UNIVERSIDADE ESTADUAL DE CAMPINAS
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ANA LUIZA OLIVEIRA CORRÊA ROZA

ESTUDO CLINICOPATOLÓGICO E EXPRESSÃO IMUNO-HISTOQUÍMICA DE BRAF E SOX9 EM FIBROMAS, FIBRO-ODONTOMAS E FIBROSSARCOMAS AMELOBLÁSTICOS

CLINICOPATHOLOGIC STUDY AND IMMUNOHISTOCHEMICAL EXPRESSION OF BRAF AND SOX9 IN AMELOBLASTIC FIBROMAS, FIBRO-ODONTOMAS, AND FIBROSARCOMAS

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Orientador: Prof. Dr. Pablo Agustin Vargas
Coorientador: Prof. Dr. Mário José Romañach Gonzalez Sobrinho

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"Faça o teu melhor, na condição que você tem,
enquanto você não tem condições melhores,
para fazer melhor ainda."

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RESUMO

Lesões odontogênicas são incomuns e possuem comportamento clínico que variam de acordo com as suas características clínicas, radiográficas e microscópicas. O fibroma ameloblástico (FA) afeta predominantemente a mandíbula posterior de crianças e adolescentes, apresentando-se como uma lesão radiolúcida bem-delimitada e expansiva. O fibrodentinoma e fibro-odontoma ameloblástico (FDA/FOA) representam tumores odontogênicos com características semelhantes ao FA, mas que também apresentam formação de dentina e esmalte. Muitos autores acreditam que alguns desses tumores podem representar odontomas em desenvolvimento; no entanto, casos com características clínicas e radiográficas agressivas favorecem uma contraparte neoplásica. O fibrossarcoma ameloblástico (FSA) representa a contraparte maligna do FA, na qual apenas o componente ectomesenquimal apresenta características de malignidade. Alguns FA e FDA/FOA apresentam mutação patogênica no BRAF p.V600E, um achado molecular não encontrado em odontomas. A proteína SOX9, conhecida por seu papel na diferenciação de vários tipos celulares, ainda não havia sido estudada em tumores odontogênicos. Neste estudo, relatamos as características clinicopatológicas de uma série de FA, FDA/FOA e FSA, e analisamos a expressão imuno-histoquímica de BRAF p.V600E e SOX9, com o objetivo de fornecer novos achados sobre o comportamento biológico desses tumores e evidências adicionais para uma futura classificação. Dados clínicos e radiográficos foram obtidos dos arquivos de quatro serviços de Patologia Oral entre 1991 e 2024. Foram identificados 72 casos, incluindo 30 FA, 32 FDA/FAO e 10 FSA. Trinta e oito pacientes eram do sexo masculino e 34 do sexo feminino. As idades médias para FA, FDA/FOA e FSA foram 15,3, 12,3 e 30 anos, respectivamente. Os tumores afetaram predominantemente a mandíbula posterior, como lesões radiolúcidas ou mistas, uniloculares com tamanho médio de 5,4 cm, causando impactação dentária e expansão de corticais ósseas. Microscopicamente, todos os casos apresentaram componentes mesenquimais celulares semelhantes à papila dentária, contendo cordões e ilhas de epitélio odontogênico. FDA/FOA também apresentaram tecido dentário mineralizado. FSA exibiu hipercelularidade estromal com pleomorfismo celular e menos ilhas ameloblásticas. Tumores raros foram identificados, incluindo associações de FA com cistos odontogênicos calcificantes, fibroma cemento-ossificante e lesão central de células gigantes. A expressão imuno-histoquímica de BRAF p.V600E foi observada em 81% dos FA, 54% dos FDA/FOA e 60% dos FSA, enquanto SOX9 apresentou expressão difusa nos componentes epitelial e mesenquimal em 94% dos casos. Este estudo representa uma das maiores casuísticas bem documentadas de FA, FDA/FOA e FSA, contribuindo achados clinicopatológicos e imuno-histoquímicos adicionais.

Palavras-chave: Tumores odontogênicos. Mandíbula.

ABSTRACT

Odontogenic lesions are uncommon and demonstrate variable clinical behavior depending on their clinical, radiographic, and microscopic characteristics. Ameloblastic fibroma (AF) predominantly affects the posterior mandible of children, presenting as a well-defined expansile radiolucency. Ameloblastic fibrodentinoma and fibro-odontoma (AFD/AFO) represent odontogenic tumors with similar features to AF that also show dentin and enamel formation. Many authors believe that some of them may be developing odontomas; however, cases with aggressive clinical and radiographic features support a neoplastic counterpart. Ameloblastic fibrosarcoma (AFS) represents the malignant counterpart of AF in which only the ectomesenchymal component shows features of malignancy. A subset of AF and AFD/AFO harbor the pathogenic BRAF p.V600E mutation, a molecular finding not expressed in odontomas. SOX9, known for its role in differentiating various cell types, has not been previously studied in odontogenic tumors. Herein, we report the clinicopathologic features of a large series of AF, AFD/AFO, and AFS and analyze their immunohistochemical expression of BRAF p.V600E and SOX9 to provide insight into their biologic behavior and additional evidence for further classification. Clinical and radiographic data were retrieved from the archives of four Oral Pathology services between 1991 and 2024. Seventy-two cases were identified, including 30 AF cases, 32 AFD/AFO cases, and 10 AFS. Thirty-eight patients were male and 34 were female. The average ages for AF, AFD/AFO, and AFS were 15.3, 12.3, and 30, respectively. Tumors predominantly affected the posterior mandible as asymptomatic unilocular radiolucent or mixed lesions with an average size of 5.4 cm, causing tooth impaction and cortical expansion. Microscopically, all cases presented with cellular mesenchymal components resembling dental papilla, containing branching strands and islands of odontogenic epithelium. AFD/AFO also showed dental hard tissue. AFS exhibited increased stromal hypercellularity with marked pleomorphism and fewer ameloblastic islands. Rare tumors were identified, including the association of AF with calcifying odontogenic cysts, cemento-ossifying fibroma, and central giant cell granuloma. BRAF p.V600E immunohistochemistry expression was observed in 81% of AF, 54% of AFD/AFO, and 60% of AFS, while SOX9 showed diffuse nuclear expression in both epithelial and mesenchymal components in 94%. This study represents one of the largest well-documented series of AF, AFD/AFO, and AFS, contributing additional clinicopathologic and immunohistochemical findings.

Keywords: Odontogenic tumors. Mandible.

SUMÁRIO

1 INTRODUÇÃO

Tumores odontogênicos (TO) compreendem um grupo complexo de lesões que acometem a região oral e maxilofacial que se originam a partir de remanescentes do germe dentário (Kramer *et al.*, 1992; Neville *et al.*, 2024). Em geral, TO são lesões benignas relativamente raras, representando menos de 1% do total de tumores de cabeça e pescoço, e possuem comportamentos clínicos e diversidade microscópica de ampla variação (Takata & Slootweg, 2017). Clinicamente, os TO manifestam-se como aumentos de volume assintomáticos de crescimento lento e progressivo, provocando expansão de corticais ósseas, deslocamento dentário e reabsorção radicular. O diagnóstico é baseado na análise histopatológica, os quais podem mostrar variações dos componentes epitelial e ectomesenquimal.

Tumores odontogênicos mistos (TOM) correspondem a um grupo de lesões compostos por epitélio odontogênico proliferando em um ectomesênquima semelhante à papila dentária. De forma semelhante à odontogênese, TOM demonstram diversas interações indutoras entre o epitélio e ectomesênquima odontogênico, podendo levar à formação de quantidades variáveis de esmalte e dentina e, consequentemente, apresentar desafios na classificação dessas lesões (Neville *et al.*, 2024). Algumas dessas lesões representam anomalias de desenvolvimento (hamartomas [odontomas]), enquanto outras parecem ser neoplasias verdadeiras (fibroma, fibrodentinoma, e fibro-odontoma ameloblástico), que raramente desenvolvem transformação maligna (fibrossarcoma ameloblástico). Em alguns casos, os achados microscópicos não são suficientes para distinguir entre hamartomas e lesões neoplásicas, necessitando da correlação das características clínicas e radiográficas em cada caso. O tratamento da maioria dos casos é cirúrgico variando de cirurgias conservadoras até resseções parciais dos ossos gnáticos (Takata & Slootweg, 2017; Neville *et al.*, 2024).

Padrões internacionais apropriados para classificações foram desenvolvidos pela primeira vez pela Organização Mundial da Saúde (OMS) em 1952 (Sobin, 1971). As classificações foram desenvolvidas por grupos de especialistas internacionais que revisaram vários casos e concordaram com

uma terminologia uniforme, descrevendo critérios práticos e clinicamente relevantes para o diagnóstico dessas lesões (Speight, 2025). A primeira classificação da OMS de Cistos e Tumores Odontogênicos foi realizada em 1971, sendo uma classificação abrangente com descrições claras e concisas das características clínicas, radiográficas e histopatológicas. Desde então, a categoria dos TOM permanece um tópico controverso até os dias atuais. Devido à sua semelhança microscópica com áreas de odontomas em desenvolvimento e à evidência de que alguns TOM amadurecem em odontomas, fibrodentinoma ameloblástico e fibro-odontoma ameloblástico foram removidos da 4ª edição da Classificação de Cistos e Tumores Odontogênicos da OMS (2017) após um consenso de que, uma vez formado tecido dentário mineralizado, a maioria dessas lesões amadureceriam em odontomas, e assim evitar tratamentos agressivos nestes tumores (Takata & Slootweg, 2017).

1.1 Fibroma ameloblástico

O fibroma ameloblástico (FA) é considerado um TOM verdadeiro, composto por epitélio e ectomesênquima odontogênico neoplásicos, sem a formação de tecido mineralizado (Vered *et al.*, 2023). FA é considerado um tumor benigno incomum, correspondendo a 2% de todos os TO, contudo é difícil avaliar os dados epidemiológicos a respeito da sua frequência devido à relatos antigos, diagnosticados erroneamente como FA, representarem odontomas em estágios iniciais de desenvolvimento (Vered *et al.*, 2023; Neville *et al.*, 2024). Embora o FA tenha sido relatado em pacientes com ampla faixa etária, sua predileção por pacientes jovens é bem estabelecida na literatura, com pico de incidência na segunda década de vida, tendo uma leve predileção pelo sexo masculino (Chrcanovic *et al.*, 2018; Vered *et al.*, 2023). O FA caracteriza-se clinicamente como uma lesão assintomática podendo causar aumento de volume e assimetria facial quando atinge tamanhos exuberantes. Dor e sensibilidade são achados incomuns.

No geral, o tumor é diagnosticado aproximadamente em 70% dos casos na região posterior de mandíbula (Takeda, 1999; Chrcanovic *et al.*, 2018; Vered *et al.*, 2023; Neville *et al.*, 2024). FA frequentemente exibe

características radiográficas de uma lesão odontogênica benigna, apresentando-se como uma imagem radiolúcida unilocular ou multilocular com bordas corticalizadas. Cerca de 75% dos FAs estão associados a coroa de um dente não-erupcionado, mais comumente um molar (Buchner & Vered, 2013; Neville *et al.*, 2024). O FA exibe um padrão de crescimento radiográfico distinto quando comparado ao ameloblastoma, crescendo de forma expansiva e não tende a infiltrar trabéculas ósseas adjacentes, de modo que as bordas da lesão frequentemente encontram-se lisas (Takeda, 1999). Embora possa haver expansão do osso sobrejacente, a cortical óssea permanece intacta na maioria dos casos, eventualmente sofrendo destruição. O diagnóstico diferencial clínico e radiográfico do FA inclui lesões odontogênicas e não-odontogênicas como cisto dentígero, queratocisto odontogênico, cisto odontogênico calcificante, ameloblastoma, mixoma odontogênico, tumor odontogênico primordial, lesão central de células gigantes e miofibroma central.

A variante extra-óssea ou periférica do FA é considerada rara, com aproximadamente 10 casos reportados na literatura (Ide *et al*., 2008; Chrcanovic *et al.*, 2018). FAs periféricos afetam pacientes 10 anos mais jovens quando comparados à sua contraparte intra-óssea e apresentam-se como nódulos normocrômicos, sendo mais prevalente na gengiva superior, localizados na superfície oclusal de um dente não-erupcionado (Ide *et al.*, 2008). Mesmo com poucos casos reportados na literatura para realizar uma análise formal, é sugerido que o dente em erupção estava associado à um FA que migrou para uma localização subgengival superficial ao invés de surgir primariamente da gengiva. Alguns autores questionam a suposta natureza neoplásica do FA periférico e favorecem que seja mais provavelmente um hamartoma odontogênico (Ide *et al.*, 2008).

A peça cirúrgica do FA exibe características macroscópicas de uma massa tumoral de tecido mole com coloração esbranquiçada, consistência fibroelástica e superfície lisa ou lobulada (Cohen & Bhattacharyya, 2004; Vered *et al.*, 2023). A superfície de corte demonstra uma lesão sólida homogênea e uma aparência levemente mixóide (Vered *et al.*, 2023). Microscopicamente, o tumor é composto por um tecido mesenquimal celularizado, semelhante à papila dentária primitiva, e epitélio odontogênico

em proliferação. O componente epitelial é arranjado geralmente em dois padrões microscópicos, em quantidade e densidade variáveis (Takeda, 1999; Vered *et al.*, 2023; Neville *et al.*, 2024). O padrão epitelial mais comum consiste em cordões longos e estreitos de epitélio odontogênico, frequentemente em um arranjo anastomosado. Esses cordões geralmente têm apenas duas células de espessura e são compostos por células cuboidais ou colunares, semelhante à lâmina dentária. No outro padrão, as células epiteliais formam pequenas ilhas discretas que se assemelham ao estágio folicular do órgão do esmalte em desenvolvimento. Estas apresentam células colunares periféricas ameloblásticas, que circundam células epiteliais frouxamente dispostas que se assemelham ao retículo estrelado (Takeda, 1999; Vered *et al.*, 2023; Neville *et al.*, 2024). O componente ectomesenquimal do FA é composto por células estreladas e ovoides dispersas em uma matriz frouxa, que se assemelha à papila dentária em desenvolvimento (Vered *et al.*, 2023). A presença de colágeno geralmente é discreta, enquanto hialinização justaepitelial é ocasionalmente observada. Raros exemplos de tumores híbridos de FA com cisto odontogênico calcificante têm sido relatados na literatura (Lin *et al.*, 2004). Esses achados histopatológicos são característicos, porém não específicos do FA, uma vez que podem ser vistos em odontomas em desenvolvimento, portanto, o diagnóstico final do FA é definido pela correlação das características clínicas, radiográficas e microscópicas (Speight & Takata, 2018).

O manejo adequado do FA tem sido um tópico de debate constante. Embora inicialmente acreditasse que o FA fosse uma lesão inócua que raramente recorria após enucleação ou curetagem, relatos subsequentes indicaram um aumento do risco de recorrência após terapia conservadora. A maior taxa de recorrência (43,5%) foi registrada em uma casuística da *Armed Forces Institute of Pathology*, composta por lesões grandes e agressivas, enquanto relatos de casos na literatura reportaram recorrência em aproximadamente 19% dos casos (Trodahl, 1972). Com base nesses dados, recomendações recentes enfatizaram a terapia inicial conservadora para FA, seguida de monitoramento à longo prazo. Excisões cirúrgicas mais agressivas devem ser reservadas para lesões recorrentes e destrutivas (Vered *et al.*, 2023; Neville *et al.*, 2024). Embora transformações

sarcomatosas sejam consideradas raras, aproximadamente 50% dos fibrossarcomas ameloblásticos se originam a partir de FAs recorrentes (Vered *et al.*, 2023; Wright & Chi, 2023).

1.2 Fibrodentinoma/Fibro-odontoma ameloblástico

O fibrodentinoma ameloblástico (FDA) e fibro-odontoma ameloblástico (FOA) são tumores com características gerais de FA, mas que também contêm a presença de dentina e esmalte, respectivamente. Alguns patologistas acreditam que o FDA/FOA seja apenas um estágio no desenvolvimento do odontoma e não consideram como uma entidade separada (Takata & Slootweg, 2017). Certamente, as características histopatológicas de odontoma em desenvolvimento podem se sobrepor ao FDA/FOA. No entanto, existem exemplos bem documentados destes tumores exibindo características de neoplasias verdadeiras incluindo crescimento progressivo, atingindo tamanhos exuberantes, causando deformidade e destruição óssea (Buchner *et al.*, 2013; Best *et al.*, 2024). Entretanto, diferenciar odontoma em desenvolvimento e FDA/FOA pode ser desafiador apenas com critérios histopatológicos, e é bem provável que alguns exemplos de odontoma em desenvolvimento tenham sido relatados na literatura como FDA/FOA.

FDA/FOA geralmente ocorrem em crianças com média de idade de 10 anos e raramente acomete adultos. Assim como o FA, os FDA/FOA ocorrem principalmente nas regiões posteriores dos ossos gnáticos, a maioria das vezes acometendo a mandíbula. Pacientes masculinos são afetados com um pouco mais de frequência do que pacientes femininos, em uma proporção de 3:2 observada na literatura (Takeda, 2005; Chrcanovic & Gomez, 2017). A lesão é comumente assintomática e descoberta em radiografias de rotina para determinar a razão da falha na erupção de um dente. Exemplos exuberantes podem causar assimetria facial. Recentemente, uma pesquisa analisou casos publicados de odontoma e FOA e constatou que lesões com tamanho igual ou superior a 2,1 cm e em pacientes com menos de 13,5 anos de idade tinham maior probabilidade de apresentar características consistentes com uma neoplasia (Soluk-Tekkesin & Vered, 2021).

De maneira similar ao FA, FDA/FOA apresenta características radiográficas de uma lesão odontogênica benigna como uma imagem radiolúcida unilocular ou multilocular bem-delimitada, que contém quantidade variável de focos radiopacos no seu interior (Chrcanovic & Gomez, 2017). Esses focos radiopacos apresentam radiodensidade semelhante à estrutura dentária e podem se apresentar como múltiplas radiopacidades pequenas ou uma massa conglomerada. Aproximadamente 5% dos casos contêm uma quantidade mínima de esmalte calcificado, apresentando-se principalmente como lesões radiolúcidas e não podem ser diferenciadas de outras lesões radiolúcidas que acometem os ossos gnáticos (Neville *et al.*, 2024).

A variante periférica de AFD/AFO é considerada uma lesão extremamente rara com menos de 10 casos reportados na literatura e apresenta-se clinicamente como um nódulo normocrômico ou eritematoso localizado na gengiva superior anterior de pacientes pediátricos (Chrcanovic & Gomez, 2017; Alramadhan *et al.*, 2022). O diagnóstico diferencial clínico inclui granuloma piogênico, fibroma ossificante periférico, lesão periférica de células gigantes e hiperplasia gengival (Alramadhan *et al.*, 2022). O tratamento consiste na excisão cirúrgica conservadora e recidiva não é esperada (Alramadhan *et al.*, 2022).

O componente de tecido mole do FDA/FOA é microscopicamente idêntico ao FA, apresentando-se com cordões e ilhas de epitélio odontogênico em meio a um tecido conjuntivo primitivo semelhante à papila dentária (Takeda, 2005). Interações indutoras entre o epitélio e ectomesênquima odontogênico resultam na formação de tecido dentário mineralizado, composto por dentina e esmalte, em íntimo contato com o epitélio odontogênico. As lesões mais calcificadas podem apresentar estruturas dentárias maduras na forma de pequenos dentes rudimentares ou massas conglomeradas de esmalte e dentina.

Devido à lesão se destacar com facilidade do osso adjacente, enucleação cirúrgica conservadora é o tratamento de escolha para a maioria dos pacientes diagnosticados com FDA/FOA. Entretanto, lesões mais agressivas e destrutivas podem exigir ressecções cirúrgicas mais radicais (Buchner *et al.*, 2013). A recorrência do FDA/FOA não é esperada, com taxa de recorrência após a remoção conservadora estimada em cerca de 7%, e o

prognóstico é bom. O desenvolvimento de fibrossarcoma ameloblástico após um tratamento conservador de FDA/FOA é extremamente raro.

1.3 Fibrossarcoma ameloblástico

O fibrossarcoma ameloblástico (FSA) corresponde a um TOM maligno, representando a contraparte maligna do FA, onde apenas o componente ectomesenquimal apresenta características microscópicas de malignidade (Richardson & Muller, 2014; Wright & Chi, 2023). Apesar de décadas de documentação, o FSA é considerado um tumor raro, com aproximadamente 120 casos reportados na literatura (Chrcanovic *et al.*, 2018; Agaimy *et al.*, 2020; Neville *et al.*, 2024). Embora casos de FSA tenham sido observados surgindo *de novo*, em aproximadamente 50% dos casos o tumor maligno representa uma recorrência de um tumor previamente diagnosticado como FA ou FOA (Takeda, 1999; Wright & Chi, 2023).

FSA geralmente ocorrem em pacientes adultos, com média de idade de 28 anos e uma ligeira prevalência em homens. Embora tanto a maxila quanto a mandíbula possam estar envolvidas, cerca de 75%-80% dos casos ocorrem na mandíbula com predileção pela região posterior (Muller *et al.*, 1995; Chrcanovic *et al.*, 2018, Wright & Chi, 2023). Dor e aumento de volume associados ao rápido crescimento clínico são queixas comuns. Lesões iniciais podem parecer benignas radiograficamente, com características que refletem um tumor precursor benigno. No entanto, com o tempo, as lesões geralmente desenvolvem margens mal definidas com destruição de corticais ósseas, características sugestivas de um processo maligno. A maioria das lesões são radiolúcidas uniloculares ou multiloculares, mas ocasionalmente podem apresentar-se como lesões mistas radiolúcidas-radiopacas (Chrcanovic *et al.*, 2018, Wright & Chi, 2023).

Microscopicamente, FSA apresentam um componente epitelial semelhante ao observado no FA, com características de benignidade e ausência de atipia celular, embora seja menos proeminente quando comparado ao FA. No entanto, o componente ectomesenquimal é caracterizado por hipercelularidade, apresentando graus variáveis de atipia e pleomorfismo celular. As células malignas ectomesenquimais frequentemente

demonstram núcleos hipercromáticos e células pleomórficas bizarras (Muller *et al.*, 1995; Wright & Chi, 2023). As mitoses são geralmente proeminentes. Em alguns casos reportados com múltiplas recorrências, o componente epitelial torna-se progressivamente menos evidente, de modo que o tumor perde suas características odontogênicas e eventualmente apresenta-se como um fibrossarcoma pouco diferenciado (Takeda, 1999). Independentemente de o componente epitelial estar presente ou ausente no tecido ectomesenquimal sarcomatoso, o FSA parece apresentar um comportamento clínico diferente do fibrossarcoma convencional. Em contraste com casos de fibrossarcoma convencional, metástases geralmente não ocorrem nos casos de FSA, apesar de apresentar características histopatológicas de alto grau. Acredita-se que o componente epitelial do tumor exerça um efeito organizacional sobre o componente ectomesenquimal, tanto em TOM benignos quanto em malignos (Reichart & Zobl, 1978, Takeda, 1999). Em aproximadamente 10% dos casos, a formação de dentina displásica ou pequenas quantidades de matriz de esmalte é observada e tais tumores são denominados como fibrodentinossarcomas ameloblásticos ou fibro-odontossarcomas ameloblásticos, e são incluídos na categoria de sarcomas odontogênicos (Richardson & Muller, 2014; Wright & Chi, 2023).

Uma vez confirmado o diagnóstico de FSA, a ressecção cirúrgica radical é o tratamento de escolha, com taxas de recorrência de aproximadamente 35% (Chrcanovic *et al.*, 2018, Wright & Chi, 2023). Curetagem ou excisão local conservadora geralmente é seguido por recorrências locais rápidas com o tumor infiltrando estruturas adjacentes. Metástase não é uma característica do FSA, ocorrendo apenas em 5% dos casos, e os casos fatais geralmente estão associados à infiltração local incontrolável após numerosas recorrências (Chrcanovic *et al.*, 2018, Wright & Chi, 2023).

1.4 BRAF p.V600E em tumores odontogênicos mistos

As interações entre as células epiteliais e ectomesenquimais conduzem o desenvolvimento dentário durante a odontogênese. Os

mecanismos moleculares associados à odontogênese envolvem cascatas de sinalização intracelular incluindo as vias MAPK, Hedgehog e Wnt, e alterações nessas vias têm sido associadas à patogênese de lesões odontogênicas (Diniz *et al.*, 2017; Guimarães *et al.*, 2017).

As vias de sinalização MAPK correspondem a '*master regulators*' de múltiplas respostas celulares e sua desregulação é comumente encontrada em diversos tumores no corpo humano (Guimarães *et al.*, 2017). O gene BRAF é o único membro da família de genes RAF frequentemente ativado por mutações em neoplasias humanas. A mutação no BRAF, que resulta na substituição de valina (V) por ácido glutâmico (E) no códon 600 (BRAF p.V600E), ativa constitutivamente a via MAPK/ERK (Ikawa *et al.*, 1988; Lavoie *et al.*, 2020). Além de estimular a sinalização MAPK/ERK, o BRAF p.V600E induz a proliferação celular e é capaz de promover a transformação maligna em alguns tumores, justificando a sua classificação como um oncogene (Pratilas *et al.*, 2012).

Estudos recentes demonstraram que TOM também foram incluídos no espectro de tumores induzidos pela via MAPK com mutações no BRAF p.V600E em FA, FDA/FOA e FSA (Coura *et al.*, 2020; Agaimy *et al.*, 2020; Oh *et al.*, 2021). Coletivamente, esses estudos relataram que 45,8% dos FA, 60% dos FDA e 34,6% dos FOA mostraram mutações patogênicas no gene BRAF p.V600E, enquanto todas as amostras de odontomas investigadas revelaram mutações do tipo selvagem para BRAF p.V600E. Esses resultados sugerem que pelo menos alguns FA, FDA e FOA de fato são entidades patológicas distintas dos odontomas. A patogênese molecular do FSA também demonstrou a presença de mutação de BRAF p.V600E variando entre 67% a 71% dos casos analisados (Coura *et al.*, 2020; Agaimy *et al.*, 2020). Em todos os casos analisados, a mutação do BRAF p.V600E estava presente apenas no componente ectomesenquimal dos tumores.

Embora a raridade destes tumores impeça um conhecimento amplo sobre suas características moleculares, os resultados atuais apoiam a importância do papel da via MAPK/ERK na patogênese de FA, FDA/FOA e FSA, abrindo caminho para futuras investigações sobre terapia alvo.

1.5 SOX9 em odontogênese

Nas últimas décadas, foram descobertos vários genes *'master regulators'* que controlam diferentes vias de diferenciação mesenquimal. Esses genes são expressos precocemente durante a embriogênese e são responsáveis pelo comprometimento de uma linhagem celular específica. SOX9 é um fator de transcrição da família SOX e desempenha um papel importante nas fases iniciais da diferenciação e sobrevivência de condrócitos e, portanto, é considerado como um regulador mestre da condrogênese e fundamental no desenvolvimento ósseo endocondral (Lefebvre *et al.*, 2019). Além disso, SOX9 também participa na proliferação e diferenciação das células da crista neural, células neuronais e gliais e manutenção de células-tronco no pâncreas, pulmão, intestino e folículo piloso (Jo *et al.*, 2014; Schock & LaBonne, 2020; Ming *et al.*, 2022).

De maneira interessante, vários estudos sugerem a participação de SOX9 na odontogênese (Mitsiadis *et al.*, 1998; Krivanek *et al.*, 2020; Ettaki *et al.*, 2025). Durante o desenvolvimento de molares e incisivos, SOX9 é altamente expresso em células progenitoras do epitélio e moderadamente em células progenitoras ectomesenquimais e da papila dentária (Mitsiadis *et al.*, 1998; Yang *et al.*, 2017). Em um estudo recente, a inativação de SOX9 em células progenitoras dentárias apicais em camundongos levou a raízes em molares mais curtas, fornecendo evidências adicionais, porém incompletas, sobre as funções do SOX9 na odontogênese (Lav *et al.*, 2023). Até o momento, SOX9 não tem sido estudado no contexto de tumores odontogênicos.

O objetivo do presente estudo é avaliar as características clinicopatológicas de uma série multicêntrica internacional de FA, FDA/FOA, e FSA, incluindo achados incomuns e analisar o perfil imuno-histoquímico desses tumores para BRAF p.V600E e SOX9, a fim de fornecer informações sobre seu comportamento biológico e apresentar evidências adicionais para uma possível reclassificação.

2 ARTIGOS

2.1 Artigo: New Insights into Ameloblastic Fibromas, Fibrodentinomas, and Fibro-odontomas: Findings from an International Multicenter Study

Artigo aceito para publicação no periódico *Head and Neck Pathology, 2025, Volume 19, Article number 57, DOI: 10.1007/s12105-025-01792-0* (Anexo 3)

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Presentation: Preliminary results of the present study were presented as an oral presentation during the American Academy of Oral & Maxillofacial Pathology (AAOMP) Annual Meeting (April 13-16th, 2024) in Orlando, FL – USA.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Abstract

Introduction: Ameloblastic fibroma (AF), ameloblastic fibrodentinoma (AFD), and ameloblastic fibro-odontoma (AFO) are rare mixed odontogenic tumors. While some authors propose that some cases may evolve into odontomas, other tumors with aggressive clinical features suggest a neoplastic origin. A subset of AF and AFD/AFO harbor the pathogenic BRAF p.V600E mutation. SOX9, known for its role in the differentiation of various cell types, particularly in chondrogenesis, has not been previously studied in odontogenic tumors. In this study, we report the clinicopathologic features of a large international cohort of AF and AFD/AFO cases and analyze the immunohistochemical expression of BRAF p.V600E and SOX9. **Material and methods:** Clinical and radiographic data were collected from four Oral and Maxillofacial Pathology service archives spanning 1991 to 2024. Deidentified slides were reviewed by two independent oral pathologists. Immunohistochemical staining for BRAF p.V600E and SOX9 was performed on non-decalcified tissue samples from cases with available specimens. **Results:** A total of 62 tumors were identified, including 30 AF cases and 32 AFD/AFO cases. The cohort consisted of 33 male and 29 female patients, with average ages of 15.3 years for AF and 12.3 years for AFD/AFO. Tumors predominantly affected the posterior mandible and appeared as unilocular or multilocular radiolucent or mixed lesions, often causing tooth impaction and cortical expansion, with an average size of 3.7 cm for AF and 2.5 cm for AFD/AFO. Two cases were classified as peripheral AF/AFD. Microscopically, all cases exhibited cellular mesenchymal components resembling dental papilla, with branching strands and islands of odontogenic epithelium. AFD/AFO cases also displayed dental hard tissue, and occasional chondromyxoid differentiation was observed within the stroma. Rare hybrid tumors were identified, including associations with calcifying odontogenic cysts, cemento-ossifying fibroma and central giant cell granuloma. BRAF p.V600E showed cytoplasmic positivity in the mesenchymal component of AF (81%) and AFD/AFO (54%). SOX9 exhibited diffuse nuclear immunoreactivity in both epithelial and mesenchymal components (92%). **Conclusion:** This study represents one of the largest well-documented series of AF and AFO/AFD, providing valuable clinicopathologic and immunohistochemical insights. Additionally, the diffuse expression of SOX9 in

both epithelial and mesenchymal components suggests a potential role in odontogenic differentiation, a novel finding that may have implications for understanding the histogenesis of these lesions. The aggressive behavior of some AFs and AFD/AFOs in our study supports their classification as odontogenic neoplasms rather than hamartomas.

Keywords: Odontogenic tumors, Ameloblastic fibroma, Ameloblastic fibro-odontoma, BRAF p.V600E, SOX9

Introduction

The ameloblastic fibroma (AF) is a benign mixed odontogenic tumor composed of ameloblastic epithelium with a primitive mesenchymal stroma, that maintains the potential for interaction between them, potentially forming mineralized odontogenic tissues such as dentinoid (ameloblastic fibrodentinoma [AFD]) and enamel-like matrix (ameloblastic fibro-odontoma [AFO]) [1-3]. Due to its microscopic similarity to areas of developing odontomas and evidence that some mixed odontogenic tumors continue to mature into odontomas, AFD and AFO were removed from the 2017 WHO Classification following a consensus that, once dental hard tissue is formed, most (greater than 50%) of these lesions would mature into odontomas [4-5].

Although clinical and radiographic criteria are still used for the diagnosis of these lesions, an increasing number of publications, including molecular studies, have focused on understanding the neoplastic nature of AF and AFD/AFO. These studies have revealed pathogenic mutations in the MAPK/ERK signaling pathway, notably in the proto-oncogene serine/threonine kinase B-Raf (BRAF), which is absent in odontomas [6-8]. These findings suggest that a subset of AF and AFD/AFO are, in fact, distinct pathological entities [6-8], although few studies have validated the use of immunohistochemistry for BRAF p.V600E in small samples [9]. Recently, dental pulp cells showed expression of SOX9, a transcription factor particularly involved in chondrogenesis [10-12] and to our knowledge, it has not been previously described in odontogenic tumors.

The aim of this study is to contribute with the clinicopathological characteristics of a large series of AF and AFD/AFO, including uncommon findings and their immunohistochemical profile for BRAF p.V600E and SOX9 in order to provide insight into their biological behavior and present additional evidence for a potential reclassification.

Materials and methods

This retrospective cross-sectional study analyzed a total of 62 cases of AF and AFD/AFO, retrieved from the archives of four oral pathology diagnostic centers across three countries, spanning from 1991 to 2024. Of these, 34 cases were from the USA (Texas A&M University), 17 cases from

Brazil (9 cases from the University of Campinas, and 8 cases from the Federal University of Rio de Janeiro), and 11 cases from South Africa (University of Pretoria). Demographic, clinical, and radiographic data were collected from patient records for subsequent analysis. All cases were reviewed by two or more board-certified oral pathologists and confirmed based on the histopathologic diagnostic criteria for AF, as outlined in the current WHO Classification of Head and Neck Tumors [1]. Cases exhibiting the deposition of dentinoid and/or enameloid material were classified as AFD and AFO, respectively. Cases lacking sufficient clinical and radiographic information, unconfirmed histopathologic diagnoses, or absent histopathologic slides were excluded from the study.

Immunohistochemical analyses were conducted on formalin-fixed paraffin-embedded tissues (FFPET) from diagnostic biopsies or surgical specimens of cases with available tissue. The following antibodies were used: BRAF p.V600E (ready-to-use, clone VE1, Ventana), SOX9 (1:2000 dilution, clone EPR14335, Abcam), pan-cytokeratin AE1/AE3 (1:300 dilution, clone AE1/AE3, Dako), CD68 (1:300 dilution, clone KP1, Dako), and vimentin (ready-to-use, clone v9, Dako). For the immunohistochemical reactions, 3µm sections were deparaffinized and rehydrated prior to antigen retrieval, which was performed in an EDTA/Tris solution (pH 9.0) or Citrate (pH 6) for 15 minutes in an electric pressure cooker. Endogenous peroxidase activity was blocked using 6% H2O2 for 15 minutes. Subsequently, sections were incubated with the primary antibody for 2 hours at room temperature. Immunohistochemical staining was performed using the EnVision detection system (Dako, Carpinteria, CA), according to the manufacturer's protocol, followed by exposure to diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St Louis, USA) for 5 minutes or with EnVision Labelled Polymer (Dako, Glostrup, Denmark). Finally, slides were counterstained with Carazzi's hematoxylin. All cases selected for immunohistochemical staining were non-decalcified. Positive reactions for BRAF p.V600E, AE1/AE3, CD68, and vimentin were confirmed by cytoplasmic staining, while SOX9 expression was confirmed by nuclear staining. Immunohistochemical reactions were classified as weak/strong and focal/diffuse. Fontana-Masson histochemical staining was performed, and the presence of brownish-black pigment was considered

indicative of melanin pigmentation. Positive controls were included in all reactions. A descriptive analysis of the clinical, radiographic, histopathologic, and immunohistochemical features is provided and discussed in this study. This research was conducted following the principles of the Helsinki Declaration for studies involving human subjects and approved by the respective local research ethics committees (FOP-UNICAMP, protocol number 43944721.1.0000.5418; University of Pretoria, Research Ethics Committee No 629/2021; Texas A&M University, IRB protocol number 24-0317).

Results

The demographic, clinical, and radiographic characteristics of the 62 cases of AF and AFD/AFO are summarized in **Table 1** and presented in detail in **Supplementary Table S1**.

A total of 30 cases were diagnosed as AF. Fifteen patients (50%) were male and fifteen (50%) were female, with an average age of 15.3 years (ranging from 3 to 38 years). Twenty-four cases (80%) affected the mandible and 4 cases (13%) the maxilla. The mandibular tumors were primarily located in the posterior regions (17 cases, 57%), with fewer cases in the anterior region (5 cases, 17%), and two cases (7%) were unspecified. All maxillary tumors (4 cases, 13%) were located in the posterior region.

Radiographically, the tumors were mostly described as well-defined unilocular (15 cases, 50%) or multilocular (6 cases, 20%) radiolucencies (26 cases, 87%) and, less frequently, as mixed radiolucent-radiopaque lesions (2 cases, 7%), with an average size of 3.7 cm. Common clinical and radiographic findings included cortical bone expansion (15 cases, 50%), cortical disruption (12 cases, 40%), and association with an impacted tooth (12 cases, 40%). Tooth displacement was observed in 7 cases (23%) and root resorption in 4 cases (13%) (**Fig 1A-C**). One patient presented with a slow-growing soft tissue nodule in the lower right gingiva, without radiographic alterations, and was classified as peripheral AF. Most patients were treated with conservative surgical excision (11 cases, 37%), while five patients (17%) underwent segmental resections.

A total of 32 cases were diagnosed as AFD/AFO. Eighteen patients (56%) were male, and 14 patients (44%) were female, with a mean age of

12.3 years (ranging from 5 to 25 years). Twenty cases (63%) affected the mandible, 18 (56%) in the posterior region and 2 (6%) in the anterior region, while 11 cases (34%) affected the posterior maxilla. One case (3%) was diagnosed as a peripheral AFD; an erythematous gingival nodule in the upper anterior gingiva with microscopic features consistent with AFD and epithelial component highlighted by AE1/AE3 (**Fig 2**).

Radiographically, most tumors were well-defined unilocular (17 cases, 53%) or multilocular (10 cases, 31%) lesions, presenting as either radiolucent (15 cases, 47%) or mixed radiolucent-radiopaque (14 cases, 44%) lesions, frequently associated with an impacted tooth (20 cases, 63%) (**Fig 1D-F**). The average size of AFD/AFO was 2.5 cm, with common findings including cortical bone expansion (18 cases, 56%) and tooth displacement (11 cases, 34%). Treatment typically involved conservative surgical excision, performed in 15 patients (47%), while two patients (6%) required segmental resections. Exuberant tumor growth after an incisional biopsy was a rare clinical manifestation observed in both AF and AFD/AFO (**Fig 3**).

Microscopically, the tumors presented as solid growths consisting of varying amounts of odontogenic epithelium embedded in primitive odontogenic mesenchymal tissue resembling dental papilla. The epithelial component in all cases appeared cytologically bland and benign, morphologically arranged in islands exhibiting enamel organ-like features, with peripheral cells demonstrating ameloblastic differentiation and stellate reticulum-like cells centrally (76%), with branching strands of bilayered epithelium resembling dental lamina (56%) (**Table 2**). Adjacent to the epithelial component, juxtaepithelial hyalinization deposits were observed in 19 cases (31%), whereas clear cells, characterized by polygonal cells with clear cytoplasm and eccentric nuclei, were present in 12 cases (19%). Focal areas of primordial odontogenic tumor-like invaginations, comprised of peripheral nodules of primitive ectomesenchymal tissue surrounded by a single layer of columnar ameloblastic epithelium, were observed in 11 cases (18%) (**Fig 4**).

Notably, AF more commonly exhibited a diffuse growth pattern (57%), while AFD/AFO predominantly presented with a lobular architecture (59%). Hypercellularity (23%) and bone infiltration (27%) were observed more

frequently in AF. Cytologic atypia and increased mitotic figures were rarely identified in either AF or AFD/AFO. Amorphous hyalinized material was present in 38 cases (61%). Furthermore, the authors observed an unusual chondromyxoid appearance of the mesenchymal tissue in 13 cases (21%) (**Fig 5**). Dental hard tissue deposition, composed of dysplastic dentinoid with or without enameloid matrix, was present in all 32 cases of AFD/AFO (100%). Occasional dystrophic calcification within the epithelial component was observed in 30% of AF cases and 34% of AFD/AFO cases.

A subset of tumors demonstrated unusual histopathologic features, including associations with ghost cell lesions (4 cases, 6%), particularly calcifying odontogenic cysts (COC), cystic compartments (4 cases, 6%), melanin pigmentation within the epithelial component (3 cases, 5%) as evidenced by Fontana-Masson staining, and stromal granular cells positive for vimentin and CD68 (2 cases, 3%) (**Fig 6**). One case of AFO demonstrated a rare association with cemento-ossifying fibroma (COF) and central giant cell granuloma (CGCG) (**Fig 7**). Detailed histopathologic features for each case are provided in **Supplementary Table S2**.

Immunohistochemical staining for BRAF p.V600E was performed in 40 cases (16 AF and 24 AFD/AFO) (**Table 2**). The mesenchymal cells showed positive cytoplasmic staining in 13 cases of AF (81%) and 13 cases of AFD/AFO (54%), with staining ranging from weak and focal expression (11 cases, 28%), weak and diffuse (6 cases, 15%), strong and diffuse (8 cases, 21%), and strong and focal (1 case, 3%) (**Fig 8**). The epithelial component was negative in all cases. The average age of patients with BRAF-positive AFs and AFD/AFOs was 17.5 and 12.6 years, respectively, while the average tumor size was 5.1 cm and 3.5 cm. No histologic differences were noted between BRAF-positive and BRAF-negative tumors. Three cases from this study had been previously reported by Coura et al. [6], all of which harbored wild-type mutations. These findings were validated in the current study with 100% concordance for BRAF p.V600E immunohistochemical expression. SOX9 staining was performed in 13 cases (5 AF and 8 AFD/AFO), with 12 cases (92%) showing strong and diffuse nuclear expression in both the epithelial and mesenchymal components. A characteristic feature of all tumors was the loss of nuclear SOX9 expression in the differentiated ameloblastic

cells (**Fig 9**). The immunohistochemical features of each case are described in **Supplementary Table S3**.

Discussion

More than fifty years have passed since the first WHO Classification of odontogenic tumors, and the mixed odontogenic tumor (MOT) category remains a contentious issue. The debate among oral pathologists regarding whether AF, AFD/AFO, and odontomas represent distinct entities or different stages of maturation of the same lesion has been extensively explored in the English-language literature [13, 14]. However, many of these discussions may arise from the imprecise use of the terms “hamartomas” and “neoplasms”, which could potentially lead to patient harm due to inadequate treatment [15, 16]. Currently, MOTs represent a challenging and controversial group of lesions in oral pathology. We present a large, well-documented series of AF and AFD/AFO from Brazil, the USA, and South Africa to help clarify the complexity surrounding the classification of these lesions.

AF and AFD/AFO demonstrate a significant predilection for pediatric patients, with peak incidence occurring in the second decade of life and a slight male predominance, with a ratio of approximately 1.4:1 [1, 17, 18]. In the present study, the average age of patients diagnosed with AF was older than those with AFD/AFO, a demographic trend also observed in previous studies, contrasting with the “maturation theory” proposed by Cahn & Blum [8, 15, 19, 20]. Like other odontogenic lesions, AF and AFD/AFO primarily affect the posterior jaws, particularly the mandible, where they typically present as unilocular or multilocular radiolucencies with varying amounts of radiopaque foci, often associated with the crown of an unerupted molar. These lesions commonly cause cortical bone expansion and thinning [1, 17-19]. Notably, none of our cases occurred in the anterior maxilla, a region typically affected by compound odontomas [19]. In this study, the average tumor size for AF and AFD/AFO was 3.7 and 2.5 cm, respectively. In a recent publication, Soluk-Tekkesin & Vered suggested that, in addition to patients younger than 13.5 years, lesions larger than 2.1 cm are more likely to represent true neoplasms rather than developing odontomas [19]. Based on these crucial clinical and radiographic features, clinicians and pathologists should consider

the possibility of odontomas, particularly in smaller lesions located in the anterior maxilla.

An interesting clinical feature observed in a subset of cases in the present study was exuberant tumor growth following a biopsy procedure. This finding was noted in both AF and AFD/AFO. After incisional biopsy, the lesions would often protrude from the surgical site and exteriorize into the oral cavity. Although this feature is not exclusive to AF or AFD/AFO, it suggests a more aggressive biological behavior than odontomas, further supporting their neoplastic nature. Additionally, while the cause of malignant transformation in AF and AFD/AFO remains unclear, multiple surgical procedures, particularly in recurrent tumors, appear to be an important contributing factor [23].

Peripheral AF and AFD/AFO are exceedingly rare, with only approximately 20 cases published in the literature [17, 18, 21, 22]. Interestingly, two patients in the present study presented with localized gingival nodules in the lower and upper gingiva, with unremarkable radiographic findings. Microscopic analysis revealed submucosal tumors with features consistent with AF and AFD, showing no connection to the overlying epithelium and only minimal chronic inflammatory infiltrate. Both patients were treated by conservative surgical excision. Notably, the peripheral AF case revealed strong and diffuse BRAF p.V600E immunohistochemical staining, suggesting a neoplastic nature rather than the previously assumed innocuous hamartomatous lesion [21]. To the best of our knowledge, this represents the first BRAF p.V600E-positive peripheral AF reported in the literature.

AF and AFD/AFO exhibit both epithelial and ectomesenchymal neoplastic components, encompassing a range of microscopic features. The epithelial component is typically organized into strands and islands, often consisting of a peripheral layer of cuboidal or columnar cells, with a central area resembling the stellate reticulum of the developing enamel organ [1, 24]. The quantity and density of the epithelial component may vary significantly within the same tumor and tend to be less prominent when compared to its malignant counterpart, ameloblastic fibrosarcoma [23, 24]. Juxtaepithelial hyalinization may occur adjacent to the epithelial component, reflecting exuberant basal lamina deposition, as previously suggested by ultrastructural studies [24].

Primordial odontogenic tumor (POT) is a recently recognized MOT characterized by primitive odontogenic ectomesenchyme surrounded by columnar odontogenic epithelium with ameloblastic differentiation, which typically invaginates into the ectomesenchymal component, forming lobular infoldings [25-27]. Interestingly, 11 cases (18%) in the present study exhibited focal POT-like areas characterized by peripheral epithelial invaginations surrounding the tumors, which have previously been reported in complex odontoma [28]. In contrast to true POT, the 11 cases with POT-like areas were characterized by ill-defined, unencapsulated tumors consisting of infiltrative islands of ameloblastic epithelium embedded in papilla-like mesenchyme, as typically seen in AF and AFD/AFO. Oral pathologists should be aware of the histopathologic overlap between AF, AFD/AFO, and POT, and exercise caution when diagnosing incisional biopsies of odontogenic lesions affecting the posterior mandible of pediatric patients. An additional uncommon microscopic feature observed in the epithelial component was melanocytic pigmentation in 3 cases (5%). While investigators have speculated on the possible origin and implications of melanin in odontogenic tumors, including a shared origin of melanocytes and odontogenic ectomesenchymal cells from neural crest cells, there is currently no evidence that pigmentation influences the biological behavior of these lesions [29, 30].

The ectomesenchymal component in AF and AFD/AFO is typically characterized by a diffuse or lobular growth of primitive cellular stroma resembling the dental papilla [1-3]. In contrast to previous publications [19], our findings revealed that AFD/AFOs more frequently exhibited a lobular architecture, while diffuse growth was more commonly observed in AF, contributing to its more infiltrative behavior. Additionally, both AF and AFD/AFO usually present with evenly hypercellular stroma, which should not be misinterpreted as an independent indicator of malignancy. In fact, bone infiltration and hypercellularity were more commonly observed in AF compared to AFD/AFO, which may account for initial concerns regarding malignant transformation.

Interestingly, 13 tumors (21%) exhibited focal to diffuse areas of chondromyxoid differentiation within the ectomesenchymal tissue, a microscopic feature rarely reported in the literature [31]. The tumor cells

displayed round-to-oval nuclei loosely arranged within the chondromyxoid mesenchyme. Recently, odontogenic tumors affecting the mandible in children have been reported under the name “reticular myxoid odontogenic neoplasm” with *STRN::ALK* fusion [32]. The authors described two mixed odontogenic tumors with dental hard tissue formation and a reticular/chondroid mesenchyme. AFO was considered a differential diagnosis, although the authors appeared unaware of the possibility of a reticular/chondroid appearance in AFO [32]. Our study demonstrates that chondromyxoid appearance falls within the morphologic spectrum of AF and AFD/AFO. Therefore, we performed SOX9 staining to investigate this unusual microscopic feature and interestingly, both epithelial and ectomesenchymal components revealed strong and diffuse nuclear positivity. SOX9, a transcription factor classically described as a master regulator of chondrogenesis, plays an essential role in the early phases of chondrocyte differentiation [38]. The association of SOX9 and odontogenesis has been scarcely reported, and this study represents the first evidence of SOX9 expression in odontogenic tumors [12]. A noteworthy immunohistochemical finding was the loss of nuclear SOX9 expression in the peripheral ameloblastic cells within the epithelial islands, similarly to what has been described previously in normal odontogenesis, where SOX9 labels all pulp cells but not odontoblasts or the dental follicle [12]

In rare cases, AF and AFD/AFO are associated with other odontogenic lesions, the most common being COC [33, 34]. While COC typically affects the anterior region, hybrid AF-COC cases tend to occur more frequently in the posterior jaws, a finding also observed in our study [34]. One AFO case in our series showed the unique association with COF and CGCG, which, to the best of our knowledge, represents the first description in the literature. COF and CGCG have been described in association with other odontogenic lesions, particularly central odontogenic fibroma (COdF) [35]. Similarly to COdF, we speculate that odontogenic ectomesenchyme in AF may contain unique stem cells capable of differentiating into osteoid-producing cells, osteoclast-like multinucleated giant cells, and fibroblast-like cells [35]. In addition, focal areas showing stromal granular cells positive for vimentin and CD68 were observed in two AF cases in the present study, a feature also

described in a variant of COdF [35]. These findings suggest a significant potential for cellular differentiation of primitive cells within the odontogenic ectomesenchyme in AF and AFD/AFO, highlighting an intriguing microscopic overlap between COdF and MOTs.

Accumulating evidence now indicates that a subset of AF and AFD/AFO harbor pathogenic BRAF p.V600E mutations [6, 8, 9]. Initially believed to be confined to the odontogenic epithelium, these mutations have been shown to primarily affect the mesenchymal component of AF and AFD/AFO [6-8, 36]. These findings support the hypothesis that tumor growth in these lesions is predominantly driven by the mesenchymal component [6]. A recent systematic review reported average percentage of BRAF p.V600E mutant expression rates of 48.1%, 60%, and 26.3% in AF, AFD, and AFO, respectively, based on molecular testing [37]. When assessed immunohistochemically, the expression rates dropped to 20% in AF and 10% in AFO. In the present study, we conducted BRAF p.V600E immunohistochemical staining in 40 tumors, detecting positive cytoplasmic expression in the mesenchymal component of AF and AFD/AFO in 81% and 54% of cases, respectively, which is significantly higher than previously reported in the literature. Three cases in this study had undergone molecular analysis [6], with results later validated by immunohistochemical staining. Interestingly, patients with BRAF-positive AFs and AFD/AFOs presented an average age of 17.5 and 12.6 years, respectively, with an average tumor size of 5.1 cm and 3.5 cm, clinical features that correspond with the attempt of characterizing true neoplasms as suggested by Soluk-Tekkesin & Vered [19]. The discrepancy between molecular and immunohistochemical results in previous publications may be due to the greater sensitivity of molecular tests, with some authors raising concerns about false-negative immunohistochemical results [9]. Indeed, several aggressive and destructive tumors in our study tested negative for BRAF p.V600E, highlighting the need for further molecular investigation in specific cases. Unfortunately, molecular investigation could not be performed in the present study, which would be ideal to confirm the presence of BRAF mutation. The higher positivity rates observed in the present study could be attributed to the larger sample size used for evaluating BRAF p.V600E compared to previous studies and the

possibility of false-positive staining [9]. The investigation of BRAF p.V600E mutations in odontogenic tumors remains a relatively recent topic in the literature and should be approached with caution in clinical practice, given the significant possibility of negative results in tumors with aggressive clinical features.

Conclusion

Our study represents one of the largest well-documented series of AF and AFD/AFO in the English-language literature. AFD/AFO affected younger patients when compared to AF and showed a predilection for the posterior regions of the jaws, rarely occurring as a peripheral tumor. Both epithelial and mesenchymal components of AF and AFO/AFD display a wide range of morphological variants, including melanin pigmentation, clear cells, ghost cells, mesenchymal granular cells, chondromyxoid differentiation, and the first report of AFO associated with COF and CGCG. BRAF p.V600E immunohistochemistry was frequently positive in the mesenchymal component of AF and AFD/AFO, while SOX9 was expressed in both epithelial and mesenchymal components. Additionally, the diffuse SOX9 expression in AF and AFD/AFO suggests a potential role in odontogenic differentiation, a novel finding that may have implications for understanding the histogenesis of these lesions. These results contribute to the molecular characterization of mixed odontogenic tumors and may aid in diagnostic differentiation and the development of new therapeutic approaches. The aggressive behavior of some AFs and AFD/AFOs in our study supports their classification as odontogenic neoplasms rather than hamartomas.

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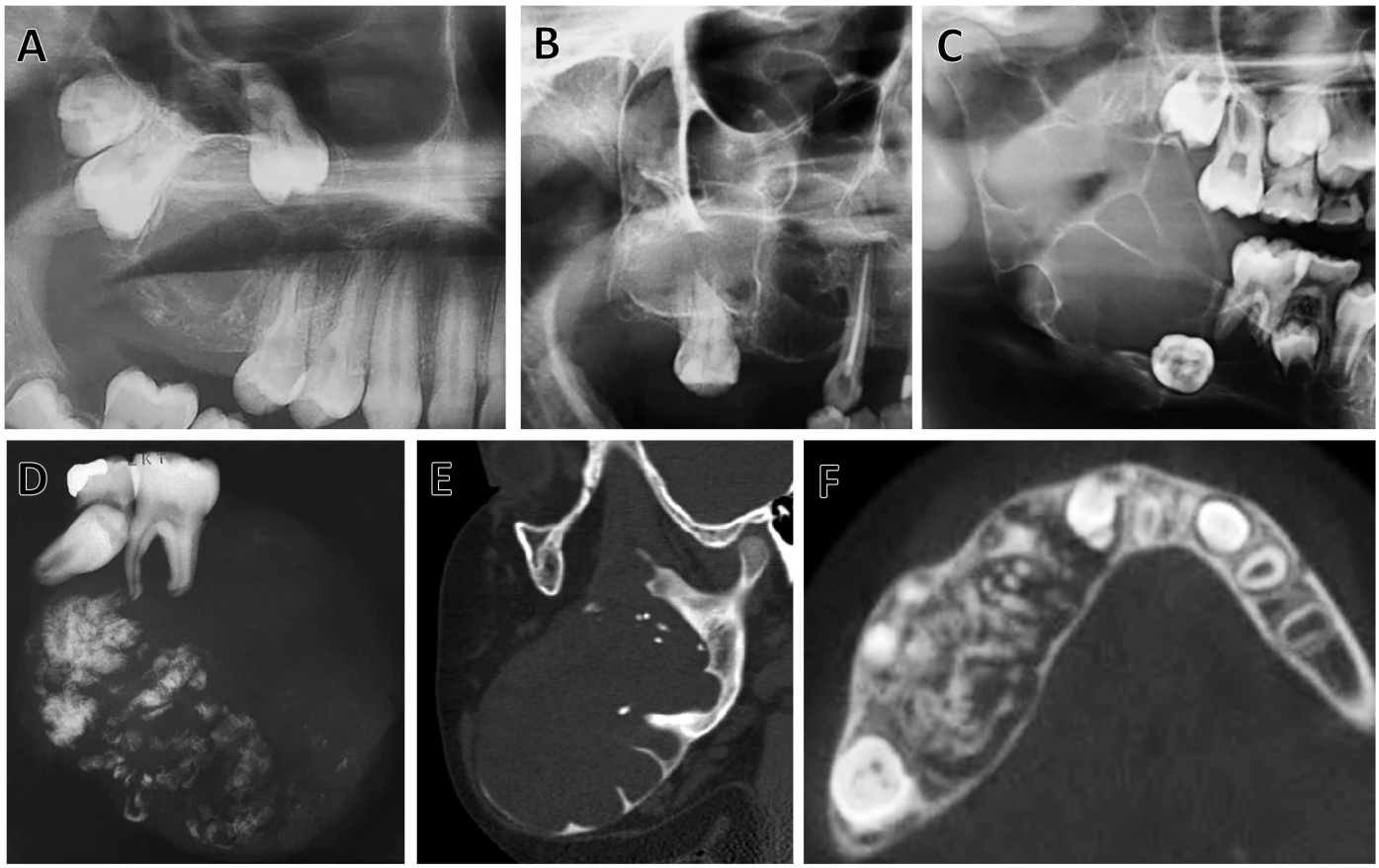

Figure 1. Radiographic features of AF and AFD/AFO. A-C. AF commonly presented as well-defined multilocular radiolucencies associated with unerupted molars, causing cortical bone expansion and root resorption. **D-F.** AFD/AFO were aggressive tumors showing varying amounts of radiopaque foci within the lesions and exuberant sizes.

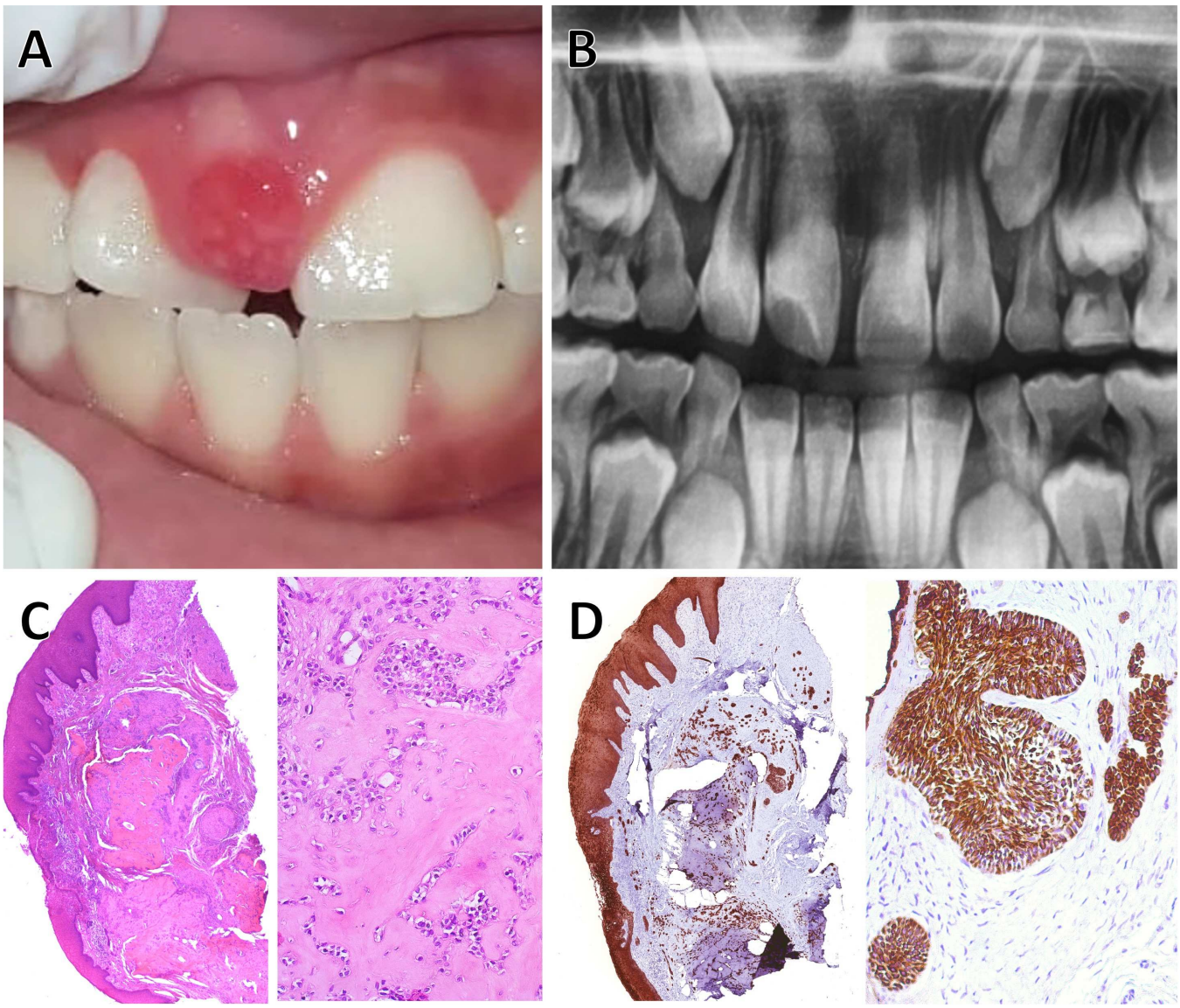

Figure 2. Clinical, histopathologic, and immunohistochemical features of peripheral AFD. A-B. An erythematous gingival nodule in the upper anterior gingiva showing no signs of intraosseous involvement. **C.** Microscopic features revealed a submucosal tumor consisting of odontogenic epithelium and primitive cellular ectomesenchyme with abundant dentinoid deposition. **D.** The odontogenic epithelium was highlighted by AE1/AE3. (**H&E, C** x50, x200; **IHC, D** x50, x200)

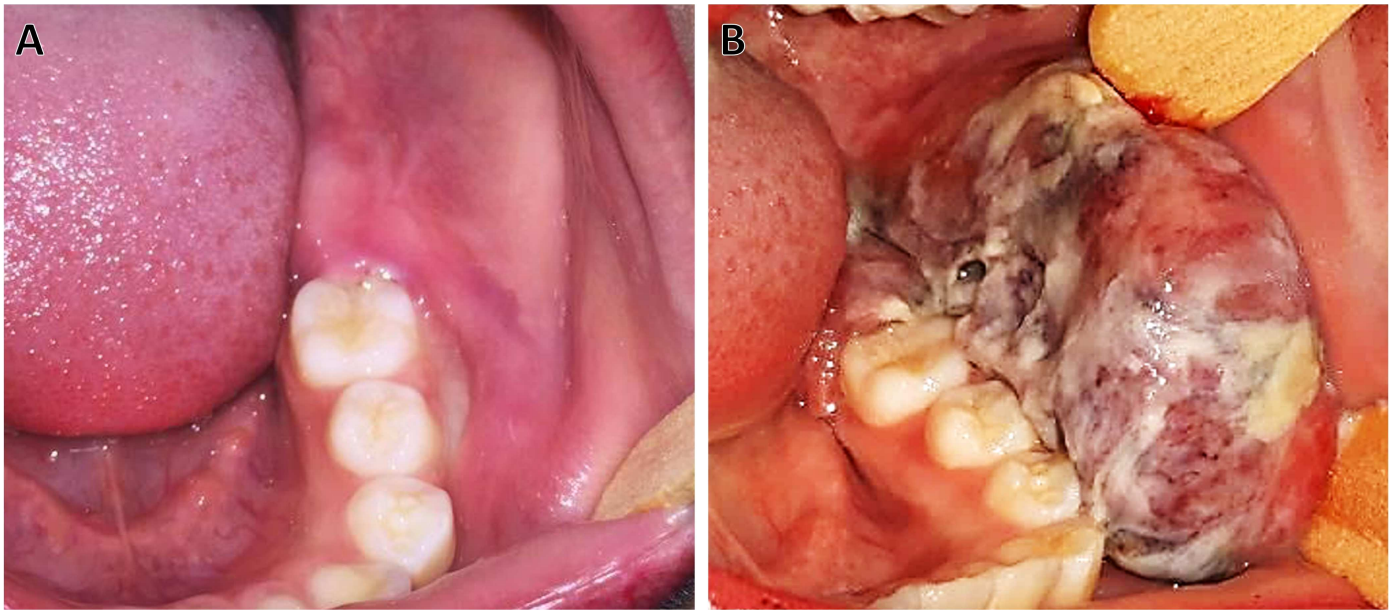

Figure 3. Exuberant tumor growth preceding an incisional biopsy. A. Initial intraoral aspect of a 12-year-old Brazilian girl diagnosed with AFD. **B.** A week after the incisional biopsy, the tumor revealed an exuberant growth protruding from the surgical site and exteriorizing into the oral cavity. No signs of malignancy were observed in the surgical resection.

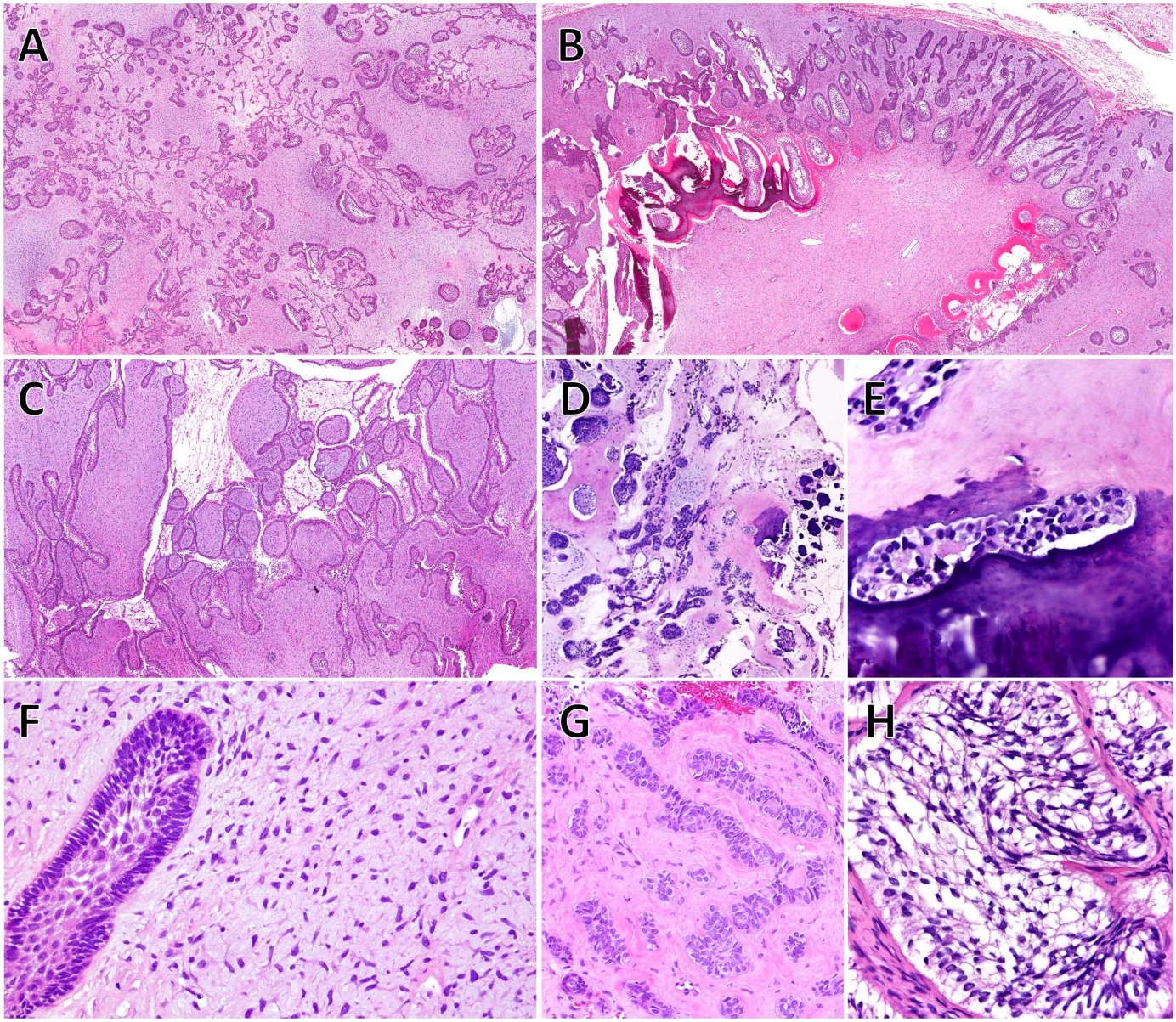

Figure 4. Histopathologic features of AF and AFD/AFO. The tumors presented as solid neoplasms consisting of varying amounts of odontogenic epithelium embedded in primitive odontogenic mesenchymal tissue resembling dental papilla. AF more commonly exhibited a diffuse growth pattern (**A**), while AFD/AFO predominantly presented with a lobular architecture (**B**). **C.** POT-like invaginations may be seen in a subset of cases. **D-E.** Dental hard tissue deposition was intimately associated with the epithelial islands. **F.** The epithelial component in all cases appeared cytologically bland, morphologically arranged in islands exhibiting enamel organ-like features, with peripheral cells demonstrating ameloblastic differentiation and stellate reticulum-like cells centrally. **G.** Branching strands of bilayered epithelium resembled dental lamina. **H.** Clear cells were occasionally observed. (**H&E, A-C** x25; **D** x50, **E** x400, **F-G** x200, **H** x400)

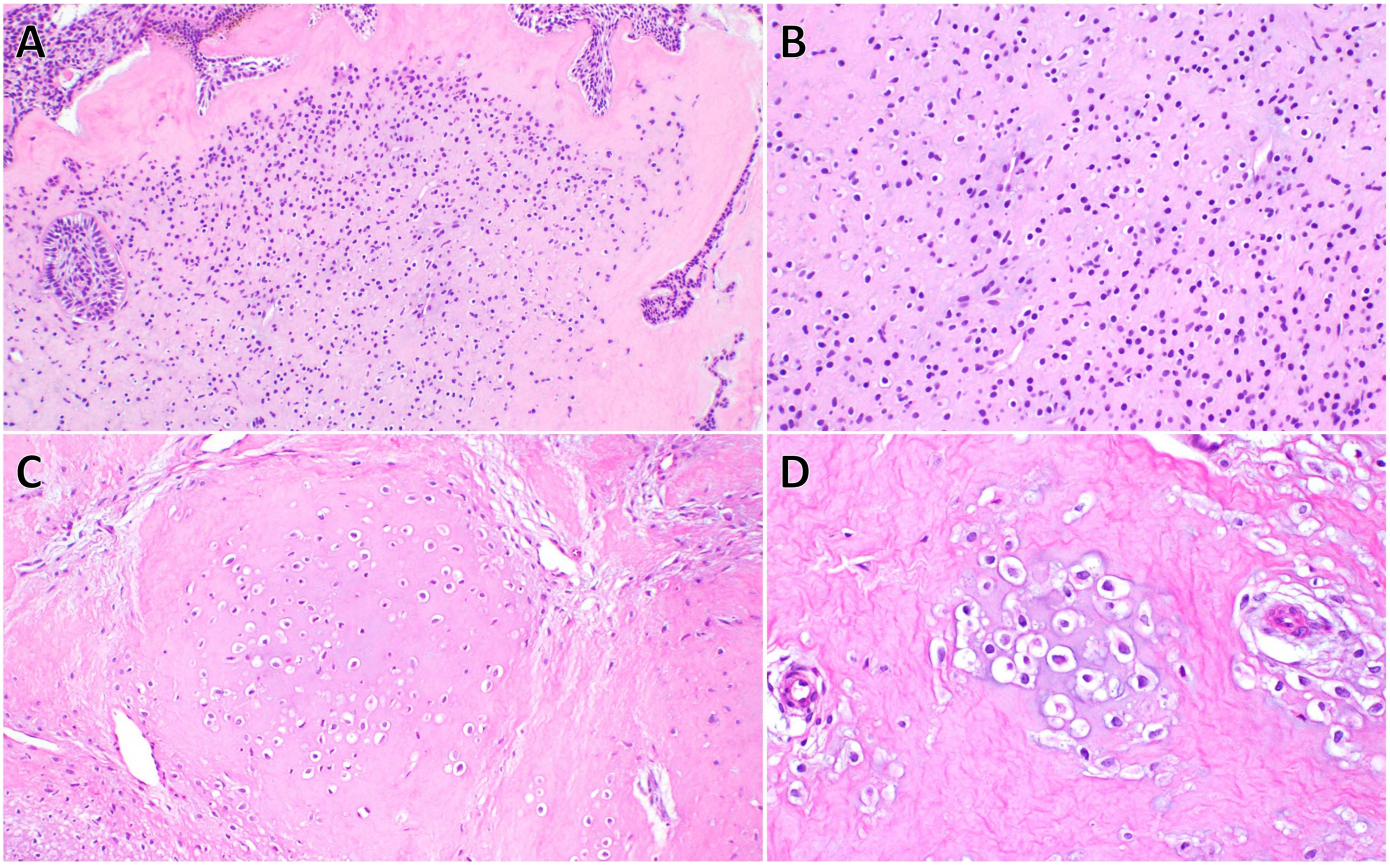

Figure 5. Chondromyxoid differentiation in the ectomesenchymal tissue. Chondroid-like areas were observed in the ectomesenchymal component of both AF and AFD/AFO. The cells displayed round-to-oval nuclei, loosely arranged within the chondromyxoid mesenchyme. (**H&E, A,C** x100; **B** x200; **D** x400)

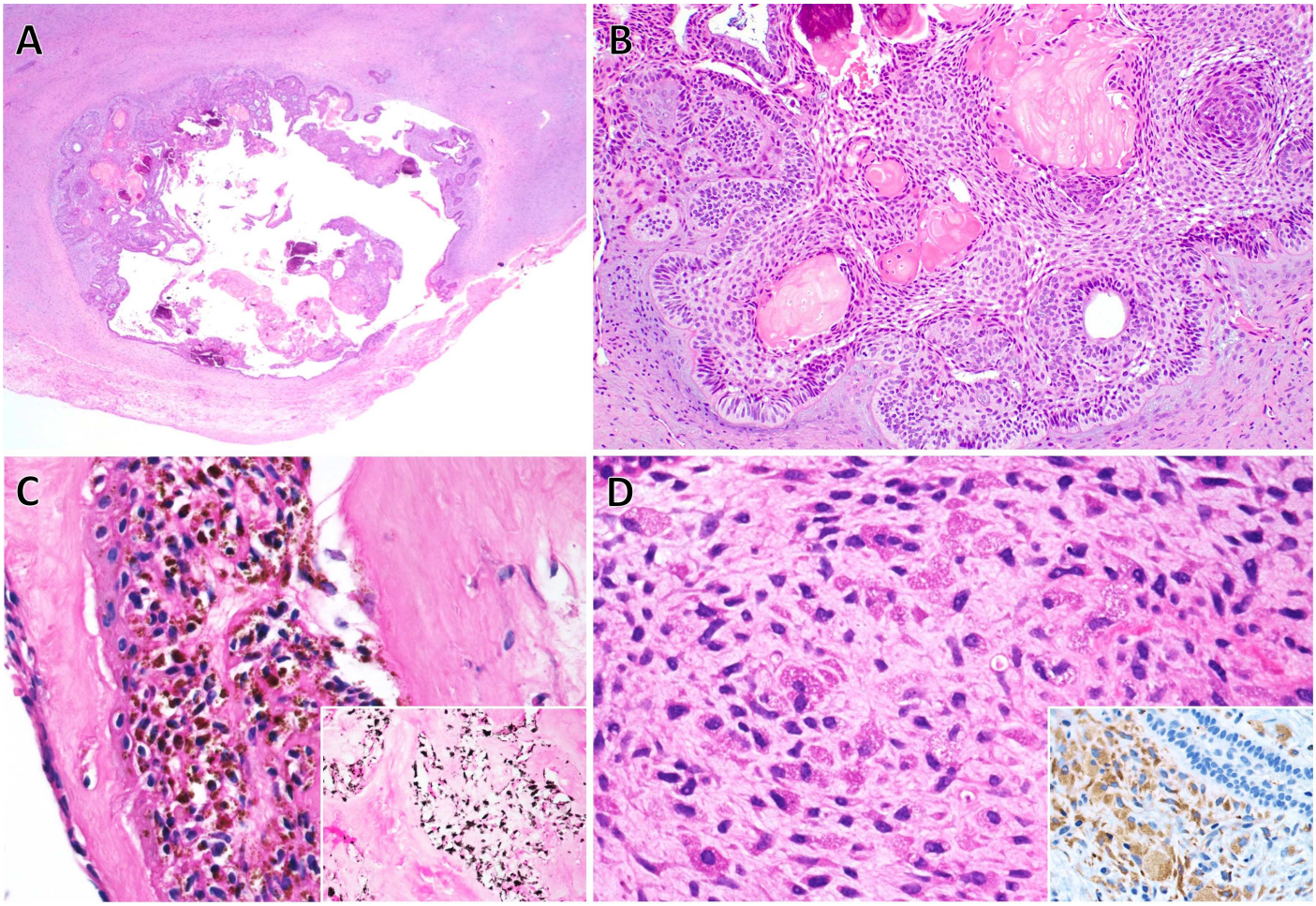

Figure 6. Uncommon histopathologic features of AF and AFD/AFO. A-B. AF and AFD/AFO may occasionally be associated with COC, showing cystic cavities lined with ameloblastic epithelium and numerous ghost cells that often undergo calcification. **C.** Melanocytic pigmentation is rarely observed in the epithelial component, which is confirmed by Fontana-Masson histochemical staining (Insert). **D.** Focal areas showing granular cells in the stromal component were positive for CD68 (Insert). (**H&E, A** x50; **B** x200, **C-D** x400; **Fontana-Masson,** x400; **IHC,** x400)

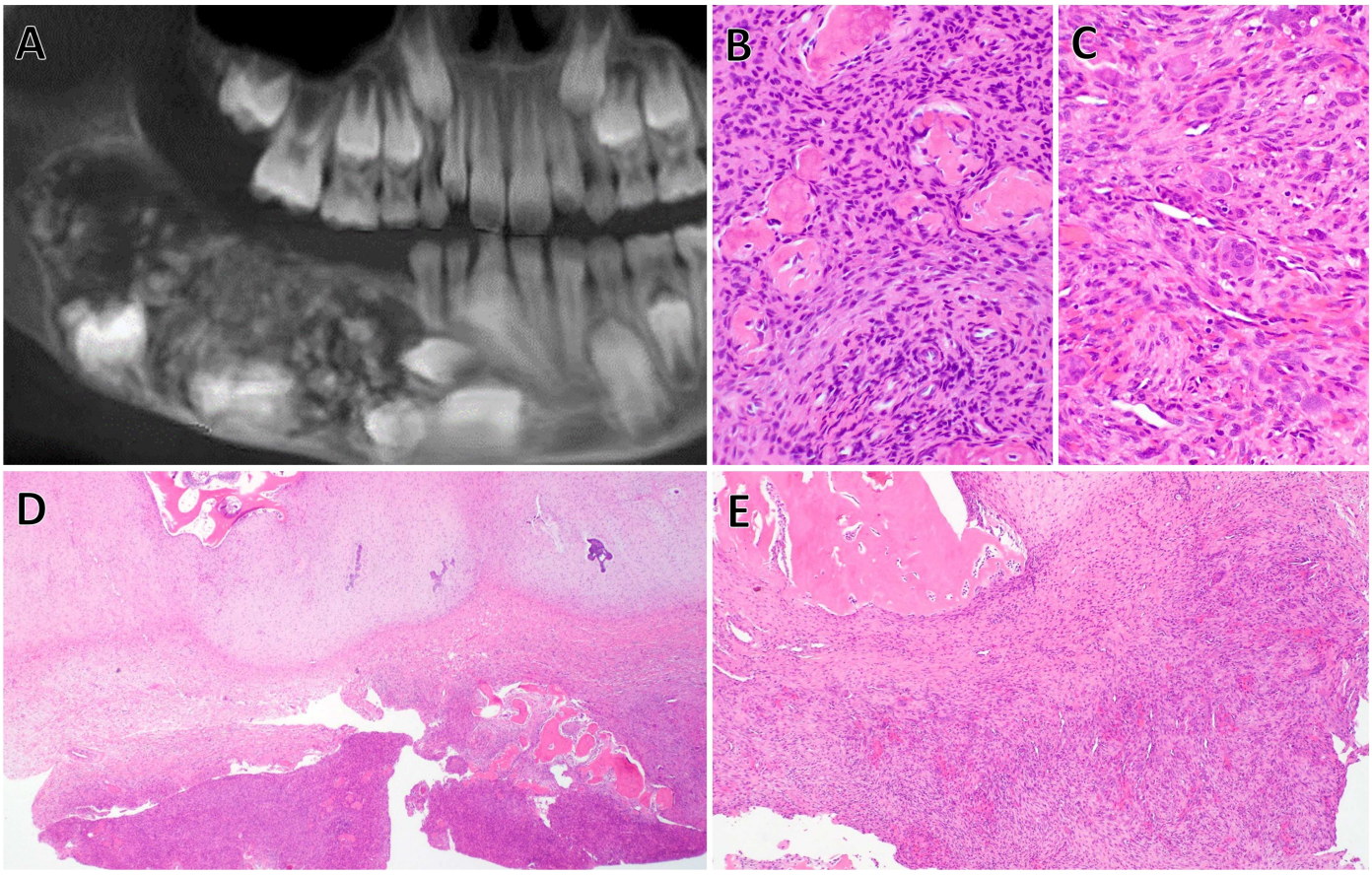

Figure 7. AFO with cemento-ossifying fibroma and central giant cell granuloma. A. An 8-year-old boy presented with an aggressive mixed lesion causing multiple teeth impaction and cortical bone disruption. **B-E.** Microscopic analysis revealed adjacent to the AFO, areas of highly spindled stroma containing bone and cementum-like deposits consistent with cemento-ossifying fibroma and a vascularized fibrous stroma suspending numerous multinucleated giant cells, compatible with central giant cell granuloma were observed. (**H&E, B-C** x200; **D** x50; **E** x100)

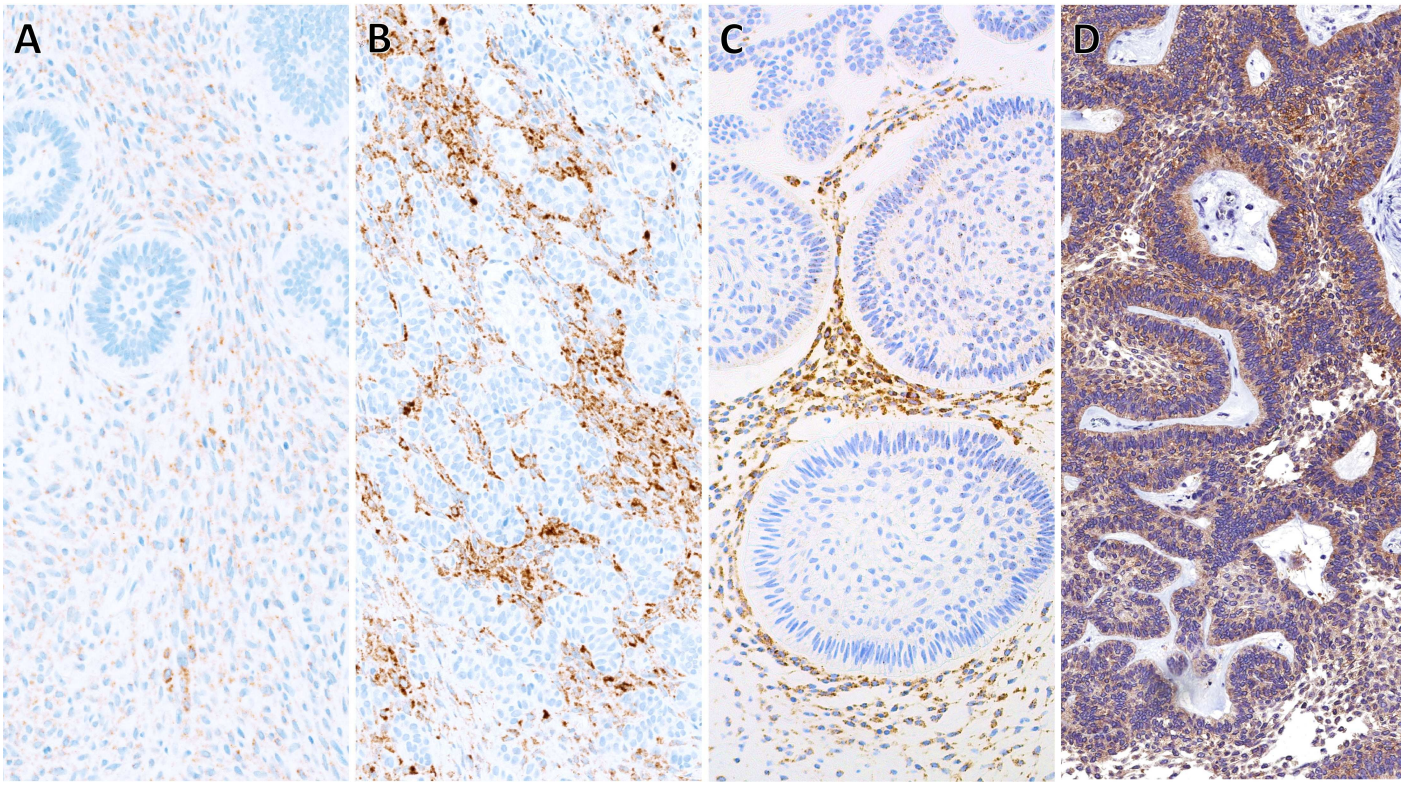

Figure 8. Immunohistochemical expression of BRAF p.V600E in AF and AFD/AFO. Positive BRAF p. V600E ranged from weak (**A**) to strong (**B-C**) cytoplasmic expression in the mesenchymal cells in AF and AFD/AFO. The epithelial component in all cases was negative. **D.** The positive control showed diffuse cytoplasmic staining in the epithelium of a conventional ameloblastoma. (**IHC, A-D** x200)

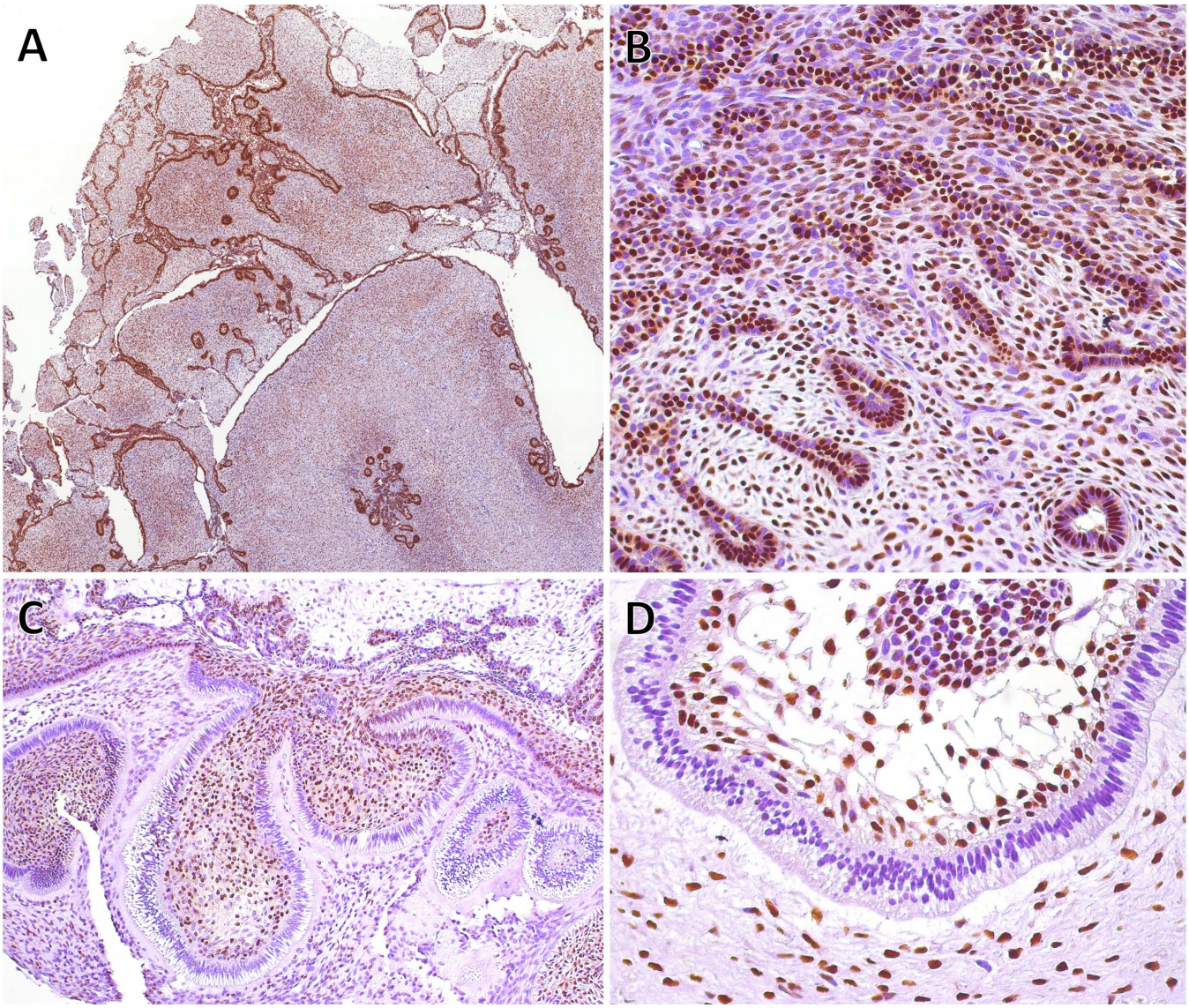

Figure 9. Immunohistochemical expression of SOX9 in AF and AFD/AFO. A-B. The tumors expressed strong and diffuse nuclear SOX9 expression in the epithelial and mesenchymal tissue. **C-D.** A characteristic immunohistochemical finding was the loss of nuclear expression of SOX9 in the peripheral ameloblastic cells within the epithelial islands. (**IHC, A** x50; **B** x200; **C** x100; **D** x400)

Table 1. Summarized demographic, clinical, and radiographic features of AF and AFD/AFO

Features	***Results (%)***	
	AF (n=30)	**AFD/AFO (n=32)**
Age (range)	15.3 (3-38)	12.3 (5-25)
Gender	15F / 15M	14F / 18M
Site		
Mandible		
Anterior / Posterior	5 (17%) / 17 (57%)	2 (6%) / 18 (56%)
Maxilla		
Anterior / Posterior	0 (0%) / 4 (13%)	0 (0%) / 11 (34%)
Peripheral	1 (3%)	1 (3%)
Radiographic appearance		
Radiolucent / Mixed	26 (87%) / 2 (7%)	15 (47%) / 14 (44%)
Loculation		
Unilocular / Multilocular	15 (50%) / 6 (20%)	17 (53%) / 10 (31%)
Cortical bone		
Expansion / Disruption	15 (50%) / 12 (40%)	18 (56%) / 11 (34%)
Impacted tooth	12 (40%)	20 (63%)
Root resorption	4 (13%)	4 (13%)
Tooth displacement	7 (23%)	11 (34%)
Size (avg)	3.7 cm	2.5 cm
> 2 cm	14 (47%)	19 (59%)
< 2 cm	6 (20%)	2 (6%)
Treatment		
Conservative excision / Segmental resection	11 (37%) / 5 (17%)	15 (47%) / 2 (6%)

AF, ameloblastic fibroma; ***AFD,*** ameloblastic fibrodentinoma; ***AFO,*** ameloblastic fibro-odontoma

Table 2. Summarized histopathologic and immunohistochemical features of AF and AFD/AFO

Features	***Results (%)***	
	AF (n=30)	**AFD/AFO (n=32)**
Epithelium		
Islands	EO = 20 (67%); DL = 10 (33%)	EO = 27 (84%); DL = 5 (16%)
Strands	EO = 10 (33%); DL = 17 (57%)	EO = 14 (44%); DL = 18 (56%)
Clear cells	3 (10%)	9 (28%)
Juxtaepithelial hyalinization	11 (37%)	8 (25%)
POT-like invaginations	4 (13%)	7 (22%)
Mesenchyme		
Growth pattern	Dif = 17 (57%); Lob = 13 (43%)	Dif = 13 (41%); Lob = 19 (59%)
Hypercellularity	7 (23%)	5 (16%)
Amorphous hyalinized material	19 (63%)	19 (59%)
Bone infiltration	8 (27%)	3 (9%)
Chondroid differentiation	6 (20%)	7 (22%)
Atypia	3 (10%)	1 (3%)
Mitotic figures	1 (3%)	2 (6%)
Hard tissue		
Dentinoid	0 (0%)	32 (100%)
Enameloid	0 (0%)	14 (44%)
Dystrophic calcifications	9 (30%)	11 (34%)
BRAF p.V600E*	**N=16**	**N=24**
Positive	**13 (81%)**	**13 (54%)**
Strong and diffuse	3 (19%)	5 (21%)
Strong and focal	0 (0%)	1 (4%)
Weak and diffuse	4 (25%)	2 (8%)
Weak and focal	6 (38%)	5 (21%)
Negative	**3 (19%)**	**11 (46%)**
SOX9	**N=5**	**N=8**
Epithelial component		
Strong and diffuse	5 (100%)	5 (63%)
Strong and focal	0 (0%)	2 (25%)
Mesenchymal component		
Strong and diffuse	5 (100%)	6 (75%)
Weak and diffuse	0 (0%)	1 (13%)
Negative	**0 (0%)**	**1 (13%)**

AF, ameloblastic fibroma; ***AFD,*** ameloblastic fibrodentinoma; ***AFO,*** ameloblastic fibro-odontoma; ***POT,*** primordial odontogenic tumor; ***EO,*** enamel organ-like; ***DL,*** dental lamina-like; ***Dif,*** diffuse; ***Lob,*** lobular
*Only mesenchymal component stained positive

Supplementary table S1. Clinical and radiographic features of AF and AFD/AFO from Brazil, USA, and South Africa

N	*Dx*	*Country*	*Age, Gnd*	*Site*	*Clinical/Radiographic features*	*Impacted tooth*	*Root resorption*	*Size (cm)*	*Treatment*	*Recurrence, Follow-up*
1	AF	Brazil	3, M	Mand, Post (Ramus)	ML RL, cortical expansion and disruption	Yes (Mol)	No	6	Conservative excision	NI, NI
2	AF	Brazil	7, M	Mand, Post (Mol)	UL RL, cortical expansion and disruption	Yes (Mol)	No	1.2	NI	NI, NI
3	AF	Brazil	14, M	Mand, Ant	RL, cortical expansion and disruption	NI	NI	NI	NI	NI, NI
4	AF+GCT	Brazil	16, F	Max, Post	UL RL, cortical expansion and disruption	Yes (Mol)	Yes	6	Seg ressection	NI, NI
5	AF atypical	Brazil	21, M	Mand, Post (Body)	RL	NI	NI	5	Conservative excision	NI, NI
6	AF	Brazil	37, F	Max, Post	ML RL, cortical expansion	No	Yes	6	NI	NI, NI
7	AF	South Africa	4, F	Mand	RL	NI	NI	NI	NI	NI, NI
8	AF	South Africa	10, M	Mand, Post (Body-Ramus)	ML RL, cortical expansion and disruption	Yes	NI	8	NI	NI, NI
9	AF	South Africa	12, M	Max, Post	ML RL, cortical expansion	Yes (Mol)	No	5	NI	NI, NI
10	AF	South Africa	13, F	Mand, Post (Mol)	RL	No	NI	NI	NI	NI, NI
11	AF	South Africa	20, M	Mand, Ant and Post	ML RL, cortical expansion and disruption	NI	NI	12	Hemimandibulectomy	NI, NI
12	AF	South Africa	36, F	Mand, Post (Body-Ramus)	RL, cortical expansion	NI	NI	9	Hemimandibulectomy	NI, NI
13	AF	South Africa	NI, M	NI	NI	NI	NI	NI	NI	NI, NI
14	AF	USA	7, M	Gingiva, Lower	No radiographic alterations	No	No	NI	Conservative excision	NI, NI
15	AF	USA	7, F	Mand, Post (Mol)	UL RL, cortical disruption	Yes (Mol)	No	1.5	NI	NI, NI
16	AF	USA	8, F	Mand, Post (Mol)	UL RL	No	No	1	Conservative excision	NI, NI
17	AF	USA	8, F	Mand, Post (Mol)	UL RL, cortical expansion and disruption	Yes (Mol)	No	2	NI	NI, NI
18	AF	USA	8, F	Max, Post	UL RL	Yes (Mol)	NI	1.5	NI	NI, NI
19	AF+COC	USA	10, M	Mand, Post (Mol)	UL MX, cortical expansion	Yes (Mol)	Yes	4	NI	NI, NI
20	AF	USA	12, M	Mand, Post (Body)	UL MX	No	Yes	2	Conservative excision	NI, NI
21	AF+COC	USA	13, F	Mand, Ant	UL RL, cortical expansion and disruption	No	No	1	Conservative excision	NI, NI
22	AF+COC	USA	13, M	Mand, Post (Body-Ramus)	UL RL, cortical expansion	No	No	5	NI	NI, NI
23	AF	USA	13, F	Mand, Post (Mol)	UL RL	Yes (Mol)	NI	NI	NI	NI, NI
24	AF	USA	14, F	Mand, Post (Mol)	UL RL	Yes (Mol)	No	NI	Conservative excision	NI, NI
25	AF	USA	16, M	Mand, Ant	UL RL, cortical disruption	No	NI	NI	Conservative excision	NI, NI
26	AF	USA	16, M	Mand, Post (Body-Ramus)	UL RL, cortical expansion and disruption	Yes (Mol)	NI	4	Conservative excision	NI, NI
27	AF	USA	27, F	Mand, Ant	UL RL	No	No	1	Conservative excision	NI, NI
28	AF	USA	28, M	Mand	RL, cortical expansion and disruption	NI	NI	NI	Hemimandibulectomy	NI, NI
29	AF	USA	28, F	Mand, Ant	RL	No	NI	NI	Conservative excision	NI, NI
30	AF	USA	38, F	Mand, Post (Body-Ramus)	ML RL	No	No	2.5	Marginal ressection	NI, NI
31	AFO	Brazil	7, M	Max, Post	UL MX, cortical expansion and disruption	Yes (Mol)	NI	8	NI	NI, NI
32	AFO	Brazil	8, M	Mand, Post (Body-Ramus)	UL RL, cortical expansion and disruption	Yes (Mol)	No	6	Conservative excision	NI, NI
33	AFD	Brazil	9, M	Mand, Post	Cortical expansion	NI	NI	5	Conservative excision	NI, NI
34	AFD	Brazil	9, F	Mand, Post (Ramus)	ML RL, cortical expansion and disruption	Yes (Mol)	Yes	7	Conservative excision	No, 3 years
35	AFD	Brazil	9, M	Gingiva, Upper	Erythematous nodule, no radiographic alterations	No	No	1	Conservative excision	NI, NI
36	AFD	Brazil	12, F	Mand, Post (Ramus)	ML MX, cortical expansion	Yes (Mols)	No	6	Seg mandibulectomy	NI, NI
37	AFD	Brazil	14, M	Max, Post	ML MX, cortical expansion	Yes (Mol)	No	3	NI	NI, NI
38	AFD	Brazil	14, M	Mand, Post (Mol)	ML MX	Yes (Mol)	No	3	NI	NI, NI
39	AFO	Brazil	15, M	Mand, Post (Mol)	ML MX, cortical expansion and disruption	No	No	3	NI	NI, NI
40	AFO	Brazil	15, M	Max, Post	ML MX, cortical expansion and disruption	Yes (Mol)	Yes	2.5	NI	NI, NI

41	AFD	Brazil	17, F	Max, Post	Cortical expansion	Yes (P)	NI	NI	NI	NI, NI
42	AFO	South Africa	11, M	Max, Post	UL MX, cortical expansion	NI	NI	5.5	NI	NI, NI
43	AFD	South Africa	16, F	Max, Post	UL MX, cortical expansion	Yes (Mol)	No	4	Conservative excision	NI, NI
44	AFD	South Africa	17, F	Mand, Post (Body-Ramus)	ML RL, cortical expansion	NI	Yes	NI	NI	NI, NI
45	AFD	South Africa	23, F	Max, Post	UL MX	Yes (Mols)	NI	NI	NI	NI, NI
46	AFD	USA	5, M	Mand, Post (Mol)	RL	No	NI	NI	Conservative excision	NI, NI
47	AFD	USA	8, M	Max, Post	UL RL	Yes (C)	Yes	2	Conservative excision	NI, NI
48	AFD	USA	8, M	Mand, Post (Mol)	UL RL, cortical disruption	No	No	2.5	Conservative excision	NI, NI
49	AFO	USA	8, F	Mand, Post (Body-Ramus)	ML RL, cortical disruption	Yes (Mol)	No	2.5	Conservative excision	NI, NI
50	AFO+COF +CGCG	USA	8, M	Mand, Post (Body-Ramus)	UL MX, cortical expansion and disruption	Yes (Mols)	No	9.5	NI	NI, NI
51	AFO	USA	10, M	Max, Post	UL RL, cortical disruption	Yes (Mol)	No	2	Conservative excision	NI, NI
52	AFD	USA	10, F	Mand, Post (Mol)	UL RL	Yes (Mol)	NI	NI	NI	NI, NI
53	AFD	USA	10, F	Mand, Post (Mol)	ML RL, cortical expansion	No	NI	3	Seg mandibulectomy	NI, NI
54	AFD	USA	11, F	Mand, Post (Mol)	UL RL, cortical expansion	No	NI	NI	Conservative excision	Yes, 1 year
55	AFD	USA	11, M	Mand, Post (Mol)	UL RL	Yes (Mol)	No	3	Conservative excision	NI, NI
56	AFO	USA	13, F	Max, Post	MX	Yes (Mol)	NI	NI	NI	NI, NI
57	AFO	USA	13, M	Max, Post	UL RL	Yes (Mol)	NI	NI	NI	NI, NI
58	AFO	USA	14, M	Mand, Post (Mol)	UL MX	Yes (P)	NI	NI	Conservative excision	NI, NI
59	AFO	USA	14, M	Mand, Post (Mol)	UL MX, cortical expansion	Yes (Mol)	No	2.5	Conservative excision	NI, NI
60	AFD	USA	14, F	Mand, Ant	UL RL	No	No	1.5	Conservative excision	NI, NI
61	AFO	USA	15, F	Mand, Post (Body-Ramus)	ML MX, cortical expansion and disruption	No	NI	NI	NI	NI, NI
62	AFD	USA	25, F	Mand, Ant	UL RL	No	NI	NI	NI	NI, NI

Dx, diagnosis; ***Gnd,*** gender; ***F,*** female; ***M,*** male; ***AF,*** ameloblastic fibroma; ***AFD,*** ameloblastic fibrodentinoma; ***AFO,*** ameloblastic fibro-odontoma; ***COC,*** calcifying odontogenic cyst; ***GCT,*** ghost cell tumor; ***COF,*** cemento-ossifying fibroma; ***CGCG,*** central giant cell granuloma; ***Mand,*** mandible; ***Max,*** maxilla; ***Ant,*** anterior; ***Post,*** posterior; ***UL,*** unilocular; ***ML,*** multilocular; ***RL,*** radiolucent; ***MX,*** mixed; ***C;*** canine; ***Mol,*** molar; ***P,*** premolar; ***Seg,*** segmental; ***NI,*** not informed

Supplementary table S2. Histopathologic features of AF and AFD/AFO from Brazil, USA, and South Africa

		Epithelium						*Mesenchyme*				*Hard tissue*		
N	***Dx***	***Islands, Strands***	***Clear cells***	***Juxtaepithelial hyalinization***	***POT-like areas***	***Growth pattern***	***Hypercellularity***	***Amorphous hyalinized material***	***Bone infiltration***	***Chondroid differentiation***	***Atypia, Mitotic figures***	***Dentinoid, Enameloid***	***Dystrophic calcification***	***Additional findings***
1	AF	EO, DL	No	No	Yes	Diffuse	No	No	No	Yes	No, No	No, No	No	
2	AF	DL, DL	No	No	No	Lobular	No	Yes	No	Yes	No, No	No, No	Yes	
3	AF	EO	No	No	No	Diffuse	No	No	No	No	No, No	No, No	No	
4	AF+GCT	EO, EO	Yes	Yes	Yes	Diffuse	Yes	No	Yes	No	No, Yes	No, No	No	Associated with ghost cell lesion
5	AF atypical	EO, EO	No	Yes	No	Diffuse	Yes	No	No	No	Yes, No	No, No	No	
6	AF	EO, DL	No	No	No	Lobular	Yes	Yes	Yes	No	Yes, No	No, No	No	
7	AF	EO, EO	No	Yes	No	Diffuse	No	No	No	No	No, No	No, No	No	
8	AF	EO, DL	No	No	No	Diffuse	Yes	Yes	No	No	Yes, No	No, No	No	
9	AF	EO, EO	No	Yes	No	Diffuse	Yes	Yes	Yes	No	No, No	No, No	Yes	Stromal granular cells
10	AF	EO, DL	No	Yes	No	Diffuse	No	Yes	Yes	No	No, No	No, No	No	
11	AF	EO, EO	No	No	No	Diffuse	No	No	Yes	Yes	No, No	No, No	No	
12	AF	EO, EO	No	Yes	No	Diffuse	No	Yes	Yes	No	No, No	No, No	No	
13	AF	EO, EO	No	Yes	No	Diffuse	No	No	No	No	No, No	No, No	No	Cystic component
14	AF	EO, DL	Yes	No	No	Lobular	No	Yes	No	Yes	No, No	No, No	No	Peripheral AF
15	AF	DL, EO	No	No	Yes	Lobular	No	Yes	No	No	No, No	No, No	Yes	
16	AF	DL, DL	No	No	No	Lobular	No	Yes	No	No	No, No	No, No	No	
17	AF	EO, DL	No	No	No	Lobular	No	Yes	No	Yes	No, No	No, No	Yes	
18	AF	DL, DL	No	No	No	Lobular	No	Yes	No	No	No, No	No, No	Yes	
19	AF+COC	DL, DL	No	Yes	No	Diffuse	No	Yes	No	No	No, No	No, No	Yes	Associated with COC
20	AF	DL, DL	No	Yes	No	Lobular	No	No	Yes	No	No, No	No, No	No	
21	AF+COC	EO	No	No	No	Lobular	No	Yes	No	No	No, No	No, No	Yes	Associated with COC
22	AF+COC	DL, DL	No	No	No	Lobular	No	No	No	No	No, No	No, No	No	Associated with COC
23	AF	EO, DL	No	No	Yes	Lobular	Yes	Yes	No	No	No, No	No, No	No	
24	AF	DL, DL	No	No	No	Lobular	No	Yes	No	No	No, No	No, No	Yes	
25	AF	DL, DL	No	No	No	Diffuse	No	No	No	Yes	No, No	No, No	No	
26	AF	EO, EO	Yes	Yes	No	Diffuse	No	Yes	No	No	No, No	No, No	No	Cystic component and squamous metaplasia
27	AF	EO, DL	No	No	No	Lobular	No	Yes	No	No	No, No	No, No	No	
28	AF	EO, EO	No	Yes	No	Diffuse	Yes	Yes	Yes	No	No, No	No, No	Yes	
29	AF	EO, DL	No	No	No	Diffuse	No	No	No	No	No, No	No, No	No	
30	AF	DL	No	No	No	Diffuse	No	Yes	No	No	No, No	No, No	No	
31	AFO	EO, EO	No	No	Yes	Diffuse	No	No	No	No	No, No	Yes, Yes	Yes	
32	AFO	EO, DL	No	No	No	Lobular	No	No	No	No	No, No	Yes, Yes	No	Melanin pigment
33	AFD	EO, EO	No	No	No	Diffuse	Yes	No	No	No	No, No	Yes, No	Yes	
34	AFD	EO, EO	No	No	Yes	Diffuse	Yes	No	No	No	No, No	Yes, No	Yes	
35	AFD	DL, DL	No	No	No	Lobular	No	No	No	No	No, No	Yes, No	No	Peripheral AFD
36	AFD	EO, EO	No	Yes	No	Diffuse	Yes	No	No	No	No, No	Yes, No	No	Infarcted
37	AFD	EO, EO	Yes	No	No	Lobular	No	Yes	No	No	No, No	Yes, No	Yes	Cystic component, adenoid-like areas, and melanin pigment
38	AFD	EO, EO	No	No	Yes	Lobular	Yes	No	No	No	No, Yes	Yes, No	Yes	
39	AFO	EO, DL	Yes	Yes	No	Lobular	No	Yes	No	No	No, No	Yes, Yes	Yes	
40	AFO	EO, DL	No	No	No	Lobular	No	Yes	No	No	No, No	Yes, Yes	Yes	
41	AFD	EO, DL	No	No	No	Diffuse	No	No	No	No	No, No	Yes, No	No	
42	AFO	EO, EO	Yes	No	No	Diffuse	No	No	No	No	No, No	Yes, Yes	No	

43	AFD	EO, DL	Yes	Yes	No	Diffuse	No	Yes	Yes	Yes	Yes, No	Yes, No	No	
44	AFD	EO, DL	No	No	No	Diffuse	Yes	No	No	No	No, Yes	Yes, No	No	Stromal granular cells
45	AFD	EO, DL	Yes	Yes	No	Diffuse	No	Yes	No	No	No, No	Yes, No	No	
46	AFD	EO, EO	No	No	No	Lobular	No	No	No	No	No, No	Yes, No	Yes	
47	AFD	EO, EO	No	Yes	No	Diffuse	No	Yes	No	No	No, No	Yes, No	No	
48	AFD	EO, EO	No	No	Yes	Lobular	No	Yes	No	No	No, No	Yes, Yes	No	
49	AFO	EO, DL	No	No	No	Diffuse	No	Yes	No	Yes	No, No	Yes, Yes	Yes	Melanin pigment
50	AFO+COF+ CGCG	EO, EO	No	No	No	Diffuse	No	Yes	Yes	No	No, No	Yes, Yes	No	Associated with COF and CGCG
51	AFO	EO, DL	No	No	No	Lobular	No	Yes	No	No	No, No	Yes, Yes	No	
52	AFD	EO, EO	No	No	No	Lobular	No	Yes	No	Yes	No, No	Yes, No	No	
53	AFD	DL, DL	Yes	No	No	Lobular	No	Yes	No	No	No, No	Yes, No	No	
54	AFD	EO, DL	Yes	No	No	Lobular	No	Yes	No	Yes	No, No	Yes, No	Yes	
55	AFD	EO, EO	Yes	Yes	Yes	Lobular	No	Yes	No	Yes	No, No	Yes, No	Yes	Cystic component
56	AFO	DL, DL	No	No	No	Lobular	No	Yes	No	No	No, No	Yes, Yes	No	
57	AFO	EO, DL	No	No	No	Lobular	No	Yes	No	No	No, No	Yes, Yes	No	
58	AFO	EO, DL	No	No	Yes	Lobular	No	No	No	No	No, No	Yes, Yes	No	
59	AFO	EO, EO	No	No	Yes	Diffuse	No	No	No	No	No, No	Yes, Yes	No	
60	AFD	EO, DL	No	No	No	Lobular	No	Yes	Yes	No	No, No	Yes, No	No	
61	AFO	DL, DL	No	Yes	No	Lobular	No	Yes	No	Yes	No, No	Yes, Yes	No	
62	AFD	DL, DL	Yes	Yes	No	Lobular	No	Yes	No	Yes	No, No	Yes, No	No	

Dx, diagnosis; ***AF,*** ameloblastic fibroma; ***AFD,*** ameloblastic fibrodentinoma; ***AFO,*** ameloblastic fibro-odontoma; ***COC,*** calcifying odontogenic cyst; ***GCT,*** ghost cell tumor; ***POT,*** primordial odontogenic tumor; ***COF,*** cemento-ossifying fibroma; ***CGCG,*** central giant cell granuloma; ***EO,*** enamel organ-like; ***DL,*** dental lamina-like

Supplementary table S3. Immunohistochemical features of AF and AFD/AFO from Brazil, USA, and South Africa

N	*Dx*	*BRAF p.V600E*		*SOX9*	
		Epithelial	***Mesenchymal***	***Epithelial***	***Mesenchymal***
1*	AF	Neg	Neg	Strong, diffuse	Strong, diffuse
2	AF	NP	NP	Strong, diffuse	Strong, diffuse
3	AF	NP	NP	NP	NP
4	AF+GCT	NP	NP	Strong, diffuse	Strong, diffuse
5	AF atypical	Neg	Neg	Strong, diffuse	Strong, diffuse
6	AF	Neg	Weak, focal	Strong, diffuse	Strong, diffuse
7	AF	NP	NP	NP	NP
8	AF	Neg	Weak, diffuse	NP	NP
9	AF	Neg	Weak, diffuse	NP	NP
10	AF	Neg	Weak, focal	NP	NP
11	AF	NP	NP	NP	NP
12	AF	Neg	Weak, focal	NP	NP
13	AF	Neg	Weak, diffuse	NP	NP
14	AF	Neg	Strong, diffuse	NP	NP
15	AF	Neg	Strong, diffuse	NP	NP
16	AF	NP	NP	NP	NP
17	AF	Neg	Strong, diffuse	NP	NP
18	AF	NP	NP	NP	NP
19	AF+COC	NP	NP	NP	NP
20	AF	Neg	Neg	NP	NP
21	AF+COC	Neg	Weak, focal	NP	NP
22	AF+COC	NP	NP	NP	NP
23	AF	Neg	Weak, focal	NP	NP
24	AF	NP	NP	NP	NP
25	AF	Neg	Weak, diffuse	NP	NP
26	AF	NP	NP	NP	NP
27	AF	NP	NP	NP	NP
28	AF	NP	NP	NP	NP
29	AF	NP	NP	NP	NP
30	AF	Neg	Weak, focal	NP	NP
31	AFO	Neg	Neg	Strong, diffuse	Strong, diffuse
32	AFO	Neg	Neg	Strong, focal	Strong, diffuse
33*	AFD	Neg	Neg	Neg	Neg
34	AFD	Neg	Weak, focal	Strong, diffuse	Strong, diffuse
35	AFD	NP	NP	NP	NP
36*	AFD	Neg	Neg	Strong, focal	Weak, diffuse
37	AFD	Neg	Strong, diffuse	Strong, diffuse	Strong, diffuse
38	AFD	Neg	Weak, focal	Strong, diffuse	Strong, diffuse
39	AFO	Neg	Weak, focal	Strong, diffuse	Strong, diffuse
40	AFO	NP	NP	NP	NP
41	AFD	NP	NP	NP	NP
42	AFO	Neg	Neg	NP	NP
43	AFD	Neg	Neg	NP	NP
44	AFD	Neg	Weak, diffuse	NP	NP
45	AFD	NP	NP	NP	NP
46	AFD	Neg	Weak, focal	NP	NP
47	AFD	Neg	Strong, focal	NP	NP
48	AFD	Neg	Weak, diffuse	NP	NP
49	AFO	NP	NP	NP	NP
50	AFO+COF+CGCG	NP	NP	NP	NP
51	AFO	Neg	Neg	NP	NP

52	AFD	Neg	Neg	NP	NP
53	AFD	Neg	Weak, focal	NP	NP
54	AFD	Neg	Strong, diffuse	NP	NP
55	AFD	NP	NP	NP	NP
56	AFO	Neg	Neg	NP	NP
57	AFO	Neg	Strong, diffuse	NP	NP
58	AFO	Neg	Neg	NP	NP
59	AFO	Neg	Neg	NP	NP
60	AFD	NP	NP	NP	NP
61	AFO	Neg	Strong, diffuse	NP	NP
62	AFD	Neg	Strong, diffuse	NP	NP

Dx, diagnosis; ***AF,*** ameloblastic fibroma; ***AFD,*** ameloblastic fibrodentinoma; ***AFO,*** ameloblastic fibro-odontoma; ***COC,*** calcifying odontogenic cyst; ***GCT,*** ghost cell tumor; ***COF,*** cemento-ossifying fibroma; ***CGCG,*** central giant cell granuloma; ***NP,*** not performed; ***Neg,*** negative

*Cases previously reported by Coura et al. confirmed with molecular analysis for *BRAF* p.V600E

2.2 Artigo: Odontogenic Sarcomas: Clinicopathologic Analysis and Immunohistochemical Expression of BRAF and SOX9 in a Multicenter Series of 10 Cases

Artigo submetido no periódico *Head and Neck Pathology* (Anexo 4)

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Conflict of Interest: All authors have indicated they have no potential conflict of interest and no financial relationships relevant to this article to disclose.

Ethical Approval: This study was carried out according to the tenets of the Helsinki Declaration for studies involving human subjects and approved by the local research ethics committees (FOP-UNICAMP, protocol number 43944721.1.0000.5418; University of Pretoria, Research Ethics Committee No 629/2021; Texas A&M University, IRB protocol number 24-0317)

Presentation: Preliminary results of the present study were presented as an oral presentation during the American Academy of Oral & Maxillofacial Pathology (AAOMP) Annual Meeting (April 13-16th, 2024) in Orlando, FL, USA.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Abstract

Introduction: Odontogenic sarcomas (OS) are rare malignant mixed odontogenic tumors characterized by both a benign epithelial component and a malignant ectomesenchymal component. **Materials and methods:** Clinical and radiographic data from ten OS cases diagnosed between 2000 and 2024 were retrieved from seven Oral and Maxillofacial Pathology services. Histological slides were deidentified and independently reviewed by two oral pathologists. Immunohistochemical staining for BRAF p. V600E and SOX9 was performed on available non-decalcified tissue samples. **Results:** The cohort included five female and five male patients, with a mean age of 30 years (range: 9–72). Eight patients had a prior diagnosis of a benign mixed odontogenic tumor, with an average interval of 3.6 years between the initial and malignant diagnoses. All tumors were located in the mandible, presenting as aggressive, destructive lesions with an average size of 8 cm. Radiographically, tumors appeared as extensive, ill-defined radiolucencies, often causing cortical expansion or perforation, root resorption, and displacement of teeth. Two patients reported pain upon presentation. Six patients underwent wide surgical resection; one experienced recurrence within two years and later showed high-grade transformation. Histopathologic examination revealed a consistent pattern of benign odontogenic epithelium interspersed within a malignant mesenchymal stroma characterized by hypercellularity, pleomorphism, nuclear atypia, and increased mitotic figures. One case exhibited focal ghost cell formation, and another showed areas of high-grade transformation. BRAF p. V600E immunopositivity was observed in 60% of evaluable cases within the mesenchymal component, with cytoplasmic staining. SOX9 demonstrated strong and diffuse nuclear expression in both epithelial and mesenchymal components across all tested samples; however, the case with high-grade transformation showed reduced SOX9 expression relative to the initial biopsy. **Conclusion:** This study presents ten well-documented cases of OS, a rare and aggressive mandibular tumor primarily affecting young adults, frequently associated with a prior benign mixed odontogenic tumor. Despite the presence of uncommon

histological features, the biological behavior remained consistent. BRAF expression was restricted to the malignant mesenchymal component in 60% of cases, while SOX9 showed strong nuclear expression in both components, supporting its diagnostic relevance. Given the potential for malignant transformation, long-term clinico-radiological surveillance is essential. Larger multicenter studies are warranted to clarify the pathogenesis of OS and support the development of targeted therapeutic strategies.

Keywords: Odontogenic sarcomas, Ameloblastic fibrosarcoma, Ameloblastic fibrodentinosarcoma, Ameloblastic fibro-odontosarcoma, BRAF p.V600E, SOX9

Introduction

Odontogenic sarcomas (OS) are exceedingly rare malignant mixed odontogenic tumors in which the malignant component is confined to the odontogenic ectomesenchyme and are further subclassified as ameloblastic fibrosarcoma (AFS), fibrodentinosarcoma (AFDS), or fibro-odontosarcoma (AFOS). [1]. OS primarily affects the posterior mandible of young adults, often as aggressive tumors, and is characterized microscopically by islands and strands of benign, enamel organ-like epithelium embedded in a malignant, hypercellular stroma [1, 2]. Surgical resection is the most common treatment, although long-term follow-up is recommended due to recurrence rates of approximately 35% and a disease-related mortality rate of 21% [1]. Since its initial description, most studies consist of small samples of OS, with tumors often affecting patients who have a previous history of a recurrent benign mixed odontogenic tumor, usually ameloblastic fibroma (AF) or ameloblastic fibro-odontoma (AFO) (**Table 1**) [3-9].

Although the pathogenesis of OS remains poorly understood, recent studies have identified *BRAF* p.V600E mutations in the mesenchymal component of a subset of cases, suggesting potential driver mutations — findings that align with those observed in their benign counterparts [8-10]. Additional molecular alterations include *NRAS* p.Q61K mutations, *EGFR* exon 20 insertions, *MDM2* amplification, and aneuploidy [6, 8, 11]. While SOX9, a transcription factor involved in chondrogenesis, has recently been described in odontogenesis, its role in OS has not yet been reported [12]. In this study, we present ten additional well-documented cases of OS and analyze the immunohistochemical expression of BRAF p.V600E and SOX9.

Materials and methods

Ten cases diagnosed as OS were retrieved from the archives of seven oral pathology diagnostic services across five countries, spanning from 2000 to 2024. Four cases were from Brazil (two from the Federal University of Rio de Janeiro and two from the University of Campinas), two from the USA (one from Texas A&M University and one from the University of Louisville), two from South

Africa (University of Pretoria), one from Mexico (Universidad Autónoma Metropolitana Xochimilco), and one from Chile (Mayor University). This study was carried out according to the tenets of the Helsinki Declaration for studies involving human subjects and approved by the local research ethics committees (FOP-UNICAMP, protocol number 43944721.1.0000.5418; University of Pretoria, Research Ethics Committee No 629/2021; Texas A&M University, IRB protocol number 24-0317). Demographic, clinical, and radiographic data were collected from each patient's records for analysis. The cases were reviewed by two or more experienced oral pathologists and confirmed after examination of hematoxylin and eosin-stained sections, which demonstrated the diagnostic histopathologic features of OS, as outlined in the current World Health Organization (WHO) Classification of Head and Neck Tumors [1].

Immunohistochemical analyses were performed on formalin-fixed paraffin-embedded (FFPE) tissue sections derived from diagnostic biopsies or surgical specimens of cases with available tissue. Antibodies targeting BRAF p.V600E (ready-to-use, clone VE1, Ventana), SOX9 (dilution 1:2000, clone EPR14335, Abcam), and Ki-67 (dilution 1:200, clone MIB-1, Dako) were employed. For the immunohistochemical reactions, 3-µm tissue sections were deparaffinized and then subjected to antigen retrieval, performed in either an EDTA/Tris solution (pH 9.0) or a Citrate Solution (pH 6), both for 15 minutes in an electric pressure cooker. Endogenous peroxidase activity was blocked with 6% H_2O_2 for 15 minutes. Sections were then incubated with the primary antibody for 2 hours at room temperature. Immunohistochemical staining was performed using EnVision detection system (Dako, Carpinteria, CA) according to the manufacturer's protocol, followed by exposure to diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St Louis, USA) for 5 minutes or EnVision Labelled Polymer (Dako, Glostrup, Denmark). Finally, the slides were counterstained with Carazzi's hematoxylin. All specimens selected for immunohistochemical staining were non-decalcified. BRAF p.V600E expression was confirmed by cytoplasmic staining, while SOX9 expression was identified through nuclear staining. The expression of Ki-67 (nuclear positivity) was evaluated in 2 selected areas representative of

the lesion using a 40X objective. Positive controls were included in all reactions. This study provides a descriptive analysis of the clinical, radiographic, histopathologic, and immunohistochemical features of ten cases of OS.

Results

Table 2 summarizes the demographic, clinical, and radiographic characteristics of the ten cases of OS. Five patients (50%) were female, and five patients (50%) were male, with a mean age of 30 years (range: 9-72 years). Eight patients (80%) had a prior diagnosis of benign mixed odontogenic tumors, most of which were AF (60%), while two patients (20%) had previously been diagnosed with AFO. In two patients, more than one biopsy at the time of diagnosis was necessary to observe different areas (AF/AFO and OS) within the same lesion, indicating malignant transformation from the benign tumor. Malignant transformation from the benign counterpart was also observed in the remaining six patients, with an average evolution time of 3.6 years between the two diagnoses.

Radiographic examinations revealed extensive, ill-defined multilocular (50%) and unilocular (30%) radiolucencies, often associated with cortical bone expansion and perforation, root resorption, and displacement of adjacent teeth (**Figure 1**). All tumors were in the mandible and presented as aggressive, destructive lesions with an average size of 8 cm (**Figures 2 and 3**). Two patients (20%) reported pain at the time of diagnosis. Six patients underwent extensive surgical resections, and a single patient presented with recurrent disease within two years of follow-up. This patient developed high-grade transformation and has remained free of disease six years after additional surgical resection.

Histopathologic analysis revealed neoplasms composed of benign odontogenic epithelium, ranging from delicate dental lamina–like strands to larger epithelial islands with peripheral ameloblastic differentiation, embedded within malignant odontogenic ectomesenchyme (**Figure 4**). Areas of conventional AF were noted in 7 cases (70%). The quantity of epithelial islands varied considerably, appearing compressed and less prominent in regions closely

associated with the sarcomatous ectomesenchyme. One case showed focal ghost cell formation within an epithelial island. The malignant ectomesenchymal component was typically characterized by extensive hypercellularity arranged in a diffuse growth pattern, often exhibiting bone infiltration. Mesenchymal cells displayed varying degrees of atypia, pleomorphism, and increased mitotic figures. Microcystic degeneration with accumulation of myxoid material was observed in four cases (40%) within the sarcomatous component. Additionally, two cases (20%) exhibited cytoplasmic vacuolization in the ectomesenchymal cells, resulting in a signet ring-like appearance. Both cases showed pronounced pleomorphism, including the presence of large, bizarre, multinucleated giant cells (**Figure 4**). Dysplastic dentin was identified in three cases (30%), and in two cases (20%), it was accompanied by an enamel-like matrix. However, the presence or absence of mineralized dental tissue did not appear to influence the biological behavior or aggressiveness of the tumors. Notably, one case underwent high-grade transformation two years after the initial diagnosis, characterized by minimal residual odontogenic epithelium and a stroma composed of highly pleomorphic cells with abnormal mitotic figures, hyperchromatism, and atypical dentinoid deposition (**Figure 5**).

Immunohistochemical staining for BRAF p.V600E was performed in five cases. Cytoplasmic positivity in the mesenchymal cells was observed in three cases (60%), with staining intensity ranging from weak and focal (20%) to strong and diffuse (40%) (**Figures 2 and 3**). One of these cases, previously reported by Coura et al., harbored a pathogenic BRAF p.V600E mutation and exhibited strong, diffuse immunohistochemical expression. The two BRAF-negative cases corresponded to tumors with marked pleomorphism in the malignant stromal component. Notably, the epithelial component was negative for BRAF p.V600E in all cases.

SOX9 immunostaining was performed in three cases, all of which demonstrated strong, diffuse nuclear expression in both the epithelial and mesenchymal components. A characteristic loss of SOX9 expression was noted in the peripheral ameloblastic cells. The tumor that underwent high-grade

transformation showed weaker SOX9 expression in the mesenchymal cells compared to the initial biopsy (**Figure 5**). The Ki-67 proliferation index was assessed in five cases at the time of diagnosis, revealing mitotic activity ranging from 5% to 30%. This activity was primarily focused on hypercellular regions adjacent to compressed odontogenic epithelium. Notably, cases with higher proliferative indices were linked to recurrences and had previously been diagnosed as AF or AFO.

Discussion

Despite centuries of documentation in the literature, odontogenic sarcomas in the jaws remain exceedingly rare. The earliest presumed report of a malignant mixed odontogenic tumor was published by Heath in 1887, describing a “spindle-celled sarcoma” that recurred following excision and subsequent metastasis to the humerus. Thoma later cited this case in 1949 under the term “adamantinosarcoma” [13, 14]. In 1951, Thoma introduced the term “ameloblastosarcoma” to describe a malignant odontogenic tumor resembling an ameloblastofibroma (now termed ameloblastic fibroma), characterized by a mesenchymal component that retained an embryonic appearance but exhibited malignant features [14]. The designation “ameloblastic fibrosarcoma” was later formalized and included among odontogenic sarcomas in the first edition of the WHO Classification of Odontogenic Cysts and Tumors in 1971. To date, OS is considered a well-recognized yet extremely rare odontogenic malignancy, with only approximately 120 cases reported in the English-language literature [2, 8].

While some cases of OS have been reported to arise de novo, numerous publications have documented malignant transformation from a pre-existing recurrent ameloblastic fibroma (AF) or ameloblastic fibro-odontoma (AFO) [2, 15–19]. OS typically affects young adults and shows a slight male predominance, with peak incidence occurring in the third decade of life. This demographic profile contrasts with its benign counterparts, AF and AFO, which are more frequently diagnosed in the second decade [1,2]. The difference in age distribution supports the hypothesis of a stepwise progression from a benign to a malignant lesion,

rather than de novo tumorigenesis. In the present study, there was no sex predilection, with patients presenting a mean age of 30 years. Notably, 80% of cases had a documented history of a prior diagnosis of AF or AFO, which subsequently underwent malignant transformation, with an average interval of 3.6 years between diagnoses. Although the precise etiology and molecular mechanisms driving this progression remain poorly understood, tumor recurrence and repeated surgical interventions have consistently been implicated as risk factors for malignant transformation [2,6,15–19]. Given the latency period observed in our series, we strongly recommend that patients diagnosed with AF or AFO undergo close clinical and radiographic follow-up every six months during the first four years following surgical treatment, and annually thereafter, due to the potential for progression to OS.

Like AF and AFO, OS most frequently arises in the mandible and typically presents as a rapidly growing, locally aggressive, expansile lesion, with or without associated pain. On imaging, OS commonly appears as a poorly defined unilocular or multilocular radiolucent or hypodense lesion, often exhibiting cortical expansion and destruction—features also observed in the present study [1, 2]. When mineralized dental hard tissues are present, the tumor may occasionally present as a mixed radiolucent-radiopaque lesion. Maxillary OS, although less common, tends to demonstrate more aggressive behavior, often infiltrating adjacent structures such as the maxillary sinus and skull base [11, 19].

Microscopically, OS is characterized by benign odontogenic epithelium embedded within a malignant ectomesenchymal stroma that exhibits marked hypercellularity, varying degrees of cytological atypia, nuclear pleomorphism, and increased mitotic activity [1]. All ten OS cases in this study displayed these hallmark microscopic features. Notably, one case demonstrated focal ghost cells within an epithelial island—a finding previously described in AF but, to our knowledge, has not yet been reported in OS. Additionally, a single case in this study showed large, multinucleated mesenchymal cells with morphological features closely resembling those of Touton giant cells. Although unusual, a few reports have mentioned the microscopic similarity between OS and lesions of

histiocytic lineages, describing it as a ‘malignant fibrous histiocytoma appearance’ [4]. This finding may be explained by ultrastructural studies identifying the predominant cell type present in the sarcomatous component, which closely resembles fibroblasts, histiocytes, and undifferentiated mesenchymal cells [20].

Interestingly, prior studies have shown that as malignancy progresses, the epithelial component often becomes less prominent and may eventually disappear, likely due to degenerative changes. This phenomenon results in histologic features resembling those of a conventional fibrosarcoma of the jaws, thereby obscuring the odontogenic origin [6, 15]. OS with high-grade transformation is typically marked by pronounced anaplastic features, including bizarre tumor giant cells, atypical mitoses, and areas of necrosis [6]. In contrast to conventional fibrosarcoma or other high-grade sarcomas of the jaws, metastasis in OS is extremely rare, occurring in fewer than 5% of reported cases, even in the presence of high-grade histologic features [1]. Some authors have postulated that the odontogenic epithelial component may exert an organizational influence on the ectomesenchymal component of OS, potentially modulating its differentiation and limiting its metastatic potential [18]. Previous studies have reported Ki-67 labeling indices in the mesenchymal components of recurrent AF and OS ranging from 9.8% to 13.5%, percentages significantly higher than those observed in non-recurrent AF and AFO, which typically range from 1.5% to 2.9% [21]. In the present study, Ki-67 expression ranged from 5% to 30% across the evaluated cases, indicating a moderately elevated proliferative activity. Although lower than that reported for non-odontogenic sarcomas, these findings align with the proliferative behavior typically seen in OS.

Recent molecular discoveries have significantly advanced our understanding of odontogenic tumors. Mixed odontogenic tumors have frequently been found to harbor genetic alterations affecting the MAPK/ERK signaling pathway, most notably the pathogenic *BRAF* p.V600E mutation [9, 10]. According to a recent systematic review, AFS has demonstrated pathogenic *BRAF* mutations within the malignant mesenchymal component in approximately 50%

to 82% of cases, as detected by immunohistochemical and molecular-based methods, respectively [22]. In our study, among the five cases in which *BRAF* p.V600E immunohistochemistry was performed, 60% demonstrated positive cytoplasmic expression within the sarcomatous cells—a finding consistent with previously reported data in the literature.

Interestingly, the two BRAF-negative tumors in our series were those that demonstrated vacuolated cells with signet-ring-like morphology and marked pleomorphism within the mesenchymal component. These findings suggest that in highly pleomorphic malignant odontogenic sarcomas, the diagnostic utility of immunohistochemical detection of the BRAF p.V600E mutation may be limited. Therefore, molecular testing may be required to accurately determine BRAF mutation status in such cases. SOX9, a transcription factor best known for its role in chondrogenesis, has also been identified in dental pulp cells [12, 23, 24]. Our group has recently demonstrated SOX9 expression in both the epithelial and mesenchymal components of AF and AFD/AFO [25]. Curiously, in the high-grade transformation case, the sarcomatous mesenchymal cells exhibited reduced SOX9 expression compared to the initial biopsy. This finding may suggest that as malignant transformation progresses, neoplastic cells gradually lose odontogenic characteristics, including lineage-specific markers such as SOX9.

In most cases, patients diagnosed with OS have been successfully managed with segmental surgical resection, although recurrence rates of up to 35% have been reported [2]. While distant metastasis may occur in approximately 5% of cases, fatal outcomes are typically associated with uncontrolled local invasion, often following multiple recurrences [1, 2, 15]. A novel therapeutic avenue has emerged with the use of personalized, targeted therapies, particularly BRAF inhibitors, which have shown promising results in the treatment of ameloblastomas and ameloblastic carcinomas. These targeted approaches have demonstrated the potential to significantly reduce surgical morbidity and improve clinical outcomes [26]. Moreover, recent studies have identified SOX9 as a novel therapeutic target and potential biomarker associated with therapy resistance in various cancers [27]. These insights hold promise for

developing less invasive therapeutic strategies, particularly for extensive and aggressive tumors in young patients. Unfortunately, mixed odontogenic tumors have not yet been studied in this context; however, this may change shortly as further research explores their molecular profiles and therapeutic responses. Pathologists should be aware that, although no standardized staging system currently exists for OS, the use of dataset reporting from the International Collaboration on Cancer Reporting (ICCR) is strongly encouraged to ensure consistent documentation and data collection in clinical practice [28].

Conclusion

This study adds ten well-documented cases of OS to the English-language literature, highlighting its presentation as a clinically aggressive mandibular tumor predominantly affecting young adults. Most patients had a prior diagnosis of a benign mixed odontogenic tumor, with an average progression interval of 3.6 years to malignancy. Consistent microscopic features of OS were observed across all cases, and one patient exhibited high-grade transformation within two years of the initial diagnosis, reinforcing the importance of early detection and long-term follow-up. Despite the presence of unusual microscopic features—such as focal ghost cells, Touton-like multinucleated giant cells, cytoplasmic vacuolization, microcystic myxoid degeneration, and mineralized dental tissue—the biological behavior of OS does not appear to be affected. Additionally, BRAF immunohistochemistry was positive in 60% of the cases, with expression restricted to the malignant mesenchymal component, while SOX9 demonstrated uniform and strong nuclear staining in both epithelial and mesenchymal components, supporting its role in tumor differentiation. Most patients underwent segmental mandibular resection, with tumor recurrence observed in one case within a two-year follow-up. Considering the risk for malignant transformation, patients diagnosed with AF or AFO should undergo close clinico-radiological surveillance every six months for the first four years following surgery and annually thereafter. Further multicenter studies are essential to elucidate the molecular pathogenesis of OS, refine prognostic

biomarkers, and guide the development of more effective, targeted therapeutic approaches.

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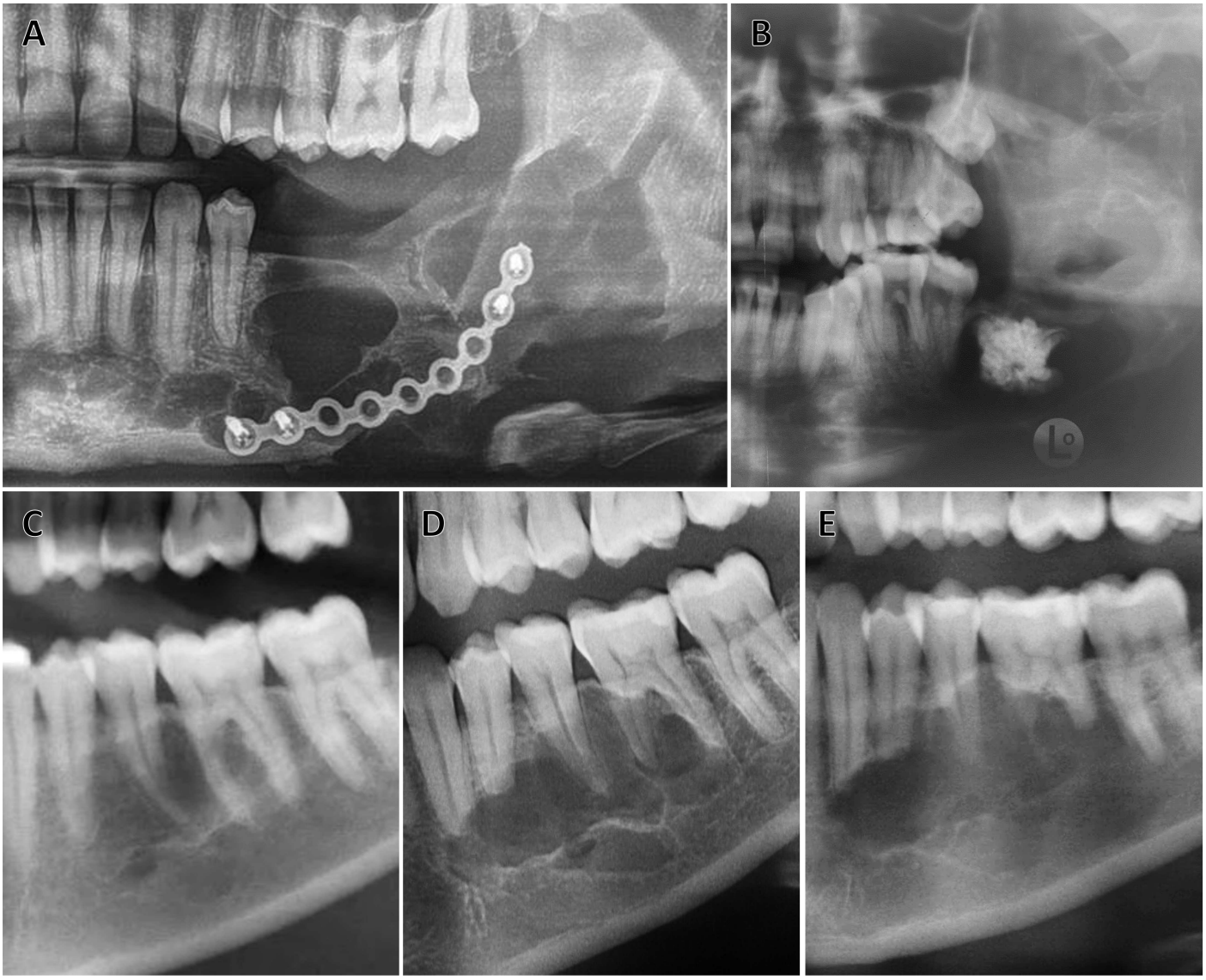

Figure 1. Radiographic features of odontogenic sarcomas. A. AFS in a 25-year-old woman (case 9), identified 27 months after marginal resection of a previously diagnosed ameloblastic fibroma. **B.** AFOS in an 18-year-old man (case 5), with a previous diagnosis of ameloblastic fibro-odontoma two years earlier. **C-E.** Radiographic progression of AFDS in the mandible of a 41-year-old woman (case 8), showing progressive growth over 7 years with unilocular radiolucency, root resorption, and bone perforation (**C:** initial; **D:** 6 years; **E:** 7 years; [**E**] panoramic radiograph).

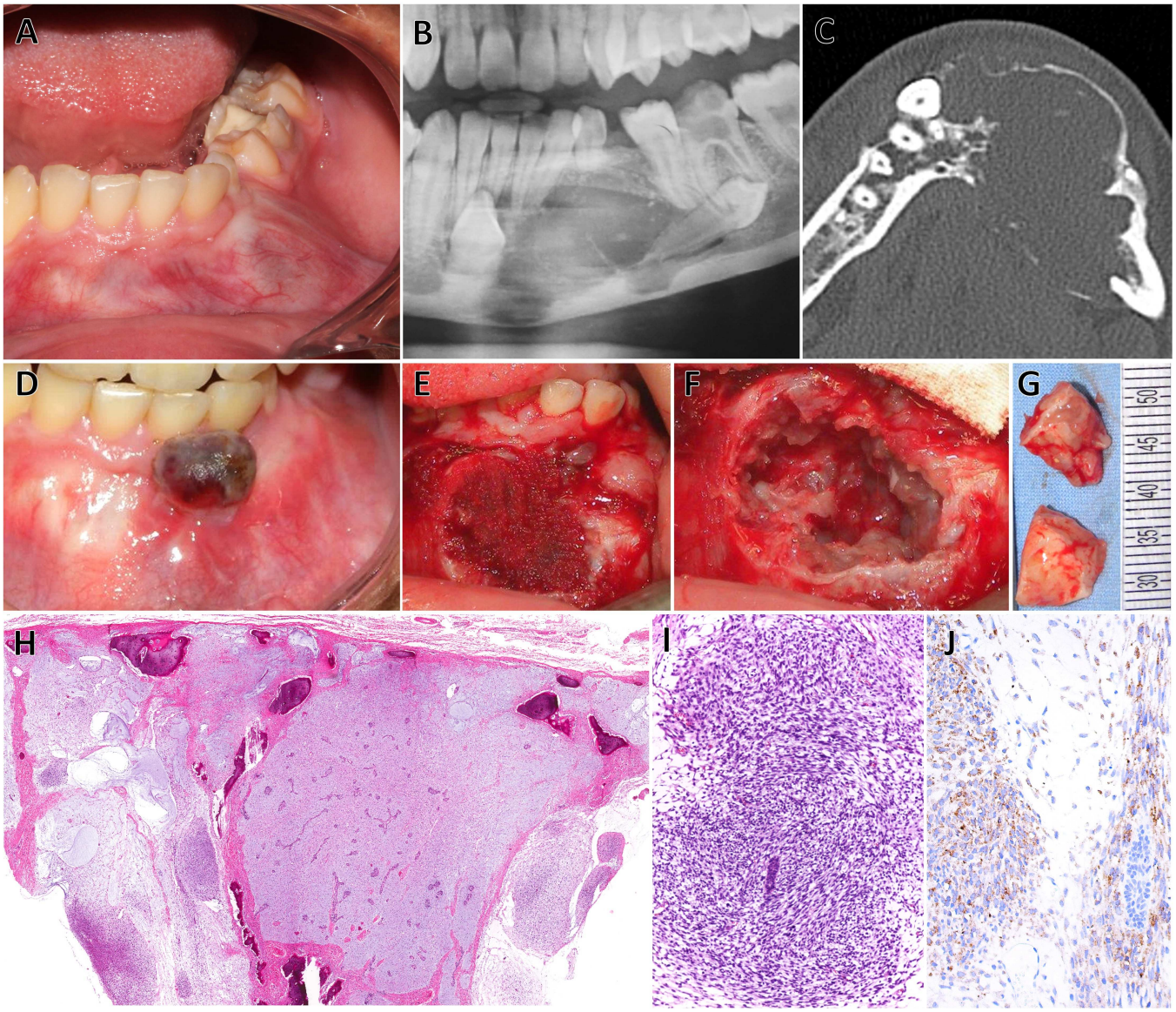

Figure 2. Clinico-radiographic, macroscopic, microscopic, and immunohistochemical features of ameloblastic fibrosarcoma (Case 2). A-C. An exuberant swelling located in the anterior mandible of a 14-year-old girl, radiographically appearing as a non-corticated multilocular radiolucency with radiopaque foci, causing tooth impaction and cortical bone expansion. **D-F.** After an incisional biopsy, the lesion exteriorized into the oral cavity, and the patient underwent another biopsy to retrieve a deeper specimen, which **(G)** macroscopically revealed a solid whitish soft-tissue tumor with soft consistency. **H-I.** Microscopic analysis revealed a mixed odontogenic tumor infiltrating bone with areas of hypercellularity within a malignant ectomesenchymal stroma, compressing islands of benign odontogenic epithelium. **J.** BRAF immunohistochemistry showed diffuse positive cytoplasmic expression in the ectomesenchymal tissue and negativity in the epithelium. (**H&E: C,** x25; **D,** x100; **IHC: E,** x200)

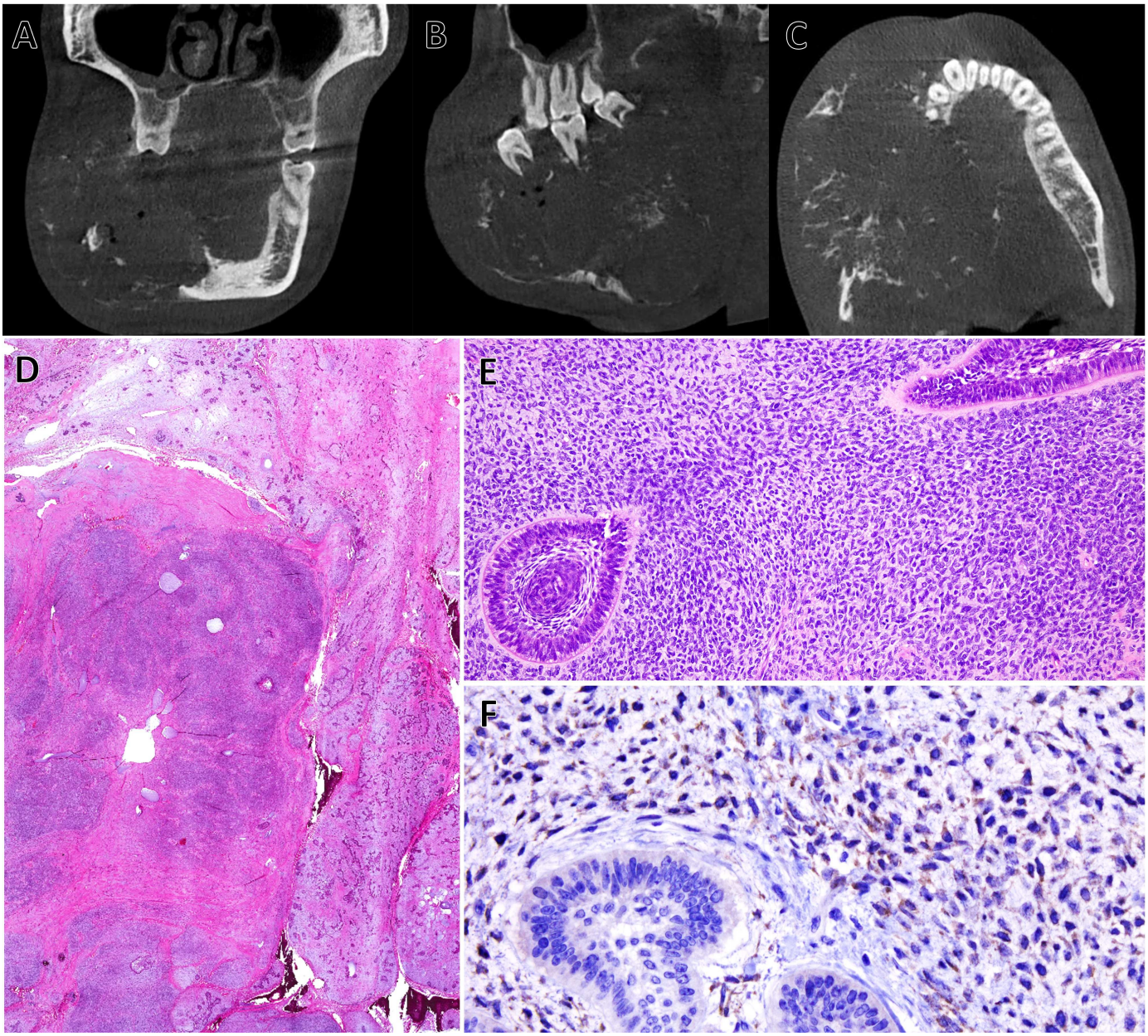

Figure 3. Clinico-radiographic, microscopic, and immunohistochemical features of ameloblastic fibrosarcoma (Case 6). A–C. Expansive and aggressive mandibular tumor in a 28-year-old male, characterized by extensive bone destruction and cortical expansion. **D.** Histological examination revealed an infiltrative malignant mixed odontogenic neoplasm adjacent to areas of conventional ameloblastic fibroma. **E.** The mesenchymal component exhibited sarcomatous features, including marked hypercellularity and cytologic atypia, while the epithelial component retained benign morphology. **F.** Immunohistochemistry for BRAF showed positive cytoplasmic staining in the malignant ectomesenchymal component and negative expression in the epithelial islands (**H&E**: **D**, x25; **E,** x100; **IHC: F**, x200).

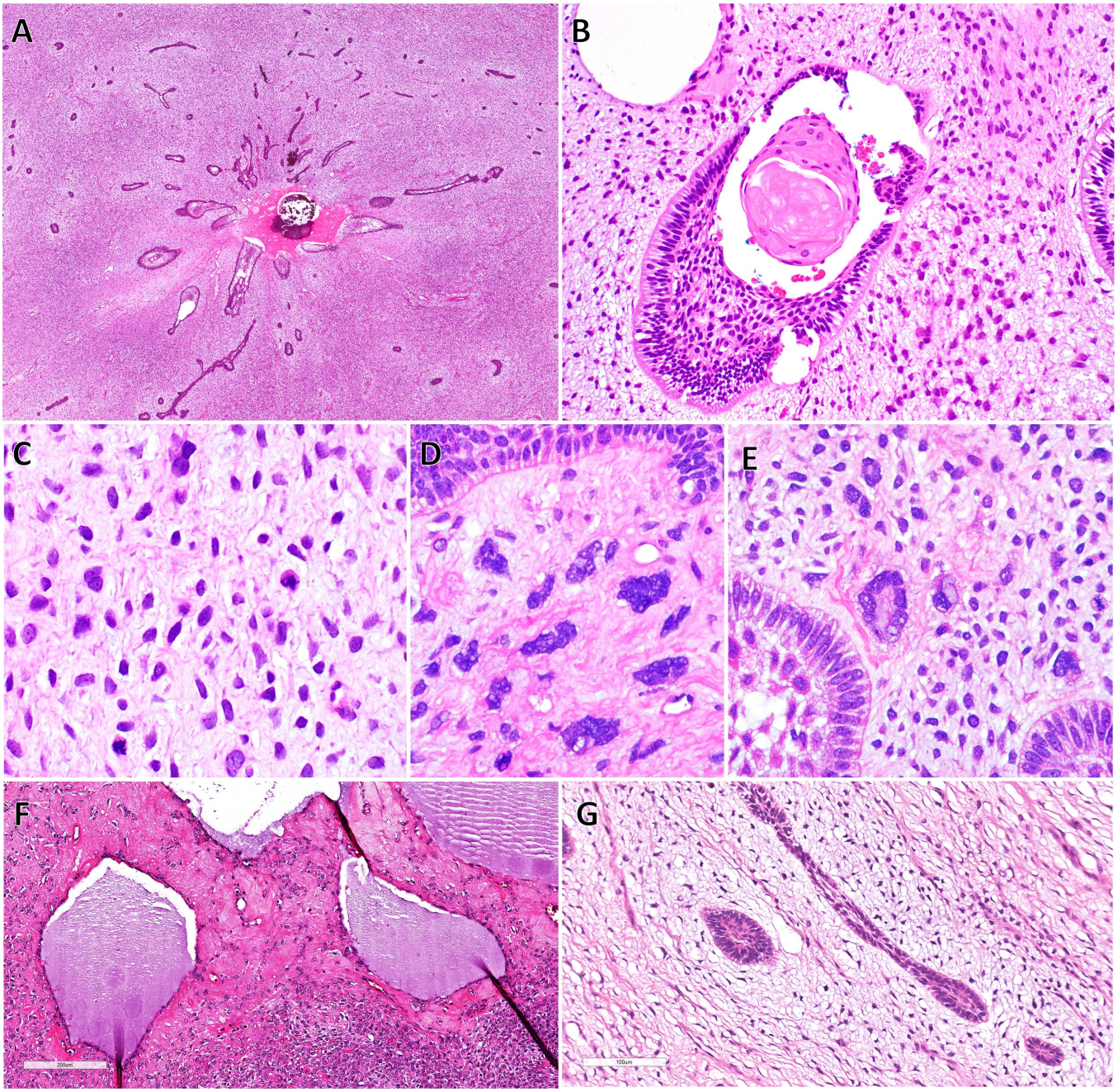

Figure 4. Microscopic features of odontogenic sarcomas. A. Malignant mixed odontogenic tumor composed of a hypercellular ectomesenchymal component compressing odontogenic epithelial islands and strands, associated with dysplastic dentin and enamel matrix formation. **B.** An unusual finding was the presence of ghost cells within an epithelial island. **C.** High mitotic activity was observed within the malignant mesenchymal component. **D.** The epithelial component appeared cytologically benign, while the ectomesenchymal component showed marked pleomorphism. Multinucleated cells with nuclear pseudoinclusions were observed, **[E]** some resembling Touton-like giant cells. **F.** Microcystic spaces filled with myxoid material were occasionally observed, as well as the presence **[G]** of cytoplasmic vacuolization resulting in a signet ring-like appearance of the sarcomatous cells (**H&E**: **A**, ×25; **B**, ×200; **C–E**, ×400).

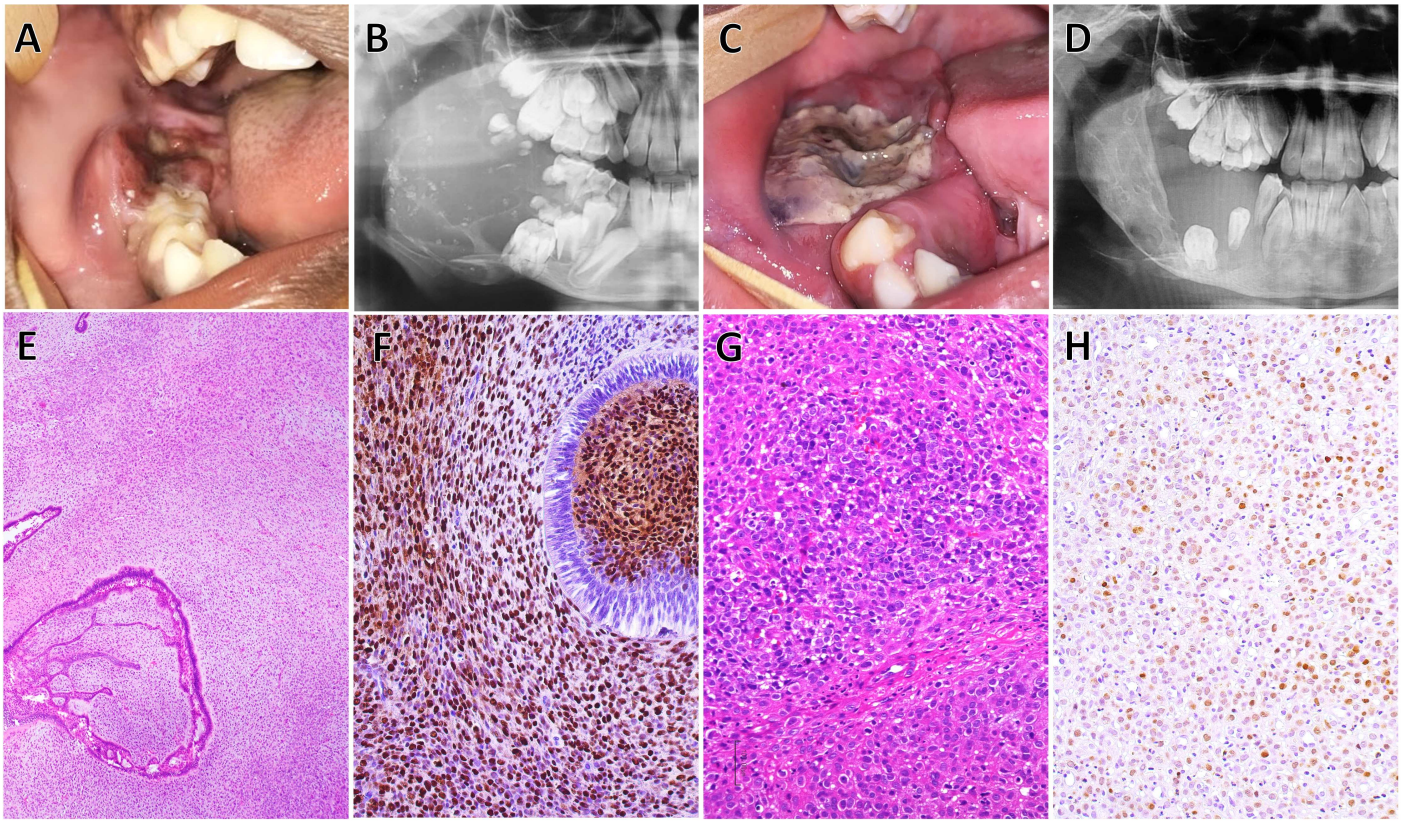

Figure 5. Clinico-radiographic, microscopic, and immunohistochemical features of ameloblastic fibro-odontosarcoma with high-grade transformation (Case 1). A–B. A 9-year-old boy presented with a painful, ulcerated lesion in the posterior mandible. Radiographic examination revealed a large, ill-defined mixed radiolucent–radiopaque lesion associated with multiple impacted and displaced teeth, along with cortical bone disruption. **C–D.** Tumor recurrence after a 2-year follow-up. **E.** Microscopic analysis of the initial biopsy showed a sarcomatous component confined to the odontogenic ectomesenchyme, with scattered islands of benign odontogenic epithelium. **F.** Strong and diffuse nuclear expression of SOX9 in both mesenchymal and epithelial components. Note the loss of SOX9 expression in peripheral ameloblastic-like cells. **G.** After 2 years, the tumor recurred with high-grade transformation, showing an undifferentiated, pleomorphic ectomesenchymal component with increased mitotic activity. **H.** SOX9 expression was reduced and less diffuse (**H&E: E,** ×50; **G,** ×200; **IHC: F** and **H ×200**).

Table 1. Summarized data of series comprising of more than 3 cases of Odontogenic Sarcomas published in the English-language literature.

	Authors, year	*Country*	*N*	*Gnd (M / F)*	*Avg age (range)*	*Site*		*Previous history of benign MOT / Arising de novo*	*Treatment*	*Follow-up (N)*
						Max	*Mand*			
1	Leider et al., 1972	USA	6	2 / 4	22.5 (9-43)	2	4	4 / 2	Radical surgical resection (6)	FOD (5), LTF (1)
2	Chomette et al., 1983	France	3	2 / 0	24.6 (9-38)	1	2	3 / 0	Radical surgical resection (3)	FOD (1), Recurrence (1), Metastasis (1)
3	Dallera et al., 1994	Italy	5	3 / 2	27.8 (18-44)	0	5	NR	Radical surgical resection (2), Marginal excision (1), Curretage (1), Curretage + Resection (1)	FOD (2), Recurrence (2), DOD (1)
4	Muller et al., 1995	USA	5	2 / 3	46.2 (17-83)	1	4	4 / 1	Radical surgical resection (4), Excision (1)	FOD (4), Recurrence (1)
5	Martínez-Martínez et al., 2014	Brazil, Guatemala, and Mexico	6	2 / 4	19 (7-24)	1	5	NR	NR	NR
6	Agaimy et al., 2020	Germany, Czech Republic, Italy, Switzerland, and Saudi Arabia	7	3 / 4	30.1 (16-57)	2	4	5 / 2	Radical surgical resection (7)	FOD (5), Recurrence (1), NR (1)
7	Coura et al., 2020	Brazil	3	1 / 2	23.6 (14-32)	0	3	NR	NR	NR
8	Present study, 2025	Brazil, South Africa, USA, Mexico, and Chile	10	5 / 5	30 (9-72)	0	10	8 / 0	Radical surgical resection (6)	FOD (4), Recurrence (1), LTF (3), NR (3)

N, number; ***M,*** male; ***F,*** female; ***Max,*** maxilla; ***Mand,*** mandible; ***MOT,*** mixed odontogenic tumor; ***FOD,*** free of disease; ***DOD,*** dead of disease; ***NR,*** not reported; ***LTF,*** lost to follow-up

Table 2. Clinical and radiographic features of 10 patients diagnosed with Odontogenic Sarcoma.

N, Country	*Diagnosis*	*Age, Gnd*	*Site*	*Previous diagnosis of AF/AFO*	*Interval between benign and malignant diagnoses*	*Radiographic appearance*	*Impacted tooth*	*Pain*	*Size (cm)*	*Treatment; Recurrence, Follow-up*
1, Brazil*	AFOS	9, M	Mand, Post (Ramus)	Yes (AFO)	>1 biopsy performed at the time of diagnosis	Ill-defined ML MX, cortical expansion, and disruption	Yes (C-Mol)	Yes	7	Seg mandibulectomy; Yes, 6 years FOD
2, Brazil	AFS	14, F	Mand, Ant and Post	Yes (AF)	>1 biopsy performed at the time of diagnosis	Ill-defined ML MX, cortical expansion	Yes (C-P)	No	10	Seg mandibulectomy; No, 10 months
3, Brazil	AFS	33, F	Mand, Post	NI	NI	NI	NI	Yes	NI	NI; NI, NI
4, Brazil	AFS	72, F	Mand, Ant and Post	Yes (AF)	2 years	Ill-defined UL MX, cortical expansion	No	No	4	NI; NI, NI
5, South Africa	AFOS	17, M	Mand, Post (Body-Ramus)	Yes (AFO)	2 years	Ill-defined ML MX, cortical expansion, and disruption	No	NI	12	LTF
6, South Africa	AFS	28, M	Mand, Ant and Post	Yes (AF)	14 years	Ill-defined ML, RL, cortical expansion, and disruption	No	NI	12	Hemimandibulectomy; LTF
7, USA	AFS	28, M	Mand	Yes (AF)	1 year	RL, cortical expansion, and disruption	NI	NI	7.6	Hemimandibulectomy; NI, NI
8, USA	AFDS	41, F	Mand, Post (Body)	NI	NI	Well-defined UL RL, cortical expansion, and disruption	No	No	3.5	Seg mandibulectomy; No, 18 months FOD
9, Mexico	AFS	25, F	Mand, Post (Mol)	Yes (AF)	2 years and 3 months	Ill-defined UL RL, cortical expansion, and disruption	No	NI	5	Seg mandibulectomy; No, 8 months FOD
10, Chile	AFS	32, M	Mand, Ant	Yes (AF)	6 months	Well-defined ML MX, cortical expansion, and disruption	No	No	6	LTF

N, number; ***AFS,*** ameloblastic fibrosarcoma; ***AFDS,*** ameloblastic fibrodentinosarcoma; ***AFOS,*** ameloblastic fibro-odontosarcoma; ***Gnd,*** gender; ***M,*** male; ***F,*** female; ***Mand,*** mandible; ***Ant,*** anterior; ***Post,*** posterior; ***Mol,*** molar; ***ML,*** multilocular; ***UL,*** unilocular; ***RL,*** radiolucent; ***MX,*** mixed; ***C,*** canine; ***P,*** premolar; ***Dx,*** diagnosis; ***AF,*** ameloblastic fibroma; ***AFO,*** ameloblastic fibro-odontoma; ***Seg,*** segmental; ***NI,*** not informed; ***FOD,*** free of disease; ***LTF,*** lost to follow-up

*Patient developed high-grade transformation

3 DISCUSSÃO

Tumores odontogênicos mistos (TOM) abrangem um grupo complexo e incomum de lesões em que maioria é considerada ou de origem neoplásica, sendo subdividida em entidades benignas e malignas, ou hamartomatosa. Apesar de centenas de anos de pesquisa e investigação, a classificação e a patogênese dessas lesões ainda são pouco compreendidas e amplamente debatidas. No entanto, a origem de muitas dessas discussões pode advir do uso impreciso dos conceitos de ‘hamartoma’ e ‘neoplasia’ (Soluk-Tekkesin *et al.*, 2025). Dessas neoplasias odontogênicas, FA e FDA/FOA se enquadram na categoria benigna, enquanto sua contraparte maligna, FSA, é significativamente mais rara. Embora a maioria dos casos sejam simples, um subconjunto significativo apresenta desafios diagnósticos e terapêuticos devido às suas características microscópicas variáveis e comportamento clínico imprevisível. O presente estudo compreende uma das maiores casuísticas de FA, FDA/FOA e FSA na literatura de língua inglesa com o objetivo de ajudar a elucidar a complexidade que envolve a classificação dessas lesões.

FA e FDA/FOA tipicamente acometem pacientes pediátricos, com pico de incidência na segunda década de vida, e uma ligeira prevalência pelo sexo masculino (Takeda, 2005; Chrcanovic & Gomez, 2017; Chrcanovic *et al.*, 2018; Vered *et al.*, 2023). No presente estudo, a média de idade dos pacientes com FA foi de 15,3 anos, enquanto os pacientes com FDA/FOA tiveram média de 12,3 anos, um perfil demográfico também relatado em estudos anteriores e contrastando com a “teoria da maturação” proposta por Cahn & Blum (Buchner & Vered, 2013; Soluk-Tekkesin & Vered, 2021; Guo *et al.*, 2024). Além disso, FSA comumente ocorre em pacientes na terceira década de vida, geralmente decorrente de FA ou FOA preexistente, um achado observado em 90% dos FSA no presente estudo.

De maneira similar a outras lesões odontogênicas, FA e FDA/FOA apresentam uma forte predileção pelas regiões posteriores dos ossos gnáticos, particularmente a mandíbula, como lesões radiolúcidas uniloculares ou

multiloculares associadas à coroa de um molar incluso, causando expansão e destruição de corticais ósseas (Vered *et al.*, 2023; Wright & Chi, 2023). Curiosamente, nenhum dos casos no presente estudo ocorreu na maxila anterior, uma região tipicamente afetada por odontoma composto. Em uma publicação recente, Soluk-Tekkesin & Vered (2021) sugeriram que, além de pacientes com menos de 13,5 anos de idade, lesões maiores que 2,1 cm provavelmente representam neoplasias verdadeiras, em vez de odontomas em desenvolvimento. No presente estudo, o tamanho médio dos casos de FA e FDA/FOA foi de 3,7 e 2,5 cm, respectivamente. FSA geralmente se apresenta como uma lesão agressiva e de crescimento rápido, com ou sem dor associada, características também observadas no presente estudo (Wright & Chi, 2023).

Um achado clínico interessante observado em alguns casos do presente estudo foi o crescimento tumoral exuberante após a realização de biópsia incisional. Após biópsia incisional, as lesões frequentemente se exteriorizavam na cavidade oral. Embora essa característica não seja exclusiva de FA ou FDA/FOA, ela sugere um comportamento biológico mais agressivo quando comparado aos odontomas, reforçando sua natureza neoplásica. No presente estudo, 90% dos pacientes com FSA tinham histórico de diagnóstico prévio de FA ou FOA que posteriormente sofreu transformação maligna, com intervalo médio de 4,25 anos entre os diagnósticos. Embora a etiologia e os mecanismos moleculares que desencadeiam essa progressão sejam pouco compreendidos, a recorrência tumoral e múltiplas intervenções cirúrgicas têm sido constantemente implicadas como fatores de risco para transformação maligna (Muller *et al.*, 1995; Chrcanovic *et al.*, 2018). Dado o período de latência observado no presente estudo, recomendamos fortemente que os pacientes diagnosticados com FA ou FOA sejam submetidos a acompanhamento clínico e radiográfico rigoroso a cada seis meses durante os primeiros quatro anos após o tratamento cirúrgico e, posteriormente, anualmente, devido ao potencial de progressão para FSA.

Aproximadamente 20 casos de FA e FDA/FOA periféricos foram relatados na literatura até o momento, apresentando-se preferencialmente como um

nódulo gengival adjacente aos dentes incisivos superiores de pacientes pediátricos (Ide *et al.*, 2008; Chrcanovic *et al.*, 2018; Alramadhan *et al.*, 2022). No presente estudo, dois pacientes apresentaram nódulos gengivais localizados na gengiva anterior inferior e superior, sem alterações radiográficas. A análise microscópica revelou lesões submucosas com características consistentes com FA e FDA. Ambos os pacientes foram tratados por excisão cirúrgica conservadora. Interessantemente, o caso de FA periférico revelou expressão imuno-histoquímica forte e difusa para BRAF p.V600E, sugerindo uma possível natureza neoplásica ao invés de uma lesão hamartomatosa inócua como previamente presumida (Ide *et al.*, 2008).

FA, FDA/FOA e FSA apresentam um amplo espectro de características microscópicas de ambos os componentes epitelial e ectomesenquimal. O componente epitelial é tipicamente organizado em cordões e ilhas, frequentemente apresentando-se com uma camada periférica de células cuboidais ou colunares e uma área central que se assemelha ao retículo estrelado do órgão do esmalte em desenvolvimento (Takeda, 1999; Vered *et al.*, 2023; Wright & Chi, 2023). A quantidade e a densidade do componente epitelial variam significativamente dentro do mesmo tumor e se tornam menos proeminentes quando compara os FA e FDA/FOA com FSA, como foi observado no presente estudo. A presença de áreas semelhantes ao tumor odontogênico primordial, células claras, pigmentação melânica, células granulares estromais e associação com outras lesões odontogênicas foram achados incomuns no presente estudo e Patologistas Orais devem reconhecer a variação microscópica de FA e FDA/FOA.

FSA apresentam o componente maligno restrito ao estroma ectomesenquimal odontogênico, classicamente caracterizado por hipercelularidade acentuada, atipia celular, pleomorfismo nuclear e aumento da atividade mitótica (Wright & Chi, 2023). No presente estudo, todos os casos de FSA apresentaram as características microscópicas previamente descritas. Curiosamente, estudos anteriores demonstraram que, à medida que a malignidade progride, o componente epitelial frequentemente se torna menos

proeminente e pode eventualmente desaparecer, provavelmente devido a alterações degenerativas. O FSA com transformação de alto grau é tipicamente marcado por características anaplásicas pronunciadas, incluindo células gigantes tumorais bizarras, mitoses atípicas e áreas de necrose, as quais foram presentes em um caso no presente estudo (Muller *et al.*, 1995).

Um achado microscópico peculiar observado foi a presença de diferenciação condromixoide no tecido ectomesenquimal. Devido a esse aspecto condroide, a investigação com SOX9 foi realizada, resultando em forte e difusa expressão nuclear no componente epitelial e ectomesenquimal em FA, FDA/FOA e FSA. SOX9, um fator de transcrição classicamente descrito como um regulador mestre da condrogênese, desempenha um papel essencial nas fases iniciais da diferenciação dos condrócitos e sua associação com odontogênese tem sido pouco relatada na literatura (Lefebvre *et al.*, 2019; Krivanek *et al.*, 2020). Curiosamente, no caso de FSA com transformação de alto grau, as células mesenquimais sarcomatosas apresentaram expressão reduzida de SOX9 em comparação com a biópsia inicial. Esse achado pode sugerir que, à medida que a transformação maligna progride, as células neoplásicas perdem gradualmente características odontogênicas, incluindo marcadores específicos de linhagem, como SOX9. Este estudo representa a primeira evidência da expressão de SOX9 em tumores odontogênicos.

Avanços recentes em estudos moleculares começaram a aprimorar nossa compreensão das lesões odontogênicas e levaram a avanços no diagnóstico e no ramo das terapias em alvo. Atualmente, evidências acumuladas indicam que alguns FA e FDA/FOA apresentam mutações patogênicas de BRAF p.V600E (Coura *et al.*, 2020; Guimarães *et al.*, 2021; Guo *et al.*, 2024). Inicialmente acreditadas por estarem confinadas ao epitélio odontogênico, a presença da mutação de BRAF demonstrou afetar o componente ectomesenquimal de FA e FDA/FOA (Brunner *et al.*, 2015; Coura *et al.*, 2020; Guimarães *et al.*, 2021; Guo *et al.*, 2024). Esses achados corroboram a hipótese de que o crescimento tumoral nessas lesões é predominantemente impulsionado pelo componente ectomesenquimal (Coura *et al.*, 2020).

De acordo com uma revisão sistemática recente, FA, FDA, FOA e FSA demonstraram mutações patogênicas do gene BRAF no componente ectomesenquimal detectadas por métodos moleculares em aproximadamente 48,1%, 60%, 26,3%, e 82%, respectivamente (Severino-Lazo *et al.*, 2023). Quando avaliadas por métodos imuno-histoquímicos, as taxas de expressão diminuem para 20% em FA, 10% em FOA e 50% em FSA. No presente estudo, realizamos reações imuno-histoquímicas para BRAF p.V600E em 40 tumores benignos, detectando expressão citoplasmática positiva no componente ectomesenquimal em FA e FDA/FOA em 81% e 54% dos casos, respectivamente, um achado significativamente maior do que o relatado anteriormente na literatura. Nos FSA, entre os cinco casos testados, 60% demonstraram expressão citoplasmática positiva nas células sarcomatosas — um achado consistente com dados previamente relatados na literatura. Curiosamente, os dois FSA negativos para BRAF no presente estudo foram casos que exibiram células vacuoladas (morfologia em anel de sinete) e pleomorfismo acentuado no componente mesenquimal. Esses achados sugerem que, em sarcomas odontogênicos malignos altamente pleomórficos, a avaliação imuno-histoquímica do gene BRAF p.V600E pode ter utilidade diagnóstica limitada, e a confirmação molecular pode ser necessária para determinar com precisão o status da mutação BRAF.

A discrepância entre os resultados moleculares e imuno-histoquímicos em publicações anteriores pode ser devida à maior sensibilidade dos testes moleculares, com alguns autores sugerindo a possibilidade de resultados falso-negativos em estudos imuno-histoquímicos (Oh *et al.*, 2021). De fato, vários tumores agressivos e destrutivos em nosso estudo apresentaram resultados negativos para BRAF p.V600E, destacando a necessidade de investigação molecular adicional em casos específicos. Infelizmente, a investigação molecular não pôde ser realizada no presente estudo, o que seria ideal para confirmar a presença da mutação BRAF. As altas taxas de positividade observadas no presente estudo podem ser atribuídas ao maior tamanho amostral utilizado para avaliar o gene BRAF p.V600E em comparação com estudos anteriores e à

possibilidade de resultados falso-positivos (Oh *et al.*, 2021). A investigação de mutações no gene BRAF p.V600E em tumores odontogênicos permanece um tópico relativamente recente na literatura e deve ser abordada com cautela na prática clínica, dada a significativa possibilidade de resultados negativos em tumores com características clínicas agressivas.

A maioria dos pacientes diagnosticados com FSA é tratado com sucesso com ressecção cirúrgica segmentar, embora taxas de recorrência de até 35% tenham sido reportadas (Wright & Chi, 2023). Uma nova via terapêutica surgiu com o uso de terapias direcionadas, particularmente com o uso de inibidores de BRAF, que mostraram resultados promissores no tratamento de ameloblastomas e carcinomas ameloblásticos. Essas abordagens direcionadas demonstraram um potencial significante na redução da morbidade cirúrgica e na melhora do desfecho clínico (Bologna-Molina *et al.*, 2024). Além disso, estudos recentes identificaram SOX9 como um novo alvo terapêutico e um biomarcador potencial associado à resistência à terapia em diversos tipos de câncer (Tripathi *et al.*, 2022). Essas percepções são promissoras para o desenvolvimento de estratégias terapêuticas menos invasivas, particularmente para tumores extensos e agressivos em pacientes jovens. Infelizmente, TOM ainda não foram estudados neste contexto; no entanto, isso pode mudar no futuro próximo, à medida que novas pesquisas exploram seus perfis moleculares e respostas terapêuticas. Patologistas orais devem estar cientes de que, embora atualmente não exista um sistema de estadiamento padronizado para sarcomas odontogênicos, o uso de relatórios de conjuntos de dados da *International Collaboration on Cancer Reporting* (ICCR) é fortemente recomendado para garantir documentação e coleta de dados consistentes na prática clínica (Slootweg *et al.*, 2019).

4 CONCLUSÃO

- O presente estudo representa uma das maiores séries bem documentadas de FA, FDA/FOA e FSA na literatura. FDA/FOA afetaram pacientes mais jovens quando comparado ao FA, enquanto FSA acometeram adultos jovens com diagnóstico prévio de FA/FOA. Devido ao risco de transformação maligna, pacientes diagnosticados com FA ou FDA/FOA devem ter acompanhamento clínico-radiográfico rigoroso durante os primeiros 4 anos após o tratamento cirúrgico;
- FA, FDA/FOA e FSA mostraram predileção pela região posterior dos ossos gnáticos, raramente ocorrendo na região anterior ou como lesões periféricas;
- Os componentes epiteliais e ectomesenquimais do FA e FDA/FOA exibem espectro morfológico amplo, incluindo pigmentação melânica, células claras, células fantasmas, células granulares mesenquimais, diferenciação condromixoide e o primeiro relato de FOA associada a FCO e LCCG. FSA apresentam hipercelularidade no componente ectomesenquimal e menos epitélio odontogênico;
- A expressão imuno-histoquímica de BRAF p.V600E é frequentemente positiva em FA, FDA/FOA e FSA mostrando expressão citoplasmática confinada ao componente ectomesenquimal, enquanto SOX9 foi fortemente expressa nos componentes epitelial e ectomesenquimal. A expressão difusa de SOX9 em FA, FDA/FOA e FSA sugere um papel na diferenciação odontogênica, um achado novo que pode ter implicações para a compreensão da histogênese dessas lesões. Esses resultados contribuem para a caracterização molecular de TOM e podem auxiliar no desenvolvimento de novas abordagens terapêuticas mais eficazes e direcionadas;
- O comportamento agressivo de alguns FA e FDA/FOA nesse estudo justifica sua classificação como neoplasias odontogênicas e não como hamartomas.

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ANEXOS

Anexo 1 – Certificado do Comitê de Ética

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

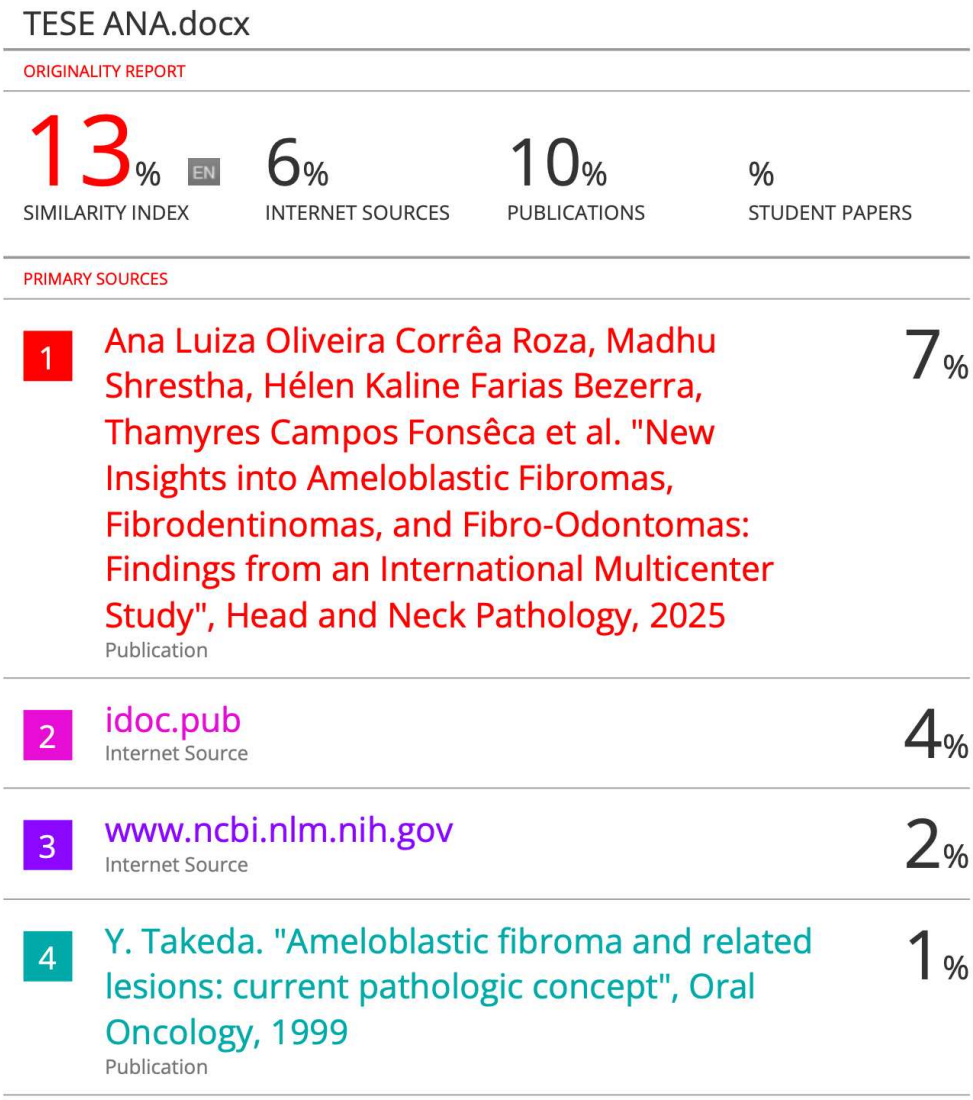
Título da Pesquisa: Estudo clinicopatológico e proteômico de fibromas e fibrossarcomas ameloblásticos

Pesquisador: Ana Luiza Oliveira Correa Roza
Área Temática:
Versão: 2
CAAE: 43944721.1.0000.5418
Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.601.106

Anexo 2 – Relatório de verificação de originalidade e prevenção de plágio



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Anexo 3 – Comprovante de publicação (Artigo 1)

Head and Neck Pathology (2025) 19:57
https://doi.org/10.1007/s12105-025-01792-0

RESEARCH

New Insights into Ameloblastic Fibromas, Fibrodentinomas, and Fibro-Odontomas: Findings from an International Multicenter Study

Ana Luiza Oliveira Corrêa Roza[1] · Madhu Shrestha[2] · Hélen Kaline Farias Bezerra[1] · Thamyres Campos Fonsêca[3] · Ciska-Mari Schouwstra[4] · Chané Smit[4] · André Caroli Rocha[5] · Michelle Agostini[3] · Aline Corrêa Abrahão[3] · Letícia Ferreira Cabido[2] · Victoria Woo[2] · Jacks Jorge Júnior[1] · Willie F. P. van Heerden[4,6] · John M. Wright[2] · Mário José Romañach[3] · Pablo Agustin Vargas[1,7]

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Abstract

Introduction Ameloblastic fibroma (AF), ameloblastic fibrodentinoma (AFD), and ameloblastic fibro-odontoma (AFO) are rare mixed odontogenic tumors. While some authors propose that some cases may evolve into odontomas, other tumors with aggressive clinical features suggest a neoplastic origin. A subset of AF and AFD/AFO harbor the pathogenic BRAF p.V600E mutation. SOX9, known for its role in the differentiation of various cell types, particularly in chondrogenesis, has not been previously studied in odontogenic tumors. In this study, we report the clinicopathologic features of a large international cohort of AF and AFD/AFO cases and analyze the immunohistochemical expression of BRAF p.V600E and SOX9.

Materials and methods Clinical and radiographic data were collected from four Oral and Maxillofacial Pathology service archives spanning from 1991 to 2024. Deidentified slides were reviewed by two independent oral pathologists. Immunohistochemical staining for BRAF p.V600E and SOX9 was performed on non-decalcified tissue samples from cases with available specimens.

Results A total of 62 tumors were identified, including 30 AF cases and 32 AFD/AFO cases. The cohort consisted of 33 male and 29 female patients, with average ages of 15.3 years for AF and 12.3 years for AFD/AFO. Tumors predominantly affected the posterior mandible and appeared as unilocular or multilocular radiolucent or mixed lesions, often causing tooth impaction and cortical expansion, with an average size of 3.7 cm for AF and 2.5 cm for AFD/AFO. Two cases were classified as peripheral AF/AFD. Microscopically, all cases exhibited cellular mesenchymal components resembling dental papilla, with branching strands and islands of odontogenic epithelium. AFD/AFO cases also displayed dental hard tissue, and occasional chondromyxoid differentiation was observed within the stroma. Rare hybrid tumors were identified, including associations with calcifying odontogenic cysts, cemento-ossifying fibroma and central giant cell granuloma. BRAF p.V600E showed cytoplasmic positivity in the mesenchymal component of AF (81%) and AFD/AFO (54%). SOX9 exhibited diffuse nuclear immunoreactivity in both epithelial and mesenchymal components (92%).

Conclusion This study represents one of the largest well-documented series of AF and AFO/AFD, providing valuable clinicopathologic and immunohistochemical insights. Additionally, the diffuse expression of SOX9 in both epithelial and mesenchymal components suggests a potential role in odontogenic differentiation, a novel finding that may have implications for understanding the histogenesis of these lesions. The aggressive behavior of some AFs and AFD/AFOs in our study supports their classification as odontogenic neoplasms rather than hamartomas.

Keywords Odontogenic tumors · Ameloblastic fibroma · Ameloblastic fibro-odontoma · BRAF p.V600E · SOX9

Anexo 4 – Comprovante de submissão (Artigo 2)

Gmail

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Head and Neck Pathology - Receipt of Manuscript 'Odontogenic Sarcomas: Clinicopathologic...'

1 message

Head and Neck Pathology <maryjenifer.ramesh@springernature.com> Mon, May 19, 2025 at 10:09 AM

To: naluroza@gmail.com

Ref: Submission ID 662ec460-e6d2-4486-8c01-083719429457

Dear Dr Roza,

Please note that you are listed as a co-author on the manuscript "Odontogenic Sarcomas: Clinicopathologic Analysis and Immunohistochemical Expression of BRAF and SOX9 in a Multicenter Series of 10 Cases", which was submitted to Head and Neck Pathology on 19 May 2025 UTC.

If you have any queries related to this manuscript please contact the corresponding author, who is solely responsible for communicating with the journal.

Kind regards,

Editorial Assistant
Head and Neck Pathology