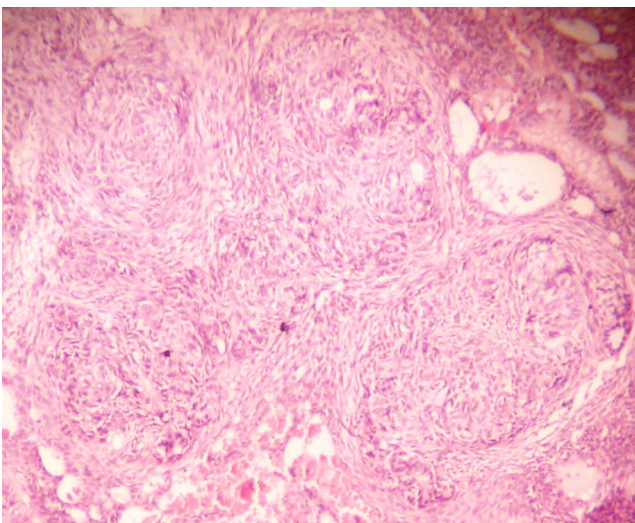
However this is a pilot study further study with larger samples and genetic and molecular biology will provide better insight into the nature of AOT.

### Conflict of interest

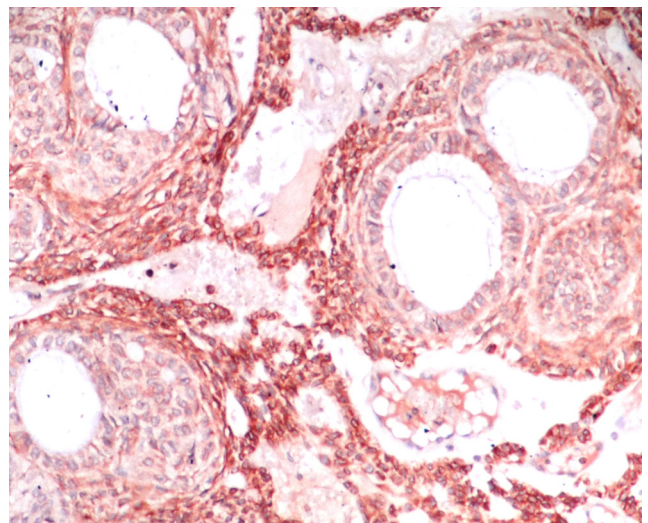
All authors declare no conflict of interests.

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1\_AOT\_H\_\_E



2\_AOT\_pos