Very large Ameloblastic Fibroma with Calcifying Odontogenic Cyst in an 8-year-old child. Histological and immunohistochemical characterisation

T. Cutilli*, G. Coletti**,
M. Walid A. Fatayer**,
S. Caruso***, S. Tecco****,
R. Gatto***, P. Leocata**

Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy
**Maxillofacial Surgery Operative Unit, San Salvatore City Hospital, L'Aquila, Italy*
***Pathology Operative Unit, San Salvatore City Hospital, L'Aquila, Italy*
****Dental School*
*****Dental School, Vita-Salute San Raffaele University, Milan, Italy*

e-mail: silvia.caruso@univaq.it

DOI 10.23804/ejpd.2019.20.01.04

Abstract

Background Ameloblastic fibroma (AF) is an uncommon odontogenic tumour that may present an aggressive behaviour and may have potential for malignant transformation. Ghost cell (GC) differentiation within AF is extremely rare. There are only seven cases in the international literature in which ghost cells are found in AF.

Caae report In this study, we report a case of a 8-year-old female child with a cystic-solid mass, measuring 3 x 1.7 x 1.2 cm, characterised by mixed odontogenic tumour, with AF in most of the lesion, with areas characterised by GC, non mi è chiaro, non so se ho corretto giusto while ameloblastic and ameloblastic fibrodontoma areas were also detected. Other histological sections showed only AF tissue, with areas of Calcifying Odontogenic Cyst. The immunohistochemical characterisation of the lesion was also performed. A comparative table of the immunoistochemical staining of the AF and COC areas revealed some differences in the expression of markers.

KEYWORD Ameloblastic fibroma, calcification, COC, ghost cells

Introduction

Ameloblastic fibromas (AFs) are neoplasms of odontogenic epithelium and mesenchymal tissues and are classified as mixed odontogenic tumours. Other mixed odontogenic lesions, such as ameloblastic fibro-odontomas and odontomas share some clinical, radiographic and histologic similarities with AF. AFs are rare and comprise approximately 1.5–4.5% of all odontogenic tumours [Regezi et al., 1978; Cohen and Bhattacharya, 2004; Philipsen et al., 1997; Cargini, 2012]. These lesions are considered tumours of childhood and adolescence and occur almost exclusively in the first and second decades of life [Slootweg, 1981]. A slight male prevalence has been noted [Philipsen et al., 1997; Slootweg, 1981]. The most common location for the tumour is the posterior mandible, followed by the posterior maxilla. An impacted tooth may be associated with the tumour in approximately 75% of the cases [Cohen and Bhattacharya, 2004; Philipsen et al., 1997; Trodahl, 1972; Nasim, 2015].

Since this lesion may potentially contain various types of tissues that might affect the final diagnosis, it is imperative to remove the tumour preserving the integrity of the lesion intact, for an accurate histological examination. This approach should be followed also when radiologically the lesion has a great extension. This case report shows the correct therapeutic approach, in accordance with the guidelines of the oncological management of this rare lesion of childhood.

Case report

An 8-year-old girl was admitted to the the Maxillofacial Surgery Department, University of L'Aquila, San Salvatore City Hospital, in December 2015, because of swelling of the right maxillary vestibule. The clinical examination revealed absence of the permanent upper right first molar. The dental X-ray showed a radiopaque area at the right maxillary sinus,

and the displacement of the first molar in a superior and posterior area (Fig. 1). Initially the patient had been admitted for an orthodontic treatment with palatal expansion to treat the case as an impacted tooth [Tecco et al., 2007]. But the patient also referred pain in the right area of the mandible, that was initially hypothised as Temporo-mandibular joint pain [Cianetti et al., 2017]. Then, a CT examination was scheduled. CT-slices showed a maxillary lesion that largely occupied the right maxillary sinus extending in the pterygoid-maxillary fossa with a serious deformation of the maxillary walls (Fig. 2). The lesion was initially hypothised also as a infiammatory granuloma [Tecco et al., 2018].

The patient underwent surgery under general anaesthesia. Dental fear and anxiety was controlled pharmacologically. [Tecco et al., 2017]. The lesion was enucleated by oral approach and by the use of optical instruments; since neither partial maxillary resection nor radical enucleation (with cytolytic liquid or simal) were performed, the specimen was sent for histopathological examination. The macroscopic examination showed a cystic-solid mass, measuring 3 x 1.7 x 1.2 cm (Fig. 3): the solid part of the lesion was white, while the cystic portion was brown-white. Another portion of white tissue measuring 1.5 x 0.6 x 0.3 cm contained a brownish polypoid formation. The permanent first molar was also removed (Fig. 4, 5).

Histological characterisation

The histological sections of the tissue embebbed in paraffin had a thickness of 4 microns. They were stained with haematoxylin and eosin (HE), and Congo red.

The solid portion of the lesion showed a cellular myxoid stroma resembling the primitive dental papilla, mixed with numerous islands of ameloblastic epithelium consisting of interconnecting strands, islands and cords of proliferating odontogenic epithelium, within dense collagenous stroma, that was often immature. The cords were usually only two cells in thickness, with columnar or cuboidal cells. The peripheral layer of cells showed reverse nuclear polarity and a distinct basal membrane, a characteristic of AF.

There were also deposits of dentinoid material, adjacent to the epithelial lining; enamel organ-like epithelial islands were observed within the primitive dental papilla, which characterised as Ameloblastic Fibro Odontoma (AFD).

Masses of Ghost cells (GC) were also observed within the ameloblastic epithelium, in the AF area. GCs are pale enucleated cells, with a homogeneous eosinophilic cytoplasm and with a very clear central area, instead of the basophilic nucleus. The calcification seems to occur in the back area of the GC. Only few odontogenic and non-odontogenic tumours exhibit the presence of these GC as a typical feature. GC are typical of Calcifying Odontogenic Cysts (COC), which are commonly associated with other odontogenic tumours, mostly odontoma, and are rarely associated with AF.

Microscopically, COC consists of numerous polyhedral masses of epithelial cells, with a well-defined layer of columnar cells, with a characteristic concentric calcification.

The final microscopic diagnosis was a mixed odontogenic tumour, characterised by AF in most of the lesion, with areas presenting GCs, while ameloblastic and AFD areas were also observed, but to a lesser extent. Other histological sections exhibited only AF tissue, with areas of COC.

Immunohistochemical characterisation

Immunohistochemical examination was performed by using EnVision FLEX and Dako detection system, with Autostainer Link 48, Dako Immunostainer (Agilent Technologies, Santa Clara, USA). The sections were dehydrated and treated at pH 6 or 9, at 97°C for 15 minutes in a thermostatic bath (PT Link Dako). Finally, the sections were cooled in Tris (EnVision FLEX Wash Buffer) and included in immunostainer, and then submitted to a computerised processing cycle. Then, they were finally stained with Carazzi's haematoxylin, rinsed in water, and dehydrated.

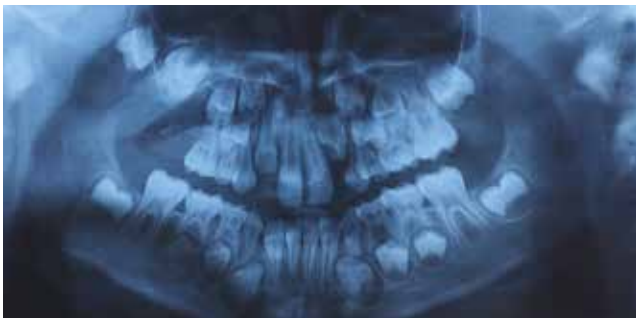

FIG. 1 Dental X-ray.

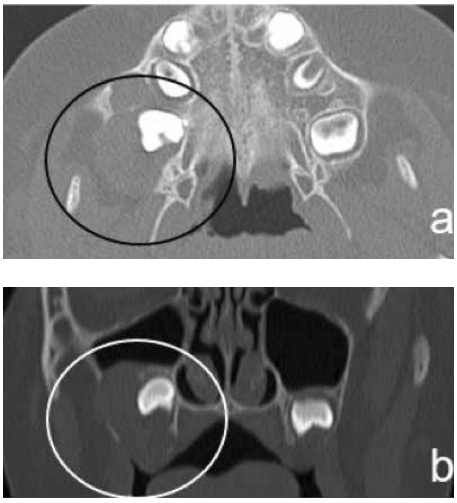

FIG. 2 Preoperative axial (A) and coronal (B) CT-slices: the very large lesion also expands in the pterygoid-maxillary fossa (black and white circles)

FIG. 3 Macroscopic aspect of the cystic-solid mass, measuring 3 x 1.7 x 1.2 cm. FIG. 4 The extracted tooth

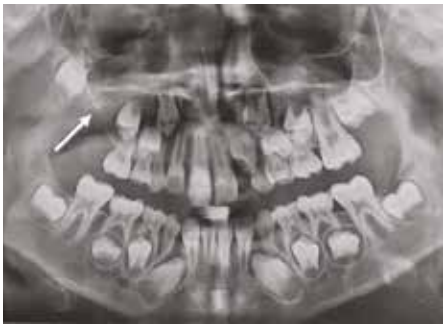

FIG. 5 Post-operative dental X-ray shows a good and regular aspect of the right profile of the maxilla (white arrow) without lesion.

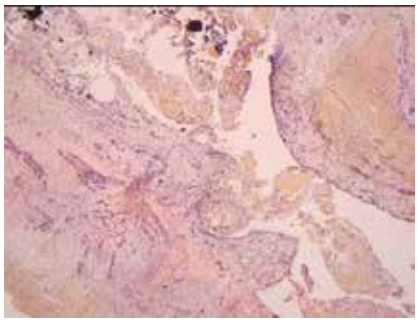

FIG. 6 AF in the main portion of the lesion, with areas of odontogenic GC tumour, ameloblastic areas and COC tumour (magnification 4x).

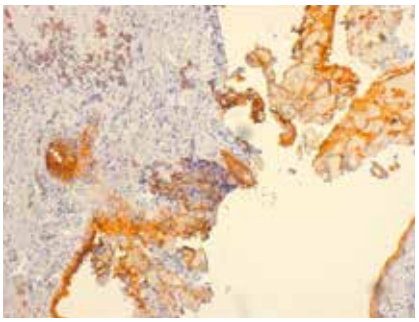

FIG. 7 A staining of the epithelial elements of AF, a stronger staining of CK19 is expressed in COC elements (magnification 10x).

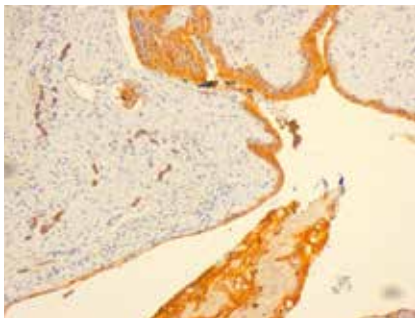

FIG. 8 CK5-6, a strong stainig is observed for the epithelial of AF, and also in COC elements (magnification 10x).

To confirm the hystological diagnosis, the following markers were used: CK14, CK19, CD138, Ki67, S-100, GFAP, CK5-6, Collagen IV, p63, EGFR, and p53. The results of the immunohistochemical staining were as follows.

- CK14: A strong staining was observed in the epithelium of AF and also in the COC elements (Fig. 6).
- CK19: Staining was observed for the epithelial elements of AF, but CK19 was more strongly expressed in COC elements (Fig. 7).
- CD138: This marker is also known as Syndecan-1. A strong staining was observed in the epithelial elements of AF, an also in COC elements.
- Ki67: The expression of this marker was positive in about 1% of ameloblastic cells and COC elements. The weak expression of this marker indicated a low proliferative rate of the lesion, further substantiating its benign nature.
- S-100: A strong staining of this marker is generally shown in Langerhans and Schwann cells. In the lesion there were a few S-100 positive cells in the foci of epithelial components, in both AFD and AF areas.
- GFAP (glial fibrillary acidic protein): no staining was shown (negative expression).
- CK5-6: A strong staining was expressed in the epithelial elements of AF, and also in the COC elements (Fig. 8).
- Collagen IV: A positive staining was expressed in the lesion.
- p63: An extremely strong staining was expressed for the epithelial elements of AF, but less staining was expressed in COC elements.
- EGFR: A strong staining was observed for the epithelial elements of AF and COC elements.
- p53: No staining was shown (negative expression).

Table 1 summarises the differences in the expression of the markers between the AF and the COC areas.

Markers of immunohistochemically	Ameloblasic Fibroma (AF)	Calcifying Odontogenic Cyst (COC)
CK14	+ +	+ +
CK19	+	+ +
CD138	+ +	+ +
S-100	+	+
GFAP	-	-
CK5-6	+ +	+ +
p63	+ +	+
EGFR	+ +	+ +
p53	-	-

TABLE 1 Comparative data of the immunohistochemical staining in the AF and the COC areas.

Discussion

This case report presents a correct therapeutic approach to the case, in accordance with the oncological management guidelines of a rare lesion of childhood, with data on histological and immunohistochemical characterisation.

The microscopic diagnosis was a mixed odontogenic tumour, characterised by AF in most of the lesion, with areas characterised by GC, while ameloblastic and AFD areas were also observed, but to a less extent. Some histological sections exhibited only AF tissue, with areas of COC.

AF was first reported by Kruse in 1891, and it is considered a rare benign mixed odontogenic tumour, with a relative frequency between 1.5% and 4.5% [Philipsen et al., 1997].

AF is generally considered a true mixed odontogenic tumour, in which both the epithelial and ectomesenchymal components are neoplastic. Indeed, these lesions show true neoplastic features respect to other mixed odontogenic lesions – as AFO or odontomas – that are better categorised as hamartomas, and, unlike the AF, have little chance of recurrence or malignant transformation [Slootweg, 1981; Nelson and Folk, 2009].

Within the limits of our knowledge, occurrence of GC in AF as those seen in COC is even a rarer event: there are only seven cases in the international literature in which GC are found in AF but all these previously reported cases were associated with typical COC [Arora et al., 2015]. In this case report, focal areas of GC differentiation are observed within the neoplastic epithelium, in the AF area. The lesion described in our study was mostly solid, with characteristic features of AF. In addition, in few areas, ameloblastic epithelium with ghost cell features and calcifications were manifested.

The literature reports a unique case in a 3.5-year-old child with a solid lesion that comprised odontogenic epithelium strands, islands, and myxoid ectomesenchyme, with focal areas of GC differentiation and calcification associated with neoplastic epithelium [Arora et al., 2015]. We present a comparative table of the immunoistochemical staining of the AF and COC areas (Table 1). The comparison revealed that the p63 marker expressed an extremely strong staining in the epithelial elements of AF, and a lower degree of staining was expressed in COC elements. In human oral mucosa, p63 is mainly restricted to basal and para-basal layers of the normal

epithelium [Gonçalves et al., 2012]. As the odontogenic cystic lesions are essentially of epithelial origin, the p63 protein may be strongly expressed during their growth and progression, indicating maintenance and integrity of cystic odontogenic epithelial lining, and so favouring the lesion persistence. It remains unknown whether those COCs which have features of the other odontogenic tumours develop those secondarily, or they are themselves secondary phenomena in pre-existing odontogenic tumours [Arora et al., 2015]. In our case, the lower staining of p63 marker in COC elements seems to indicate that it is a secondary phenomenon to the pre-existing odontogenic tumour.

In order to further characterise the lesion, the following markers were used for immunohistochemistry: CK14, CK19, CD138, Ki67, S-100, GFAP, CK5-6, Collagen IV, EGFR, and p53. The cytokeratin 14 and 19 stain odontogenic epithelium in all the stages of tooth development, including the phases of the dental lamina and the stellate reticulum, although the cytokeratin 19 is more prominent in the later stage of tooth development [Crivelini et al., 2003; Domingues et al., 2000]. Thus, the strong expression that we found for CK14 and CK19 in the epithelial cells confirmed the odontogenic nature of our lesion. The CD138 marker (also known as Syndecan-1) in our case showed a strong staining of the epithelial elements of AF, and also the COC elements. A positive expression of the Syndecan-1 is frequent in the enamel epithelium while maturing and is a common feature of both stromal cells and extracellular matrix, while the lack of Syndecan-1 expression in stromal cells and extracellular matrix could be hypothesized as a critical factor for carcinogenesis and local invasiveness of intraosseous ameloblastomas [Leocata et al., 2007]. The weak expression of Ki67 in our lesion indicated a low proliferative rate of this tumour, corroborating its benign nature [Sano et al., 1998]. There were a few S100 marker-positive cells in the foci of epithelial components in both the AFD and the AF areas, while the mesenchymal cells characterised by a dendritic or spindle shape were positive for S100 protein, both in the AFD and the AF areas [Takeda et al., 2000]. A strong staining of this marker is generally shown in Langerhans and Schwann cells. A positive staining for GFAP marker was observed only in the juxta-epithelial mesenchymal tissue, suggesting the formation of immature dentin. It was observed in various numbers of entrapped cells in AFD. In AF, no GFAP-positive cells were found [Heikinheimo et al., 1992]. However, GFAP staining was negative in other mesenchymal and epithelial tissues. In our lesion, a positive staining was expressed for the Collagen IV. The type IV collagen is one of the components of the basal membrane that is the organised extracellular matrix separating the epithelium and the adjacent connective tissue stroma. Absence or discontinuity of a basal membrane correlates with an aggressive behaviour of tumours. According to Heikinheimo et al. [1992], focal absence of laminin and type IV collagen from the basal membrane and the presence of fibronectin containing an oncofetal domain in the extracellular matrix of ameloblastoma, may correlate with their aggressive behaviour.

The epidermal growth factor receptor (the EGFR marker) is localised on the surface membranes of many cell types and is mostly involved in cell proliferation [Vered et al., 2003]. The EGFR is frequently present in the epithelial elements of human tooth germ as well as cystic odontogenic lesions and odontogenic tumours, thus implicating its participation during normal odontogenesis and development of these lesions [Li et al., 1997; Tanikawa et al., 1999].

No staining was shown in our case (negative expression) for the p53 marker. Some studies associated the high-risk HPV-16 infection (HR-HPV16) to the over-expression or mutation of the p53 gene in oral cancer [Cutilli et al., 2016(a); Cutilli et al., 2016(b)]. The COC tumour, also known as a Pindborg tumour, characterised by the presence of amyloid material, was not found in our case. Clinical, pathological and biological behaviour of the AF requires particular attention especially when it affects children. Radical surgical excision with removal of the affected teeth is the treatment of choice.

The recurrence rate varies among sources, but is considered low [Leider et al., 1992; Altini et al., 1985]. Though uncommon, the possibility of malignant transformation of AF into ameloblastic fibrosarcoma is well documented [Leider et al., 1992; Altini et al., 1985]. For this reason, periodic and accurate follow-ups are a must. The lesion can be removed by radical enucleation, enucleation with sterilization of the bony edges by chemical (liquid nitrogen, etc.) or physical means (bur).

References

- Altini M, Thompson SH, Lownie JF, Berezowski BB. Ameloblastic sarcoma of the mandible. J Oral Maxillofac Surg 1985;43:789-794.
- Arora M, Dave A, Shetty V, Saluja P. Ameloblastic fibroma with ghost cell differentiation and calcification: a unique case report. J Cancer Res Ther 2015;11(4):1033.
- Cargini P, Fidanza F, Facente MV, Sgolastra F, Gatto R, Cutilli T. Gingival myofibroma. A case report. Eur J Paediatr Dent 2012; 13 (1): 81-83.
- Cianetti S, Lombardo G, Lupatelli E, Pagano S, Abraha I, Montedori A, Caruso S, Gatto R, De Giorgio S, Salvato R.Dental fear/anxiety among children and adolescents. A systematic review.Eur J Paediatr Dent 2017 Jun;18(2):121-130.
- Cohen DM, Bhattacharyya I. Ameloblastic fibroma, ameloblastic fibro-odontoma, and odontoma. Oral Maxillofac Surg Clin North Am 2004;16:375-384.
- Crivelini MM, de Araujo VC, de Sousa SOM, de Araujo NS. Cytokeratins in epithelia of odontogenic neoplasms. Oral Dis 2003;9(1):1-6.
- Cutilli T, Leocata P, Dolo V, Altobelli E. The Association between p53 status, human papillomavirus infection, and overall survival in advanced oral cancer after resection and combination systemic treatment. Brit J Oral Maxillofacial Surg 2016 (a); 54:198–202.
- Cutilli T, Leocata P, Dolo V, Altobelli E. p53 as a prognostic marker associated with the risk of mortality for oral squamous cell carcinoma. Oncol Letters 2016 (b);12:1046-1050.
- Domingues MG, Jaeger MM, Araujo VC, Araujo NS. Expression of cytokeratins in human enamel organ. Eur J Oral Sci 2000;108(1):43-47.
- Gonçalves CK, Fregnani ER, Leon JE, Silva-Sousa YT, Perez DE. Immunohistochemical Expression of p63, Epidermal Growth Factor Receptor (EGFR) and Notch-1 in Radicular Cysts, Dentigerous Cysts and Keratocystic Odontogenic Tumor. Braz Dent J 2012;23(4):337-343.
- Heikinheimo K, Morgan PR, Happonen RP. Distribution of extracellular matrix proteins in odontogenic tumours and developing teeth. Cell Pathol 1992;61(2):101-109.
- Kruse A. On the development of cystic tumors in the mandible. Arch Path Anat 1891;124:137-148.
- Leider AS, Nelson JF, Trodahl JN. Ameloblastic fibroma. Oral Surg 1972;33:559-569.
- Leocata P, Villari D, Fazzari C, Lentini M, Fortunato C, Nicòtina A. Syndecan-I and wingless-type protein-I in human ameloblastomas. J Oral Path Med 2007;36:394-399.
- Li TJ, Browne RM, Matthews JB. Immunocytochemical expression of growth factors by odontogenic jaw cysts. Mol Pathol 1997;50:21-27.
- Nelson BL, Folk GS. Ameloblastic fibroma. Head Neck Pathol 2009;3:51-53.
- Philipsen HP, Reichart RP, Pratorius F. Mixed odontogenic tumors and odontomas: considerations on interrelationship. Review of the literature and presentation of 134 new cases of odontomas. Oral Oncol 1997;33:86-99.
- Regezi JA, Kerr DA, Courtney RM. Odontogenic tumors: analysis of 706 cases. Oral Surg 1978;36:771-778.
- Sano K, Yoshida SI, Ninomiya H et al. Assessment of growth potential by MIB-1 immunohistochemistry in ameloblastic fibroma and related lesions of the jaws compared with ameloblastic fibrosarcoma. J Oral Path and Med 1998;27(2):59-63.
- Slootweg PJ. An analysis of the interrelationship of mixed odontogenic tumors—ameloblastic fibroma, ameloblastic fibro-odontoma, and the odontomas. Oral Surg Oral Med Oral Pathol 1981;51:266-276.
- Takeda Y, Sato H, Satoh M, Nakamura SI, Yamamoto H. Immunohistochemical expression of neural tissue markers (neu-ron-specific enolase, glial fibrillary acidic protein, S100 protein) in ameloblastic fibrodentinoma: a comparative study with ameloblastic fibroma. Pathol Int 2000;50(8):610-615.
- Tanikawa Y, Bawden JW. The immunohistochemical localization of phospholipase cγand the epidermal growth-factor, platelet-derived growth-factor and fibroblast growth-factor receptors in the cells of the rat molar enamel organ during early amelogenesis. Arch Oral Biol 1999;44:771-780.
- Tecco S, Caputi S, Festa F. Evaluation of cervical posture following palatal expansion: a 12-month follow-up controlled study. Eur J Orthod. 2007 Feb;29(1):45-51. Epub 2006 Sep 6. Erratum in: Eur J Orthod. 2008 Feb;30(1):110.
- Tecco S, Nota A, Caruso S, Primozic J, Marzo G, Baldini A, Gherlone EF. Temporomandibular clinical exploration in Italian adolescents. Cranio. 2017 Oct 26:1-8. doi: 10.1080/08869634.2017.1391963. [Epub ahead of print] PMID: 29072541
- Tecco S, Caruso S, Nota A, Leocata P, Cipollone G, Gatto R, Cutilli T. Bilateral Central Giant Cell Granuloma of the mandibular angle in three females from the same family. Head Face Med. 2018 Sep 4;14(1):14.
- Trodahl JN. Ameloblastic fibroma. A survey of cases from the armed Forces Institute of Pathology. Oral Surg Oral Med Oral Pathol 1972;33:547-558.
- Vered M, Shohat I, Buchner A. Epidermal growth factor receptor expression in ameloblastoma. Oral Oncol 2003;39:13.
- Nasim VS, Perumal P. Oral epidermoid cyst. A case report. Eur J Paediatr Dent 2014; 15 (2) Suppl.: 247-249.