

Clinical, morphological and immunohistochemical findings in canalicular adenoma

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Abstract:

Canalicular adenoma (CAAd) is an uncommon benign salivary gland tumor, usually located in the upper lip. We carried out a survey of cases of CAAds among the all salivary gland tumors submitted to an Oral Pathology service over a 36-year period, and performed a comprehensive literature review of all CAAds reported from 2006 to 2016. Among 161 salivary gland tumors, only two were diagnosed as CAAds. A detailed clinical, morphological and immunohistochemical (IHC) study of the two cases was performed. Morphologically, the tumors were characterized by cuboidal to columnar neoplastic cells lining cystic spaces and forming tumor cords. The IHC reactions (positive for cytokeratin-7, AE1/AE3, S-100, p63, and negative for α -SMA) supported a luminal/ductal cell proliferation without myoepithelial cells. The clinical features of the cases were similar to that shown in the literature, while the morphological and IHC findings confirmed the epithelial nature of the tumor, and highlighted important differential diagnostic aspects.

Keywords: Salivary Gland Neoplasms; Adenoma; Immunohistochemistry.

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INTRODUCTION

Canalicular adenoma (CA_d) is a rare benign salivary gland tumor, which was only recognized as an entity separate from other monomorphic tumors in the second classification of the World Health Organization (WHO) in 1991. This tumor is believed to arise from duct reserve cells, especially those of the minor salivary glands, and commonly affects the upper lip. Canalicular adenoma accounts for 1-3% of all salivary gland tumors and is more common in female patients older than 50 years¹⁻³.

Clinically, CA_d appears as a painless, slow-growing, submucosal enlargement measuring up to 3 cm, with its color ranging from pink to blue. The tumor can manifest as a solitary nodule or as multiple nodules (multifocal). Microscopically, CA_d consists of cubic or cylindrical cells usually arranged in cords and alternate and separate rows that form anastomosed structures, called canaliculi. The stroma of this tumor is characteristically paucicellular, myxoid, and well vascularized. The differential diagnosis is made with cystic adenoid carcinoma and, especially, with basal cell adenoma. Morphological and immunohistochemical findings are useful for their differentiation. Conservative surgical excision is the most indicated treatment. Recurrences are rare, but multifocal tumors have a strong tendency to recur¹⁻³.

The objective of this study was to describe the clinical, morphological and immunohistochemical findings of two cases of CA_d in the minor salivary glands and to compare the findings with case reports published in the literature over the last 10 years.

CASE REPORTS

Clinical findings

Two CA_d were retrieved from the 161 cases of salivary gland tumors archived in one Brazilian diagnostic center in Oral Pathology (Federal University of Rio Grande do Norte) on the period spanning from January 1980 to January 2016 (36 years). The cases were treated by surgical resection and clinical data were obtained from patient's medical records.

Case 1 - Patient 75-year-old female, presenting an asymptomatic submucosal nodule, firm consistency, with smooth surface, measuring about 2.0cm, located in the upper lip region and evolution of 1 year. Given the clinical diagnosis of pleomorphic adenoma or CA_d,

the patient underwent excisional biopsy under local anesthesia.

Case 2 - Patient 56-year-old male, presenting an asymptomatic submucosal nodule, fibrous consistency, with smooth surface, measuring about 2.0cm, located in the buccal mucosa region and evolution of 6 months. Given the clinical diagnosis of fibroma the patient underwent excisional biopsy under local anesthesia.

Morphological findings

Five-micrometre-thick sections were cut, deparaffinized, and stained with haematoxylin-eosin for histological examination in light microscopy (Nikon Eclipse-E200, Tokyo, Japan). Four pathologists evaluated the samples. The slides were scanned (Pannoramic MIDI, 1:15 SPI 3D HISTECH®, Budapest, Hungary) for analysis and photomicrographs.

Microscopically CA_d showed circumscribed and encapsulated in both tumors (Figure 1A and 1C). The most distinguishing histological finding was paucicellular stroma very highly vascularised (Figure 1D). The epithelial cells are columnar, but some are cuboidal with eosinophilic cytoplasm. The nuclei are hyperchromatic round or oval. The cells are typically arranged in beaded anastomosing, often bilayered strands and cords. In one of case a supporting loose stroma exhibited some areas of sclerosing to fibrillar collagen deposition (Figure 1B). Mitotic figures were not visualized.

Immunohistochemical findings

For the immunohistochemical reactions by the polymeric biotin-free technique, 3- μ m-thick sections were mounted on organosilane-coated slides. For deparaffinization and antigen retrieval slides were submitted to pre-treatment Trilogy solution (Cell Marque, Rocklin CA, USA). The primary antibodies specifications used are described in Table 1. In amplification reaction was performed using EnVision+HRP (#K8021; Dako, Glostrup, Denmark) and chromogen diaminobenzidine for colour development. Sections were counterstained with Mayer's haematoxylin and mounted. Negative controls were obtained by substituting the primary antibodies for bovine serum albumin (BSA). A sample of normal salivary gland tissue (shown previously to be positive for the antibodies investigated) served as a positive control.

The immunoprofile of the CA_d confirm an duct origin with cells positive for AE1/AE3, cytokeratin-7 and S-100. The tumour cells are predominantly negative

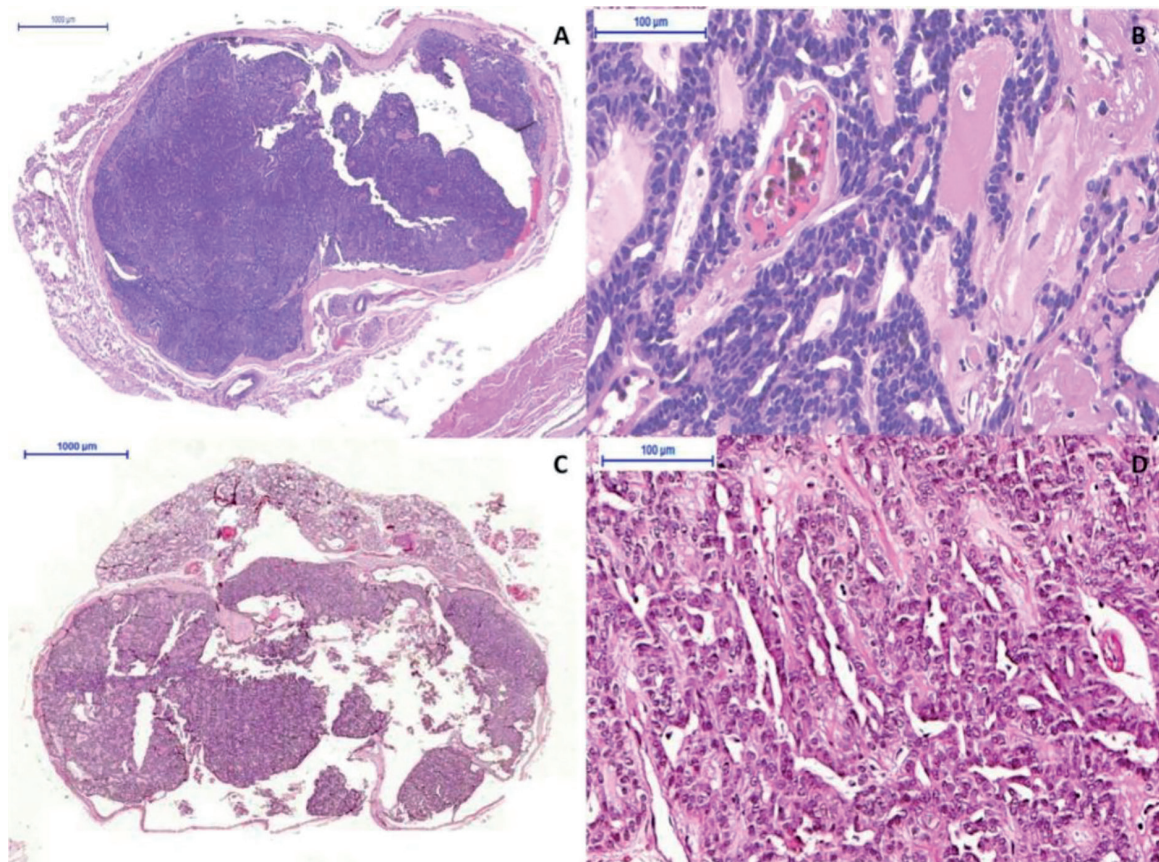


Figure 1. Morphological findings in two cases of canaliculal adenomas. (A and C) Photomicrograph showing both tumors (case 1 and 2) encapsulated (Bars indicate 1000µm). (B and D) Higher magnification showing cells typically arranged in beaded anastomosing, often bilayered strands and cords. (B) Areas of sclerosing to fibrillar collagen deposition and (D) paucicellular stroma (Bars indicate 100µm) (Hematoxylin-eosin).

Table 1. Immunohistochemical specifications and results.

Antibody	Company	Dilution and Incubation	Reaction	Localization	Pattern
Cytokeratin-7	Dako	1:100 Overnight	100% (2/2)	Membrane and Cytoplasmic	Strong and diffuse
Smooth muscle actin (SMA)	Dako	1:300 60 minutes	0% (2/2)	Negative	Negative
Cytokeratin-pan (AE1/AE3)	Dako	1:100 Overnight	100% (2/2)	Membrane and Cytoplasmic	Strong and diffuse
S100	Dako	1:2000 60 minutes	100% (2/2)	Membrane and Cytoplasmic	Strong and diffuse
p63	Leica	1:50 Overnight	100% (2/2)	Negative	Negative

or focal positive for p63 and SMA (Table 1). The lack of SMA and p63 positive cells surrounding ductal cells demonstrate absence of myoepithelial phenotype, hence differentiate CAD from a basal cell adenoma and adenoid cystic carcinoma (Figure 2A-J).

Review of published cases

A review of published cases (In English) was carried out in PubMed and MEDLINE using the descriptor “canaliculal adenoma”. Articles published in indexed periodicals between 2006 and 2016 were selected.

Eighty-eight cases of CAD were published in the scientific literature (PubMed-Medline) in the last decade (2006-2016). Tumors affecting the upper lip (n=58) and solitary nodules (n=73) were the most common. The age of the patients ranged from 26 to 85 years (mean: 68 years). Women were the most affected, with a female-to-male ratio of 3.4:1 (Table 2).

DISCUSSION

In the present study, CAD corresponded to 1.2% of all salivary gland tumors diagnosed, confirming

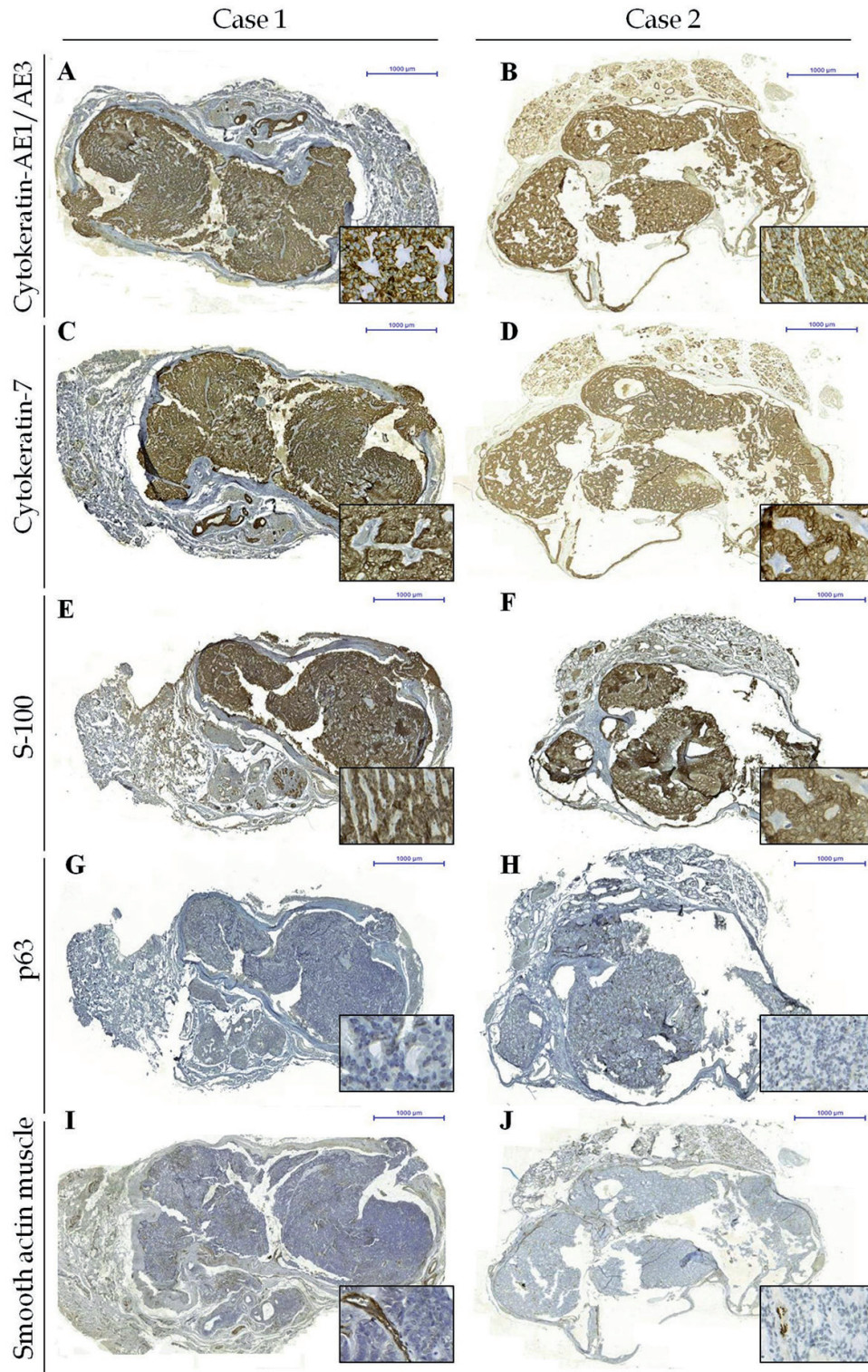


Figure 2. Immunopositivity of several markers in two cases of canaliculal adenomas. (A-F) Strong and diffuse immunopositivity for AE1/AE3, Cytokeratin-7 and S-100 in both cases. (G-J) Immunopositivity absence for p63 and Smooth muscle actin in both cases (Bars indicate 1000µm).

Table 2. Canalicular adenomas published in English in the scientific literature in the last ten years (2006-2016).

Authors	N	Gender	Age (Years)	Anatomic Site	Aspect
Present study (2016)	2	1F	75	Upper Lip	Solitary nodule
		1M	56	Buccal mucosa	
Thompson et al. ¹ (2015)	67	13 M 54 F	Mean age= 69,9	Upper Lip (n=46), Buccal mucosa (n=17), Palate (n=4)	58 Solitary nodule; 9 Multifocal;
Huebner et al. ⁴ (2014)	7	3 M 4F	Mean age= 69,0	Upper Lip (n=4) Buccal mucosa (n=2), NS (1)	Solitary nodule
Samar et al. ⁵ (2014)	1	F	61	Upper Lip	Solitary nodule
Siqueira et al. ⁶ (2013)	2	2F	72	Upper Lip	Multifocal
			79		
Sivolella et al. ³ (2013)	1	M	85	Upper Lip	Solitary nodule
Tyralik et al. ⁷ (2013)	1	F	57	Upper Lip	Multifocal
Dayisoylu et al. ⁸ (2012)	1	F	56	Intraosseous in mandible	Solitary nodule
Yüce et al. ⁹ (2012)	1	M	79	Palate	Solitary nodule
Oliveira Santos et al. ¹⁰ (2010)	1	F	70	Upper Lip	Multifocal
Butler et al. ¹¹ (2009)	1	F	85	Parotid gland	Solitary nodule
Mansueto et al. ¹² (2009)	1	F	78	Upper Lip	Multifocal
Werder et al. ¹³ (2009)	1	M	71	Palate	Solitary nodule
Pereira et al. ² (2007)	1	F	55	Upper Lip	Solitary nodule
Liess et al. ¹⁴ (2006)	1	M	26	Parotid gland	Solitary nodule
Yoon et al. ¹⁵ (2006)	1	F	76	Upper Lip	Multifocal

the prevalence of these tumors of 1-3%. Canalicular adenomas occur almost exclusively in the minor salivary glands, with the upper lip being the most commonly affected site, occurring in about 70% of the cases described^{1,3,4,9}, followed by the cheek mucosa. Involvement of the parotid gland is extremely rare^{8,10}, as can be seen in Table 2.

Although the two cases described affected different genders, CAD is more common in women, with a female-to-male ratio of 3:1. The peak incidence of CAD is in the seventh decade of life and the tumor is uncommon in patients younger than 50 years. There are no reports of CAD in pediatric patients^{1,3,5}. The age of the patients shown in Table 2 ranged from 26 to 85 years, with a mean age of 68 years.

The clinical findings of the two cases reported agree with those described in the literature. These tumors appear as slow-growing, asymptomatic, well-delimited and mobile submucosal nodules with an intact surface, occasionally exhibiting a blue color and generally measuring 0.5 to 2 cm^{5,9}. The presentation of CAD as solitary nodules is almost five times more common than that of multifocal tumors (Table 2). Cases of multifocal CAD manifest as small tumor islands adjacent or within the capsule of the main tumor, but also at the periphery

of normal salivary gland tissue, conferring a pattern of multiple nodules^{6,7}.

The architecture of CAD is usually highly specific and consists of frequently anastomosing cords of cells arranged parallel to each other, conferring the characteristic “beading” image. The tumor cells are columnar or cubic and pleomorphism is absent or very rare. The nuclear chromatin is delicate and hyperchromatic nuclei are commonly observed, but prominent nucleoli are not found. The stroma is edematous, hypocellular, and fibrillar to myxoid. Areas of collagen deposition (sclerosis) are observed in some cases. This finding can be interpreted as hyalinization and may confuse the diagnosis, especially with the membranous type of basal cell adenoma, which is characterized by several nests or islands of basaloid epithelial cells and peripheral cells arranged in a palisade surrounded by an excessive hyaline basement membrane¹⁻⁴.

The absence of an evident and diverse stroma with myxochondroid or chondroid areas and the presence of duct-like structures in a dual cell population are features that differentiate CAD from pleomorphic adenomas¹⁻³.

The immunohistochemical profile observed in our cases as well as in previous case reports supports the probable origin of CAD from the salivary gland

duct system, with strong and diffuse immunostaining for AE1/AE3 and CK7. In addition, neoplastic cells were strongly positive for protein S100, which is an important feature of these tumors. In contrast, the tumor cells were negative for myoepithelial cell markers such as SMA and p63 or exhibited non significant weak and focal immunostaining. These findings confirm the only ductal/luminal cell differentiation in cases of CA_d^{1,3,8,10}.

Immunohistochemistry can be useful in the differential diagnosis of CA_d from other salivary gland tumors⁵, especially basal cell adenoma. Basal cell adenoma consists of a dual cell population and is typically organized in two cell layers arranged perpendicular to the basement membrane. Although the tumor has a monomorphic appearance, in addition to positive staining for luminal cell markers, strong immunoexpression of myoepithelial markers such as p63 and SMA is observed, findings that confirm proliferation of a dual cell population^{3,5}.

Immunostaining for the identification of myoepithelial cells is also useful to differentiate CA_d from malignant tumors that exhibit dual cell proliferation (luminal/myoepithelial) such as cystic adenoid carcinoma. In addition to positive immunostaining for p63, SMA and other myogenic markers, this tumor lacks a capsule and a frankly invasive and infiltrative behavior, together with other morphological findings that are suggestive of the diagnosis of adenoid cystic carcinoma⁹.

Immunohistochemistry is essential to differentiate CA_d from malignancies mainly the cystic adenoid carcinoma, because this malignant tumor displays an aggressive clinical course, strong metastatic and invasive potential, and a poor prognosis in long-term^{1,2,9}. As in the cases reported here, the treatment of choice for CA_d is conservative surgical excision, which has an excellent prognosis^{3,7}. Rare cases of recurrence have been described, which can be explained by incomplete excision of the tumor and particularly by the multifocal nature in some cases. Within this context, follow-up of the patient is recommended, especially in cases of multifocal tumors. There are no reports of malignant transformation of CA_d so far^{1,7,9,10}.

CONCLUSIONS

In conclusion, we highlight that, although uncommon, canalicular adenoma exhibits peculiar

clinical, morphological and immunohistochemical features that help establish the precise diagnosis, thus minimizing errors in the therapeutic management of cases.

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