Epithelial-Myoepithelial Carcinoma of the Minor Salivary Glands: Immunohistochemical and Morphological Features

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Abstract. *Aims: Epithelial-myoepithelial carcinoma (EMC) is a rare malignant salivary gland neoplasm that most commonly occurs in the parotid gland, but can also arise in the minor salivary glands. Three cases are reported of epithelial-myoepithelial carcinoma of the minor salivary glands, with the goal of better defining this entity. Patients and Methods: All three cases showed a characteristic nodular/multinodular growth pattern and classic biphasic tubular histology. All parts of each tumor were surrounded by a myoepithelial cell rim and there was evidence of invasion. Results: Immunohistochemical analysis showed the tumor cells to be weakly positive for S100, cytokeratin (CK) CK5/6, CK7, CKAE-1/AE-3 and strongly positive for epithelial membrane antigen (EMA) and p63; they were focally positive for calponin and acute lymphoblastic leukemia antigen (CD10). The tumor cells were negative for vimentin, α-smooth muscle actin (SMA) (except one case), glial fibrillar acid protein (GFAP) and MIB1. The tumors were resected completely with wide margins and no recurrence or metastasis had occurred from 6 to 15 months after surgery. Conclusion: Three cases of minor salivary gland tumors are described and the differential diagnosis underlined in relation to benign myoepithelioma. The characteristic morphological and immunohistochemical features aided diagnosis of these biphasic tumors.*

Epithelial-myoepithelial carcinoma (EMC) is a rare neoplasm, first described in 1972 by Donath *et al*. (1), although it was recognized as early as 1956. It has been reported under a variety of names including adenomyoepithelioma, clear cell adenoma, tubular solid adenoma, monomorphic clear cell tumor, glycogen-rich adenoma, glycogen-rich adenocarcinoma and clear cell carcinoma (2-7).

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It chiefly occurs in the parotid gland, representing about 1% of all salivary gland tumors (8-10). In addition, the major sites of involvement are the maxillary sinus, trachea, larynx, hypopharynx, and minor salivary glands, although it has also been reported in the mucoserous glands of the upper and lower aerodigestive tract. Patients are mostly women in their fifth to eighth decades, the reported age range being 8-103 years (11-18).

EMC is a malignant biphasic salivary-type tumor and the diagnosis is based on conventional light microscopy, confirmed by immunohistochemical and ultrastructural investigation. Histologically, the tumor is characterized by well-defined tubules with two cell types: an outer layer of myoepithelial cells with clear cytoplasm surrounding an inner lining of eosinophilic cuboidal epithelial cells (19) resembling intercalated ducts. It has been suggested that it derives from the intercalated ducts of the salivary glands, because the tubular growth pattern of this tumor epitomizes this phenotype (3, 13).

Although the histological appearance of EMC is usually characteristic, there may be considerable morphological variation, the disease can overlap with other salivary-type tumors and differential diagnosis from many tumors of the salivary glands is necessary. The morphological features cover a wide spectrum, ranging from purely epithelial aspects such as clear cell carcinoma to the appearance of a purely myoepithelial carcinoma (20, 21). Significant overlap has also been observed between EMC and adenoid cystic carcinoma, and the two forms have been described together as the so-called "hybrid" carcinoma, suggesting yet another taxonomic link (22-27), The recent description of an oncocytic variant of EMC expands the differential diagnosis to encompass oncocytoma, oncocytic papillary cystadenoma and cystadenocarcinoma (28).

A further complexity is to differentiate the benign form, myoepithelioma, from the malignant form, EMC, it being above all difficult to establish criteria for a malignancy that lacks atypia. In the salivary gland, criteria that have been reported to be helpful in differentiating between benign and

malignant myoepitheliomas include cytological atypia, mitotic activity and tumor infiltration into surrounding salivary gland or other normal tissues (5, 29, 30). No specific mitotic-rate cut-off exists on which to base this distinction, although cytological atypia is not required for a diagnosis of myoepithelial carcinoma.

Cytological differentiation in the myoepithelial cells, increased cellularity, pleomorphism, prominent invasion and destructive growth distinguish the malignant behavior.

Three cases of EMC with minor salivary gland involvement are described, with the aim of further defining the morphological spectrum and characterizing the immunophenotype of EMC.

Patients and Methods

The clinical data are summarized in Table I. All the tumors were in the minor salivary glands. None of the patients had had any other primary malignancy at any other site and all were treated by wide surgical resection with wide margins of healthy tissue, at a depth to include the muscle layer, under local anesthesia. Neither chemotherapy nor radiotherapy was given. All the patients were alive with no evidence of recurrence local, lymph node or distant relapse from 6 to 15 months, after surgery.

Case 1. An 83-year-old healthy man was referred to the Department of Otorhinolaryngology because of an asymptomatic lesion of the hard palate, detected incidentally during a routine dental checkup. At clinical examination, a painless growth in the center of the hard palate, close to the midline and surrounded by intact mucosa, was detected. The maximum diameter of the lesion was 1.5 cm. The patient had no dental prosthesis and the oral status was otherwise normal.

Case 2. A 58 year-old woman reported intermittent pain and a mass in the palate. On examination, a fixed firm mass of 3.5×2 cm was detect in the left palate area (Figure 1a). No cervical lymphadenopathy was found. Magnetic resonance imaging (MRI) showed a soft-tissue mass in the left palate extending to the midline of the oral cavity.

Case 3. A 75 -year-old man came to our attention with a 6-month history of swelling on the right side of his cheek (Figure 1b). He reported an increasing mass over the previous 2 months with intermittent pain. At objective examination a fixed firm mass of 2.5 ×2.5 cm was observed, of hard-elastic consistency, centrally ulcerated with no mass in the laterocervical region.

Pathological findings. Specimens for surgical pathology were sent to San Gerardo Hospital, Monza, Italy. Sections were cut and prepared by the conventional routine, and stained with hematoxylin and eosin and by other appropriate methods. The cut surface in all cases showed well-circumscribed gray-white multiple nodules. Only in case 2 did the cut surface show a gray-white color, with necrosis and hemorrhage. Microscopically, all the tumors were multinodular and infiltrated adjacent tissues (Figure 2 a-c). Histologically, the specimens showed two cell types, an outer layer of myoepithelial cells and an inner layer of cuboidal eosinophilic duct-like cells (Figure 2 d). The cuboidal eosinophilic cells were surrounded by polygonal myoepithelial cells. The periepithelial stroma was partially hyalinized in some areas.

Immunohistochemistry. A panel of antibodies: cytokeratin (CK) CK5/6, CK7, CKAE-1/AE-3, epithelial membrane antigen (EMA), S100 protein, p63, calponin, CD10, vimentin, α-smooth muscle actin (SMA), glial fibrillar acid protein (GFAP), and MIB1 was applied to further sections and appropriate positive and negative controls were employed. The sources and dilutions of the antibodies are listed in Table II.

The immunohistochemistry was scored semiquantitatively, based on the proportion of immunoreactive cells and the intensity of reactivity: + positive for a limited number of cells; ++ intensely positive for numerous cells

Results

Immunohistochemistry. The findings are summarized in Table II. The ductal cells in all three tumors showed weak reactivity of all cells for CK 5/6, CK7 and strong for CK AE-1/AE-3; EMA showed strong positivity of the cell membranes and cytoplasm, and was expressed in all the cases. A nuclear reaction for p63 was seen in all the cases, staining for calponin showed weak-to-moderate and continuous cytoplasmic membranous immunoreactivity in the tumor cells (Figure 3). The average MIB1 index was low from 5 to 10%, but in each case there was a weak reaction for S100 protein and none for GFAP. In contrast, all the cells and duct cells in all three tumors were negative for vimentin and in two of the three cases for SMA. The myoepithelial cells stained positively for calponin, p63, S100 protein, and in one case for SMA (Figure 4). The keratin cocktails also weakly stained the myoepithelial components.

Discussion

EMC is a low-grade malignancy, only rarely have high-grade or dedifferentiated EMC cases been reported.

It has been observed that some morphologically low-grade myoepithelial carcinomas behave aggressively (31). Thus, in the absence of frankly malignant cytomorphology, an invasive growth pattern is the single most useful criterion for establishing malignancy in salivary EMC. In the present cases, no atypical mitoses were observed, with few atypias, but in all three cases infiltrative margins features associated with malignant behavior, were observed microscopically.

Immunohistochemical diagnosis involves various criteria and studies have commonly reported positivity for epithelial markers including CKs 7, 14 and 5/6, and for S100 protein, EMA and SMA (32). Calponin and GFAP have also been reported to be sensitive markers of myoepithelial differentiation in salivary lesions (33). Calponin is a relatively new smooth muscle-specific antibody that has been shown to be a specific and fairly sensitive marker of

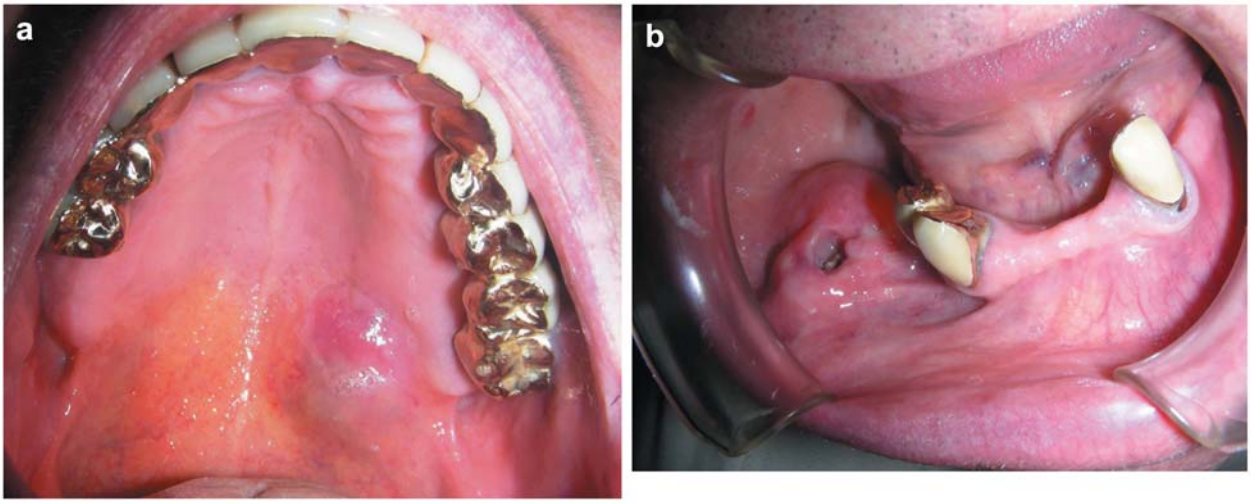

Figure 1. *Clinical view showing a fixed mass: a, left palate area; b, right cheek tumor.*

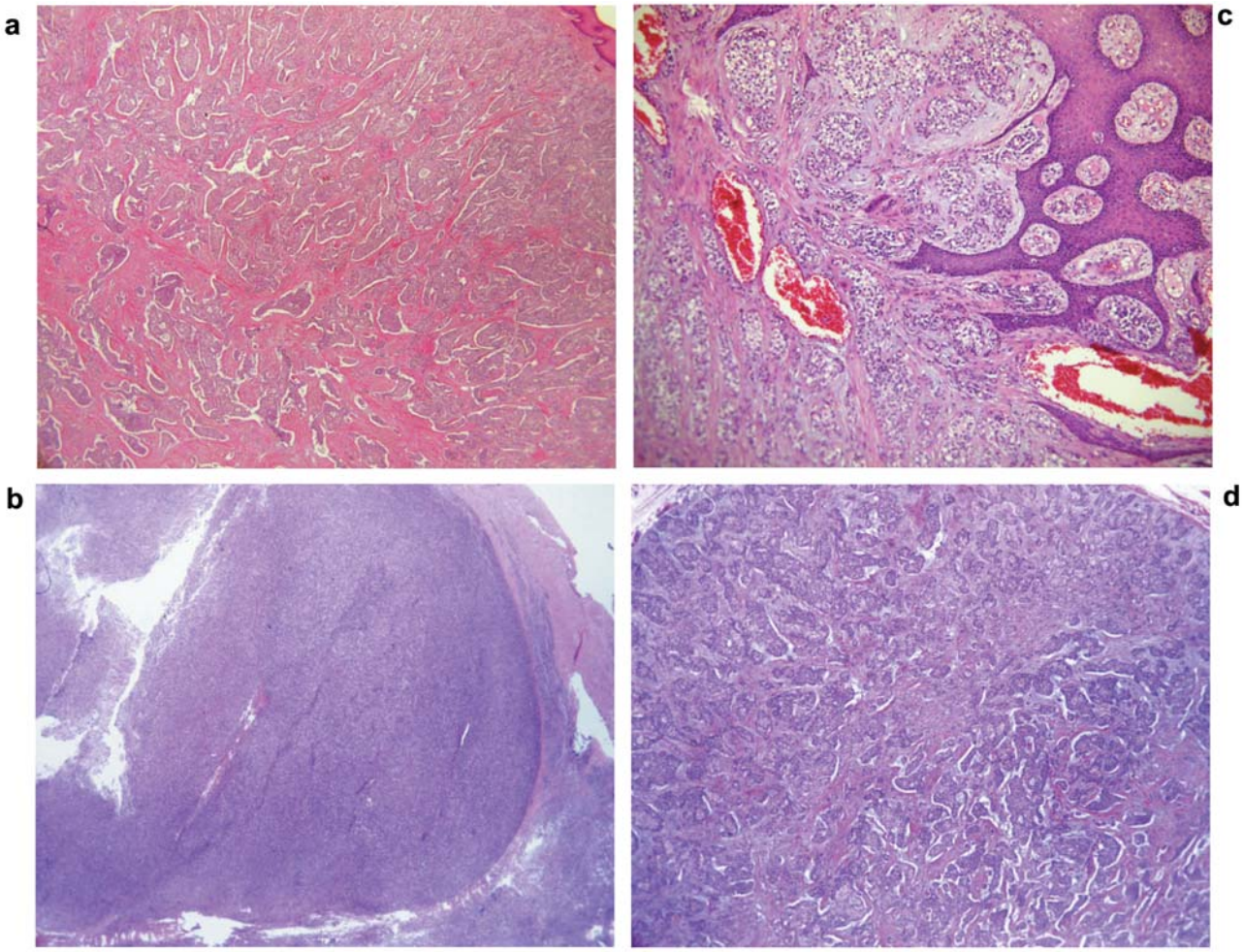

Figure 2. *a-c Epithelial-myoepithelial carcinoma, showing multinodular growth pattern with infiltrating margins in adjacent tissues (hematoxylin and eosin, ×100); d, two cell types are apparent, an outer layer of myoepithelial cells and an inner layer of cuboidal eosinophilic duct-like cells (hematoxylin and eosin, ×200).*

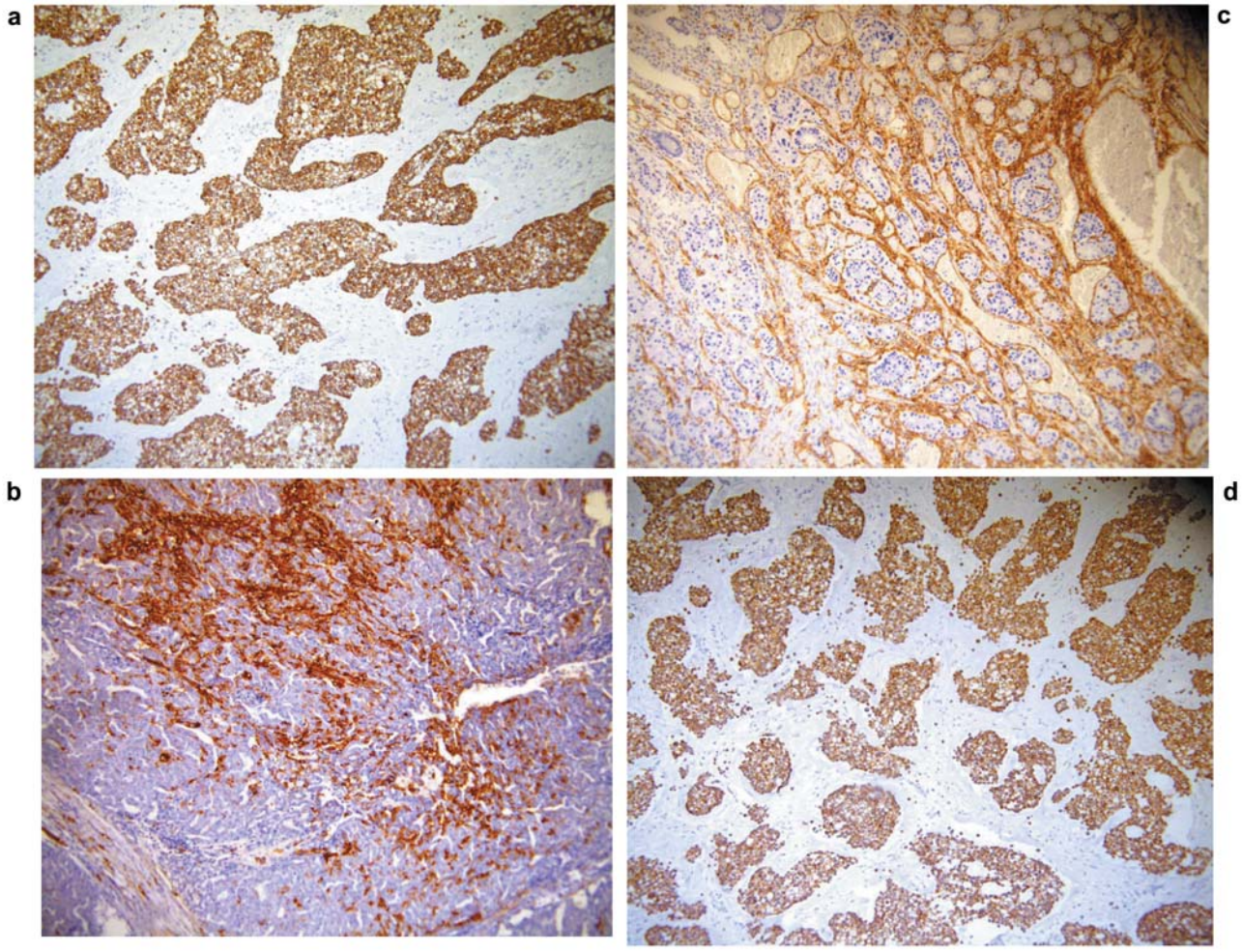

Figure 3. *Immunohistochemical expression of: a, cytokeratin (AE-1/AE-3); b, p63; c, calponin; d, CK7 (original magnification, ×200).*

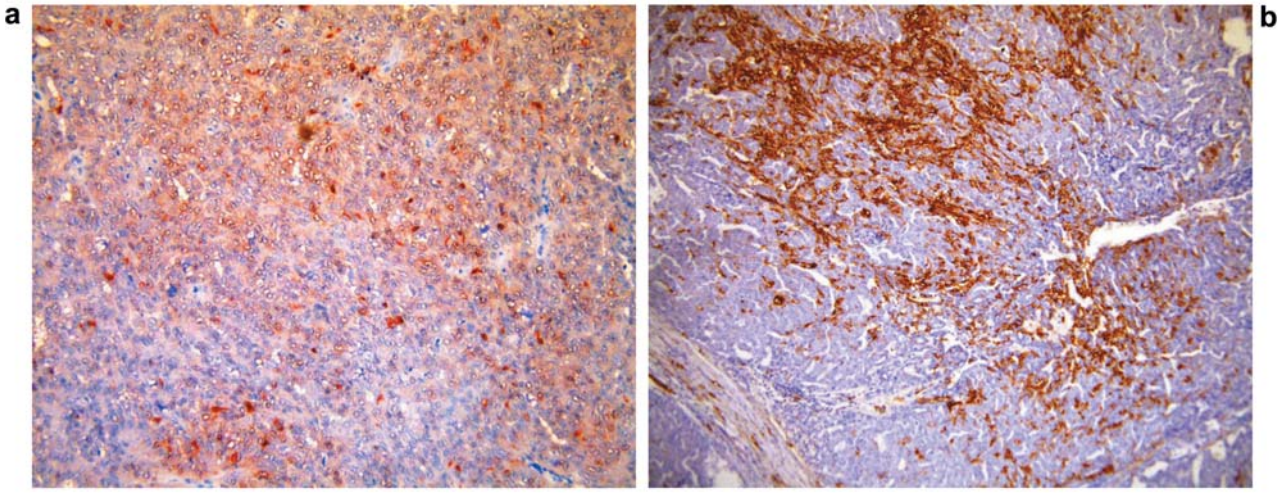

Figure 4. *Immunohistochemical staining of: a, α-smooth muscle actin (SMA); b, S100 (original magnification, ×200).*

Table I. *Clinical findings*.

	Gender/age	Site	Size (cm)	History	Outcome
Case 1	M 83	Hard palate	1.5 diameter	Hard mass; elastic consistency, no pain	NED 15 months
Case 2	F 58	Left emipalate	3.5×2	Mass , pain at times	NED 13 months
Case 3	M 75	Right cheek	2.5×2.5	Hard mass, fixed, elastic consistency, pain at times	NED 6 months

NED: No evidence of disease.

myoepithelial differentiation in salivary gland tumors, exhibiting more specificity than other smooth muscle markers of the myoepithelium. However, in the report by Savera *et al*. (34), calponin stained 75% of myoepithelial carcinomas and failed to stain 50% of the epithelioid cell type and 25% of the clear cell type. Seethala *et al*. (35) found calponin staining in only 59.1% of epithelial-myoepithelial carcinomas. However, the study by Wang *et al*. (36) also reported slight staining for calponin in one of their clear cell myoepithelial carcinomas and negative reactivity in 2 out of 4 EMC.

P63 has also recently become a widely-used marker for abluminal cells (both basal cells and myoepithelial cells) showing nuclear immunoreactivity. However, p63 is not entirely specific for myoepithelial and basal cells, since squamous cell tumors are also positive. Another marker, CD10, can also be used as a myoepithelial marker, but lacks specificity. Therefore, to assure sensitivity of detection, a panel of specific myoepithelial markers (*i.e*. calponin, SMA) and nonspecific myoepithelial markers (*i.e*. vimentin, S100, GFAP and p63) were used in this study, in line with work by other researchers (37).

In the present cases, the myoepithelial cells stained focal-positively in all cases for calponin and in only one case for SMA; the ductal cells stained for CK AE1/AE3, and for CK5/6 and 7 weakly, these keratin cocktails also weakly stained the myoepithelial components. EMA showed strong positivity in the cell membranes and cytoplasm, and was expressed in all the cases. There was a weak reaction for S100 protein. On the other hand, vimentin and GFAP were found to be negative; although some studies (34, 35) have found that these markers, despite being nonspecific, if used in conjunction with CKs may be sensitive markers of neoplastic myoepithelial cells. A nuclear reaction for p63 was found in all the present cases. The myoepithelial cell staining was weak-to-moderate for calponin, p63, S100 protein and SMA (one case only). The MIB1 index average was from 5 to 10%, but there was considerable variation from area to area.

In all three cases, the reaction for MIB1 was low: this index remains a useful marker in predicting adverse outcome in salivary gland carcinoma.

With regard to recurrence, in keeping with its low-grade malignant nature, a recurrence rate of EMC of 35-50% has been reported, with a metastatic rate of 8.1-25% (38). Seethala and colleagues found that the recurrence rate was 36.3% and survival rates were 93.5% and 81.8% for 5 and 10 years, respectively (35). The present patients have shown no sign of recurrences or metastasis during the 6 to 15 months of follow-up. EMC is characterized by a relatively good prognosis, in contrast to salivary duct carcinoma (7).

Table II. *Immunohistochemical antibodies and findings*.

Marker	Source	Dilution	Case 1	Case 2	Case 3
CK7	Dako	1:100	+	++	++
CK AE-1/AE-3	Dako	1:50	++	++	++
CD10	Novocastra	1:100	+	+	+
EMA	Dako	1:50	++	++	++
GFAP	Dako	1:4000	–	–	–
S100	Dako	1:1000	+	+	+
SMA	Dako	1:100	–	+	–
p63	Dako	1:50	++	++	++
CK5/6	Histoline	1:50	+	+	+
CALPONIN	Dako	1:800	+	+	+
MIB1	Dako	1:200	10%	5%	10%
VIMENTIN	Dako	1:100	–	–	–

For markers: –, no significant reactivity; +, positive for a limited number of cells; ++, intensely positive for numerous cells. CK, Cytokeratin; EMA, epithelial membrane antigen; SMA, smooth muscle actin; GFAP, glial fibrillar acid protein.

There is no consensus regarding the optimal treatment of this neoplasm, largely due to its rarity. Wide surgical excision with a clear margin is the treatment of choice because of the tumor's tendency to infiltrate locally. If the tumor is more than 4 cm in diameter, combined radiotherapy and surgery have been recommended. Radiotherapy may be of benefit in preventing local recurrence. The effect of chemotherapy is uncertain in this neoplasm (39).

Conclusion

Three cases of minor salivary gland tumors are described. Although our experience is limited, and despite the lack of cytological atypia (prominent nucleoli, vesicular or coarse chromatin, pleomorphism), the immunohistochemical profile,

morphology of the lesion and the local infiltrative and invasive nature were all features that enabled us to better define these tumors and made it possible to reach a diagnosis of EMC.

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