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Original Article

Adenoid 'ameloblastoma': Clinicopathological description of 4 additional BRAF-negative cases

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ABSTRACT

Objective: Adenoid ameloblastoma (AA) is an epithelial odontogenic tumor that was recognized as a separate entity in the last odontogenic classification of WHO in 2022. The etiology is unknown, and the pathogenesis remains controversial. The objective of this study is to contribute the clinicopathological features of 4 additional BRAF-negative cases to the existing literature, aiming to enhance the molecular understanding of this unique tumor in the forthcoming classification.
Materials and methods: This study consists of a case series of four patients diagnosed with AA. The patients' demographic and clinical information were collected from the universities' medical achieves. Histopathologically, all cases were reexamined according to the latest update of the WHO odontogenic tumor classification. In addition to H&E and immunohistochemical stains, cytogenetics was also evaluated.
Results: Well-defined unilocular radiolucent lesions were observed in all cases. Ameloblastoma-like components exhibited reserved nuclear polarity, suprabasal stellate reticulum-like epithelium, duct-like structure, whorls/morules, and cribriform architecture were common features. Variable immunoreactivity to CK7, CK19, CK14, p63, and p40 were determined, and proliferative activity was greater than 15%. The BRAF molecular study revealed no mutations.
Conclusions: When diagnosing AA, the essential histopathological characteristics must be rigorously applied, and a significant portion of the lesion should contain these features. Additionally, despite limited molecular data, since the BRAF mutation commonly observed in ameloblastomas is not present in the majority of AA cases, we propose changing the term "ameloblastoma" to "ameloblastic" and referring to it as "adenoid ameloblastic tumor" in the forthcoming classification.

1. Introduction

Adenoid ameloblastoma (AA) is an epithelial odontogenic tumor that was recognized as a separate entity in the last WHO classification in 2022. It is characterized by cribriform architecture and duct-like structures, ameloblastoma-like components, and epithelial whorls/morules [1,2]. AA can occur in a wide age range of 15–82 years, with an incidence peak in the 4th decade [2]. There is a slight male predilection. (M:F = 1.3:1) [2,3]. AA usually shows painless swelling, but pain and paresthesia can sometimes be seen [3,4]. Most of the lesions show unilocular, well-defined radiolucency; however, multilocularity, internal mineralization, cortical perforation, root resorption, and paranasal sinus involvement have been reported [5].

AA is considered a locally aggressive lesion with a recurrence rate of up to 70% [3,6]. Factors such as cytological atypia, hypercellularity, p53 positivity, and high Ki67 index are associated with its recurrence [2,7].

The etiology is unknown, and the pathogenesis is under debate. Based on the limited data, it seems that this unique lesion does not tend to harbor the BRAF p.V600E mutation. Herein, we report four cases of AA, highlighting their clinicopathological, immunohistochemical, and molecular features. Our aim is to increase limited molecular data in the literature and facilitate a better understanding of clinicopathologic features of this new entity by presenting 4 cases for the forthcoming classification.

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2. Material & methods

The present study is a case series of four patients with adenoid ameloblastoma retrieved from the Oral Pathology/Pathology Department of three Universities as follows: three cases from Turkey (Istanbul University and Marmara University) and one case from Iran (Tehran University of Medical Sciences). Demographic and clinical data were collected from the patients' files and they were followed up from 6 to 25 months after the treatment with periodic evaluations for disease recurrence. The clinical findings, including demographic features, symptoms, location, and size of the lesion were documented. Radiological findings were provided based on the routine workup of the patients and no additional, unnecessary imaging was prescribed. The H&E-stained microscopic slides, ancillary studies, including immunohistochemical (IHC) staining and cytogenetic studies were reviewed using light microscopy.

For the IHC study, paraffin-embedded, formalin-fixed tissue were cut and mounted on silanized glass slides. Following antigen retrieval in a water bath, endogenous tissue peroxidase was inhibited for 10 min in a solution of 3% hydrogen peroxide and distilled water that was kept out of the light. The sections were treated with the primary antibodies diluted in the background-reducing solution at 4 °C overnight for CK7, CK14, and CK19, and at 25 °C for 2 h for p63, p40, Pan CK, calretinin, SOX10, CD56 and Ki-67. Primary antibodies were substituted in all negative control reactions with phosphate-buffered saline (PBS). In a dark chamber, 3,3′-diaminobenzidine was used for five minutes to reveal the reaction. The tissue samples were counterstained with hematoxylin for five minutes before being dried and mounted.

Regarding BRAF p.V600E mutational analysis, 3 to 5 sections of formalin-fixed paraffin-embedded tissues (5 μm/section) were deparaffinized by xylene and ethanol treatment, and digested with proteinase K at 56 °C overnight. Genomic DNA was extracted using AmoyDx FFPE DNA Kit as recommended. DNA was then qualified/quantified with ND- 2000 spectrophotometer (NanoDrop Products, Thermo Scientific). In our study, we used AmoyDx BRAF Mutation Detection Kit (V2) is real-time PCR assay for qualitative detection of V600E, V600E2, V600K, V600D, V600D2, V600A, V600R mutations in BRAF gene in human genomic DNA extracted from tumor tissue sample. BRAF PCR analysis was performed according to the FAM and HEX channels. Samples were evaluated with Ct value < 26 in the FAM channel as positive and Ct value >8 in the FAM channel as negative.

3. Results

All details of clinical-radiological features are summarized in Table 1. Among the four patients presented, only one patient was referred because of changes in the routine radiological examination as the first symptom, while in other cases, most common symptom was facial swelling. The smallest and largest size of the lesion was 3.5 and 6 cm, respectively. Well-defined unilocular radiolucent lesions were observed in radiographic findings of all cases. Additionally, the CBCT scans revealed a prominent extension to the surrounding normal structures in all cases (Fig. 1). All cases underwent surgical removal, and, recurrence of the lesion was detected just in one case (case 4) after 2 years.

Table 2 demonstrates the detailed histopathologic, immunohistochemical, and molecular features of all cases. Details of histopathologic features of cases were shown in Fig. 2. In all cases, specific histopathologic features of AA, including ameloblastoma-like components showing reserved nuclear polarity, suprabasal stellate reticulum-like epithelium, duct-like structure, whorls/morules, and cribriform architecture were observed. Mitotic figures were seen in all cases, but importantly no atypical one was observed. Nuclear pleomorphism, including increased nuclear to cytoplasmic ratio and hyperchromasia, was present in three cases. Additionally, two cases showed dentinoid material, one of them demonstrated clear cell change in close approximation to dentinoid. Furthermore, no evidence of chronic inflammatory cell infiltration and ghost-cell keratinization was observed in the microscopic examination of the cases. The IHC studies showed variable positive immunoreactivity to CK7, CK19, CK14, p63, Pan CK and p40. Proliferative activity was more than 15% in all cases by Ki67 antibody (Fig. 3). No evidence of immunoreactivity was observed in calretinin, SOX10, and CD56. Additionally, the BRAF molecular study was assessed in all cases and no mutation was detected.

Table 1
Clinico-radiological features and follow-up of adenoid ameloblastoma cases.

Case	Sex and age	Symptoms	location	Size (cm)	Radiologic findings	Treatment	Recurrence	Duration of f ollow -up (month)
1	M,70	Facial swelling, non-tender	Left mandible (molar to tramus)	5	Panoramic X-ray: Ill-defined unilocular radiolucent lesion in mandibular bone extending from molar region to ramus CBCT scan: Low attenuated, perforating both buccal, and lingual plates of the bone that extended to surrounding soft tissue	Hemi mandibulectomy	NO	33
2	M,59	Facial swelling	Left Mandible (premolar to ramus)	6	Panoramic X-ray: Well-defined unilocular radiolucent lesion in left posterior mandible to ramus CBCT scan: Expansive radiolucent lesion superior to the mandibular canal that destroys the buccal and lingual cortical bones.	surgical removal	NO	16
3	F,36	Routine radiologic examination (root resorption)	Left Mandible (Premolar to molar)	3.5	Panoramic X-ray: Well-defined, unilocular radiolucent lesion causing the root resorption CBCT scan: Mild expansion with basis destruction of the mandible that extended to soft tissue	Surgical removal	NO	6
4	M,60	Facial swelling	Left Mandible (anterior to molar)	5.5	Panoramic X-ray: Well-defined unilocular expansile lesion in anterior side to posterior side of the mandible CBCT scan: Perforated lingual planet and thin expansive buccal cortical plate	Surgical removal	Yes, after 2 years	25

M:male; F:female; CBCT scan: cone beam computed tomography scan.

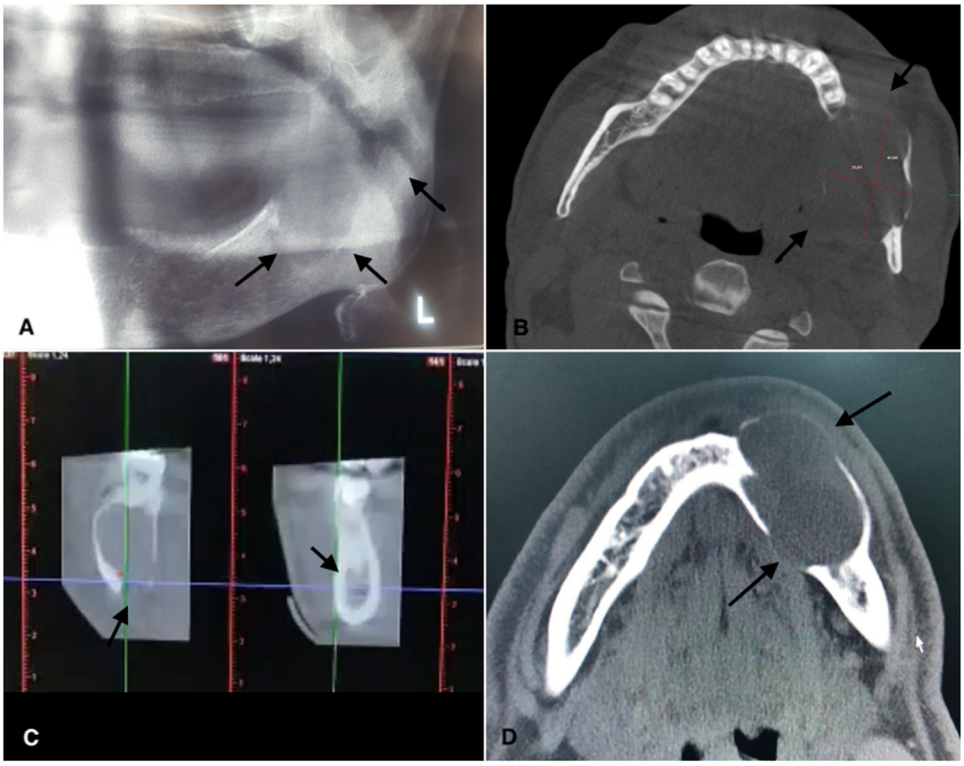

Fig. 1. A. Ill-defined destructive lesion in ascending ramus of the mandible extending to the oral cavity of case 1**;** B. An expansive radiolucent lesion destroying the buccal and lingual cortical bones of case 2; C. Mild expansive unilocular radiolucent lesion causing the root resorption of case 3**;** D. The perforated lingual planet and thin expansive buccal cortical plate of case 4. (Arrows indicate the lesions)

Table 2
Histopathologic features, IHC, and molecular results of the cases.

Histopathologic features		Case 1	Case 2	Case3	Case 4
Ameloblastoma-like component		+	+	+	+
Reserved nuclear polarity		+	+	+	+
Suprabasal stellate reticulum-like epithelium		+	+	+	+
Duct-like structure		+	+	+	+
Whorls/ morules		+	+	+	+
Cribriform architecture		+	+	+	+
Dentinoid		–	+	–	+
Clear cells		–	+	–	–
Focal ghost-cell keratinization		–	–	–	–
Mitoses		+	+	+	+
Nuclear hyperchromatism		+	+	–	+
Chronic inflammatory infiltrate		–	–	–	–
Immunohistochemistry	Positive	CK19 CK7 P63 Ki67: about 20%	CK19 (focal) p63 p40 Ki67: about 15%	CK19 (focal) p63 p40 Ki67: about10–15%	CK19 (focal) p40 p63 CK14 Pan CK Ki67: about 15–20%
	Negative	Calretinin	Calretinin SOX10	Calretinin	SOX10 Calretinin CD56 CK7
BRAF mutation	Negative	V600E V600K V600D V600R	V600E V600K V600D V600E2 V600A	V600E V600K V600D V600E2 V600A	V600E1 V600E2 V600K V600D1 V600D2 V600R

4. Discussion

This study reports on four cases of adenoid ameloblastoma that were examined to enhance our understanding of this recently acknowledged odontogenic neoplasm. Given the aggressive nature of AA, it is critically important for pathologists to be familiar with this new entity in differential diagnosis when they observe histopathological features similar to conventional ameloblastoma and adenomatoid odontogenic tumor (AOT).

AA was first introduced into the medical literature in 1959 by Waldron and was later explained in more detail by Loyola et al. in 2014 [8]. Almost 40 cases of AA have been reported in English

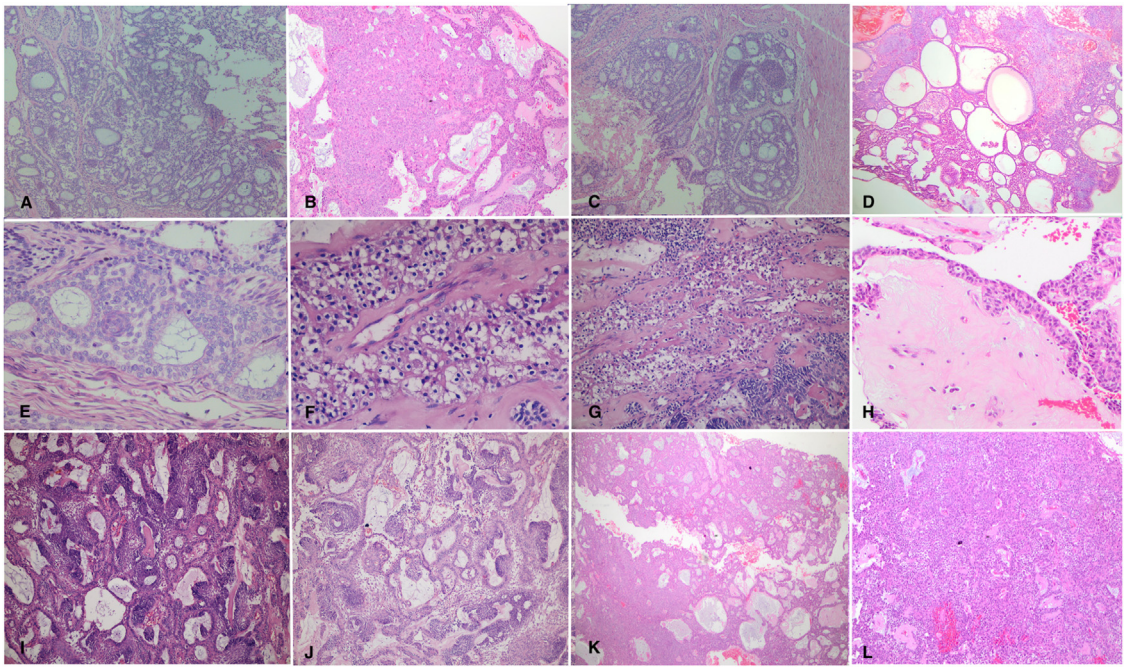

Fig. 2. A-D. Prominent various-sized duct-like structures with cribriform appearance composed of epithelial cells showing basaloid features (A-D, H&E x100); E. Areas of reverse nuclear polarity at the peripheral cells and stellate-like epithelium at the suprabasal cells and epithelial morule are seen (H&E x200); F-G. Nests and strands of tumoral cells with significant clear cell change, nuclear hyperchromatism and also dentinoid material are observed (H&E x200, x100, respectively); H. In some areas, dentinoid material aggregation is prominent (H&E x100); I-L. Ameloblastoma-like component showing microcystic structures with prominent whorls/morules formation (I-L, H&E x100).

literature so far [6]. The tumor predominantly occurs in adults with a slightly male predilection, and most cases have been reported from South America and Asia [2]. The previous studies have reported the right mandible as the most common site. [9]. However, in the fifth edition of the WHO classification of odontogenic lesions, it has stated that AA does not tend to a specific site of involvement [2]. Facial swelling is the main symptom of the patients, which is compatible with our observation [3].

In this study, our patients with facial swelling underwent radiological examination, including conventional panoramic view and CBCT scans, which ranged from unilocular and well-defined lesions to diffuse and multilocular. According to the literature review, the mean size of lesions has been reported as 3.5 cm, and despite the presence of dentinoid, about 82.4% of adenoid ameloblastomas appeared as solely radiolucent lesions, while 47.1% showed ill-defined margins and cortical perforation at the time of diagnosis [4,6].

AA is defined as an odontogenic tumor exhibiting histological characteristics of both ameloblastoma and AOT, as well as dentinoid formation and locally aggressive behavior [3]. The latest version of the WHO classification has revealed that ameloblastoma-like components, duct-like structures, whorls/ morules, and cribriform architecture are essential diagnostic criteria for AA. Additionally, the presence of dentinoid, clear cells, and focal ghost-cell keratinization are reported as desirable features [1,2]. All four cases in the current study exhibited at least three essential features. Furthermore, AOT and dentinogenic ghost cell tumor (DGCT) should be noted as differential diagnoses because of the histopathologic overlap, especially when calcified materials are present [3]. AOT is characterized by solid or sometimes cystic well-circumscribed tumors and KRAS mutations, as well as MAPK pathway activation with KRAS p.G12V and p.G12R mutations in around 70% of cases. In contrast, AA is distinguished by the combination of cribriform pattern, whorls/morules, and absence of KRAS mutations [6,10]. DGCT does not show cribriform architecture and distinct adenoid formation, whereas ghost-cell keratinization are essential feature for DGCT. Clear cell odontogenic carcinoma (CCOC) might be another important differential diagnosis when clear cells are prominent. The absence of EWSR1 rearrangements, which have been observed in over 80% of CCOC cases, facilitates the exclusion of CCOC [11,12]. Odontogenic carcinoma with dentinoid material can also show microscopic similarities to AA, especially when it is composed of clear cells with variable amounts of small round to basaloid cells [7]. Sometimes, duct-like or pseudocyst structures and columnar cells with prominent palisading are also present in odontogenic carcinomas [6]. There are still no definite criteria for differentiating AA from odontogenic carcinoma with dentinoid material [3] and clinic-radiographic correlation may help to make a definite diagnosis, but the relationship between these tumors is still controversial.

In terms of IHC findings, variable immunoreactivity to CK14, CK19, p40, p16, and p53 is reported in the literature [5]. CK14 is a typical intermediate filament of odontogenic epithelium, whereas CK19 commonly indicates odontogenic epithelium [13,14]. According to our findings, CK14 and/or CK19 positivity in the cases can explain the odontogenic origin of the tumoral cells. Additionally, CK7 positivity, which has been reported in Hertwig's epithelial root sheath and the stellate reticulum cells, was observed in the first case, but no reactivity was seen in the fourth similar to result of recently published case series by Adorno-Farias et al. [5,15]. Calretinin biomarker which is commonly considered a specific IHC marker for neoplastic ameloblastic epithelium, demonstrated no immunoreactivity in all four cases, which is in contrast to the findings of Sachdev et al. [9,16].

The vast majority of ameloblastomas are distinguished by the presence of BRAF p.V600E mutations, whereas AOTs include characteristic KRAS mutation [10,17]. Furthermore, it seems that AA does not carry the hallmark mutations of ameloblastoma and AOT, or at least, BRAF mutation can occur in an extremely small percentage of the cases [1,18]. Oh et al. suggested that AA and DGCT represent a histologic spectrum of WNT pathway–altered benign odontogenic tumors, as opposed to two separate tumors [19]. They also stated that mutations in tumor suppressor genes, such as those composing the B-catenin destruction complex or encoding E3 ubiquitin ligases,

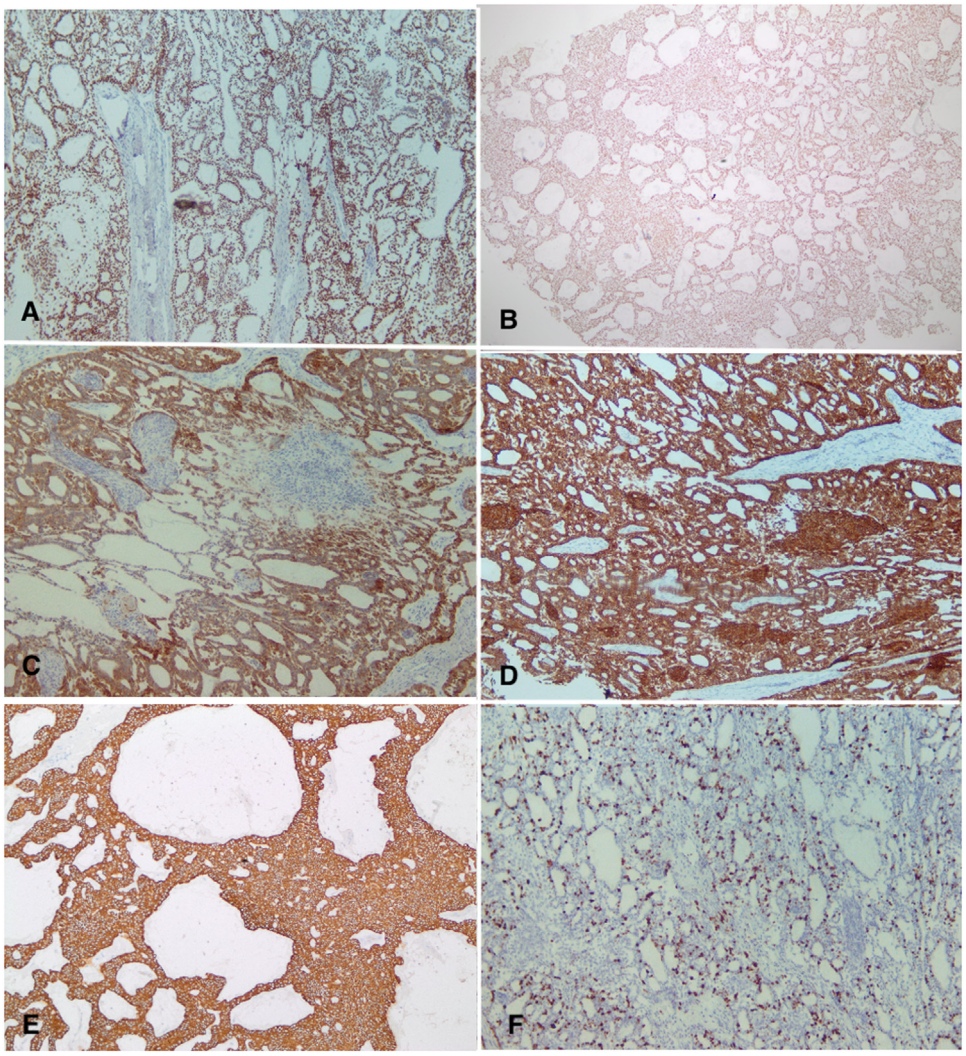

Fig. 3. IHC study of the cases shows diffuse nuclear positivity for p63 (A); p40 (B); Diffuse cytoplasmic positivity for CK7 (C); CK19 (D); CK14 (E); The proliferative activity of the tumoral cells Ki67 is up to 20% in hot spots (F). All figures, x100 original magnification.

may be common in the WNT pathway—altered benign odontogenic tumors [19]. It has been shown that nuclear-catenin is present throughout tooth development and that classical WNT activation is connected with dentin production, which may explain the frequent occurrence of dentin-like material known as dentinoid in odontogenic lesions with activated WNT pathway [19,20]. Bastos et al. revealed the presence of CTNNB1 exon 3 mutations in adenoid ameloblastoma for the first time and also supported this finding by the immunohistochemical analysis, which demonstrates the activation of the beta-catenin cellular pathway in the tumor through nuclear expression [21]. This novel insight enhances our understanding of the molecular mechanisms underlying adenoid ameloblastoma development. Therefore, the discovery of nuclear-catenin reactivity and the absence of BRAF reactivity in the vast majority of the tumors raises questions about whether it may not belong to the ameloblastoma group and whether 'adenoid ameloblastoma' is the most appropriate nomenclature for this novel tumor [1].

The limitation in these case presentations is that in such a newly attempted to define entity, other mutation pathways and genes defined in odontogenic lesions have not been examined with next-generation sequencing or Sanger sequencing. Only the BRAF mutation was investigated because it could affect treatment options.

In conclusion, the number of this recently identified odontogenic tumor remains a mystery and should be increased in the literature. The genetic studies, especially in cases that contain the specific diagnostic findings accepted by the WHO 2022 classification, including ameloblastoma-like component, duct-like structures, whorls/morules cribriform architecture, will provide more information about the origin of the tumor and contribute to the further treatment and follow-up process. In our experience, the essential histopathological features should be applied very strictly in the diagnosis of AA, and the lesion should largely contain these features. Additionally, despite limited molecular data, since the BRAF mutation commonly observed in ameloblastomas is not present in the majority of cases, we propose changing the term "ameloblastoma" to "ameloblastic" and referring to it as "adenoid ameloblastic tumor" in the forthcoming classification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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