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Salivary gland myoepitheliomas: cytological, histological, immunohistochemical and electromicroscopical studies of four cases

MB Romero-Guadarrama,* P Alonso de Ruiz,* H Cruz-Ortiz,* HA Rodríguez-Martínez*

ABSTRACT

The results of a multidisciplinary study conducted on four salivary gland myoepitheliomas diagnosed with fine needle aspiration biopsy (FAB) and treated using local excision are presented. Histologically, two cases were benign and two malignant. Smears of the benign varieties contained an uniform population of slightly spindled cells with bipolar cytoplasm and conspicuous cell membranes. The nuclei were small and oval with homogeneous chromatin. The cells were observed in groups or clusters over a proteinaceous background. There were adjacent isolated cells differing in shape with rounded eosinophilic cytoplasm, the cytoplasm was discretely granular and/or clear with round hyperchromatic centrally-located nuclei. One smear showed numerous capillaries with prominent endothelial cells on a background made up of a chondroid matrix. Two cases disclosed malignant cytological changes with a necrohemorrhagic background, discrete pleomorphism, prominent nucleoli, hyperchromatism and occasional atypical mitosis. Immunohistochemical staining was strongly positive for protein S-100 and vimentin, and focally for cytokeratin. Electron microscopy was basically composed of myoepithelial cells.

Key words: Salivary gland myoepitheliomas, fine needle aspiration biopsy.

RESUMEN

Se presentan los resultados multidisciplinarios de cuatro casos de mioepiteliomas de glándula salival diagnosticados por biopsia por aspiración con aguja fina y resecados posteriormente. Histológicamente dos casos fueron benignos y dos malignos. Los extendidos celulares de las variedades benignas contenían una población uniforme de células discretamente alargados con citoplasma bipolar y membranas celulares conspicuas. El núcleo fue pequeño y oval con cromatina homogénea. Las células se disponían en grupos y nidos sobre un fondo proteináceo. Se observaron células aisladas adyacentes que presentaban citoplasma eosinófilo, granular y claro con núcleo central, redondo e hipercromático. En uno de los extendidos se observó numerosos capilares con células endoteliales prominentes y fondo con matrix condroide. Dos casos presentaron cambios citológicamente malignos con un fondo necrohemorrágico, pleomorfismo, nucléolo prominente, hipercromatismo y mitosis atípicas ocasionales. Las reacciones de inmunohistoquímica demostraron positividad para proteína S-100 y vimentina y focalmente para citoqueratina. Los hallazgos de la microscopia electrónica correspondieron a células mioepiteliales.

Palabras clave: *Mioepiteliomas de glándula salival, biopsia por aspiración con aguja fina.*

INTRODUCTION

Myoepitheliomas are rare salivary gland tumors first described in 1943 as a variant of pleomorphic adenomas[1]. Two years later, Bauer[2] called them adenomyoepitheliomas. Following the original description, numerous reports were published discussing whether myoepitheliomas originated the novo or as part of a mixed tumor. Myoepitheliomas are considered by some authors as distinctive clinicopathological entities, with a biological behavior different from mixed tumors. Based strictly on morphology, four histological variants have been described: spindle cell, hyaline or plasmocytoid, clear cell rich in glycogen and combined or

* Pathology Unit, Hospital General de México and Faculty of Medicine, UNAM.

intermediate forms. Most clear cell myoepitheliomas occurring in the parotid gland should be considered potentially malignant, while hyaline myoepitheliomas growing in the minor salivary glands should be considered benign. Reports on myoepitheliomas primary in salivary or mammary glands[2,3,4,5], diagnosed through FAB, have been scarce. In this report, a multidisciplinary study of 3 spindle cell and one combined myoepithelioma, spindle cell and hyaline, is presented, with emphasis on the cytological, immunohistochemical and ultrastructural diagnostic findings.

MATERIAL AND METHODS

This study included 4 patients with salivary gland myoepitheliomas seen at the Oncology and Cytopathology Departments of the Hospital General de México during the last four years. Diagnosis was based on specimens obtained through FAB; the material was fixed in alcohol, air dried and stained using the Papanicolaou and Giemsa stains. Surgical tissue samples were fixed in 10% formalin and then embedded in paraffin. Sections were stained with H-E, periodic acid-Schiff and Masson's trichrome stains. The biotin-avidin-peroxidase method was used for the immunohistochemical technique. The following primary antibodies were used: S-100 protein (S-100 P, DAKO), vimentin (Vim, DAKO), epithelial membrane antigen (EMA, DAKO), cytokeratin (CK2, DAKO), carcinoembryonic antigen (CEA, DAKO), smooth muscle actin (SMA, DAKO), muscle-specific actin (MAS, DAKO) and type IV collagen (Col IV, DAKO). Tissue samples from paraffin blocks were taken for electron microscopy. These samples were deparaffinized and postfixed overnight in osmium tetroxide, and then processed using routine techniques. Thin sections were studied using a Zeiss EM9 electron microscope.

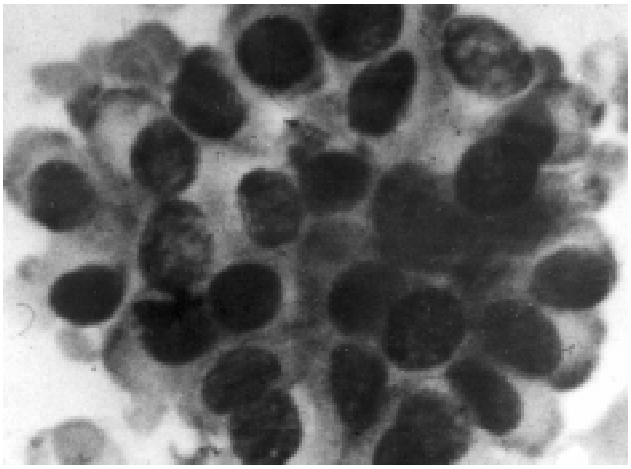

Figure 1. *Benign myoepithelioma with a group of cells of plasmocytic appearance. PAP x 250.*

RESULTS

Clinical Features. Two patients were females and two males; ages ranged from 46 to 71 years. Three tumors were located in the parotid gland and one in the submaxillary gland. The evolution time prior to surgery varied from 9 months to 6 years, and the clinical course consisted of a slow and progressive growth of the affected glands. Tumor size varied from 2 to 8 cm. Clinical follow-up, from 6 months to four years after treatment, revealed no tumoral ac-

Table I. Myoepitheliomas of salivary glands clinical course.

Case	*Sex*	*Age*	*Affected gland*	*Evolution time*	*Treatment*	*Follow up*
1	F	46	Parotid	1 year	LE, RT	4 years with out tumoral activity
2	F	61	Submaxilar	6 years	LE	6 months with out tumoral activity
3	M	63	Parotid	9 months	LE, RT, CHT	3 months after treatment with radiotherapy lost to follow up
4	M	71	Parotid	3 years	LE	3 years with out tumoral activity free of disease

LE = Local excision. RT = Radiotherapy. CHT = Chemotherapy.

Table II.

Case		*Cytological changes*	*Histological types*
Benign:			
	2	Benign myoepitheliomas spindle and ovoid cells, plasmocytoid type with regular chromatin, capillaries with prominent endothelium, clean background.	Benign spindle cell Myoepithelioma. 5% with mixochondroid matrix
	4	Spindle cell compact groups and some ovoid cells with homogeneous chromatin.	Benign spindle cell Myoepithelioma
Malignant:			
	1	Malignant myoepithelioma ovoid and spindle cell, discrete pleomorphism and clear cytoplasm, hyperchromatism, mitosis and necrohemorrhagic background.	Malignant spindle cell Myoepithelioma
	3	Ovoid cells with irregular nuclei and nucleoli, necrohemorrhagic background.	Malignant spindle cell Myoepithelioma

tivity in 3 of them *(Tables I & II)*. One of them was lost to follow up.

Cytological Findings. FAB samples taken from the benign varieties showed an uniform and predominating population of slightly spindled cells with bipolar cytoplasm and visible cell membrane. The small nuclei were round to oval with homogeneous chromatin (Cases 1 & 2) *(Figure 1)*. The cells were arranged in groups or clusters over a proteinaceous background. Other cells, showing peripheral nuclei with regular chromatin, were generally isolated and rarely seen in groups. In one case, there were numerous capillaries with prominent endothelial cells and a mixoid or chondroid matrix. The two malignant cases showed a necrohemorrhagic background and abundant cellular detritus, discrete nuclear pleomorphism, prominent nucleoli, hyperchromatism and occasional mitosis. Moreover, one of the malignant cases showed clear cells in the cytologic material *(Figures 2 and 3)*.

Histological Findings. The two benign myoepitheliomas (Cases 1 & 2) were composed of small or spindle cells arranged in bundles, showing a discrete eosinophilic cytoplasm, ovoid nuclei and regular chromatin. Tubulo-acinar structures and myxochondroid matrix were also observed in one of the specimens. The two malignant myoepitheliomas (Cases 3 & 4) showed a similar cellular pattern, as well as a clear cell component. Malignant changes, such as nuclear atypia and abnormal mitotic figures were also present. Three cases were considered as of the spindle cell type and the remainder case was a combined spindle and hyaline cell myoepithelioma *(Table II)*.

Immunohistochemical and Ultrastructural Findings. Immunohistochemical reactions for S-100 pro-

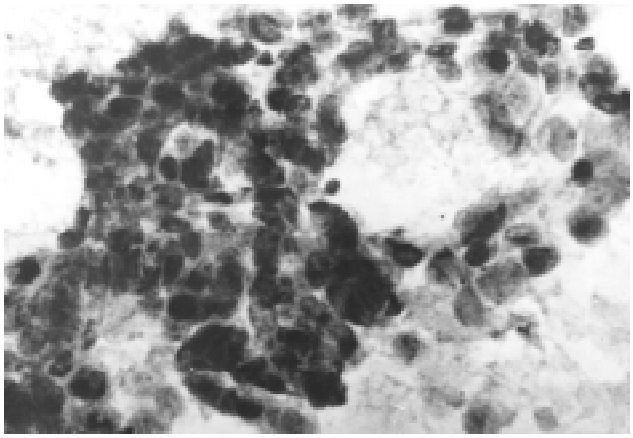

Figure 2. *Malignant myoepithelioma with clusters or conglomerates of ovoidal and spindle cells with atypical nuclei. Pap x 150.*

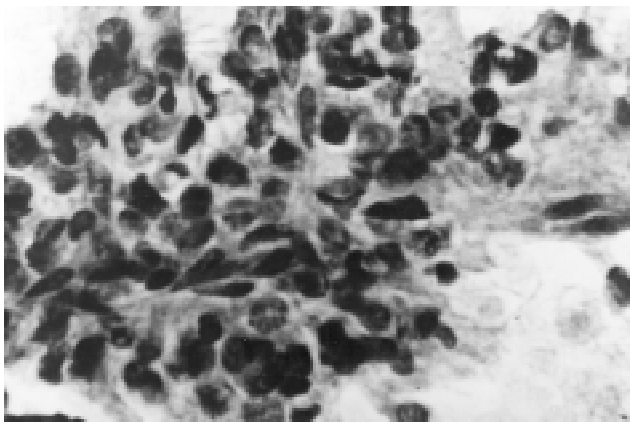

Figure 3. *Malignant myoepithelioma with clear cells with moderate atypia and mitosis. HE x 150.*

tein and vimentin were positive in all four cases, although vimentin was strongly positive in two cases and focally positive in the other two. Cytokeratin showed focal positivity in the cytoplasm of the neoplastic cells in all the cases *(Table III)*. Ultrastructurally, the cells showed cytoplasmic microfilaments and intermediate filaments as well as desmosome type intercellular junctions *(Figure 4)*.

DISCUSSION

The cytomorphology of myoepitheliomas in FABs depends on the histological variant of the tumor[6], nevertheless in most cytological smears the cell population is composed mainly of slightly spindled cells with central nuclei and rounded cells with more or less abundant cytoplasm[3]. In the benign varieties, most cells are arranged in irregular groups or clusters of several layers with preserved polarity, as well as in small groups or isolated plasmocytoid cells. Cells with clear cytoplasm are mostly found in the malignant varieties of myoepitheliomas[2]. In these tumors, it is also frequently observed certain degree of cellular pleomorphism, nuclear hyperchromatism and prominent nucleoli. In one of our cases, besides the prior findings, there were also mitotic figures. The necrohemorrhagic background found in all of our cases did not interfere with identification of the cellular details. For the differential diagnosis of myoepitheliomas, it has to be taken into consideration that there are other salivary gland tumors that also contain myoepithelial cells, although they have other features which allow a correct diagnosis[1]. Pleomorphic adenomas or mixed tumors show a mixture of epithelial and myoepithelial cells with or without tubule formation immersed in a myxochondroid stroma[7]. Adenoid cystic carcinomas characteristically show basal membrane-like globules surrounded by rather bland myoepithelial cells, with hypercromatic nuclei and clear cytoplasm, arranged in cribriform, tubular or solid patterns. Terminal duct carcinomas are reminiscent of ductal breast carcinomas[2], although they have myoepithelial cells in their structure. On the other hand, clear cell myoepitheliomas morphologicaly are somewhat similar to acinic cell carcinomas, sebaceous adenomas and oncocytomas[8], which form another important group of salivary gland tumors. A less frequent tumor with a myoepithelial cell component is the low grade polymorphous adenocarcinoma. Finally, there is the myoepithelial epithelial carcinoma with several histological patterns including a dimorphous one with an aggressive growth[6].

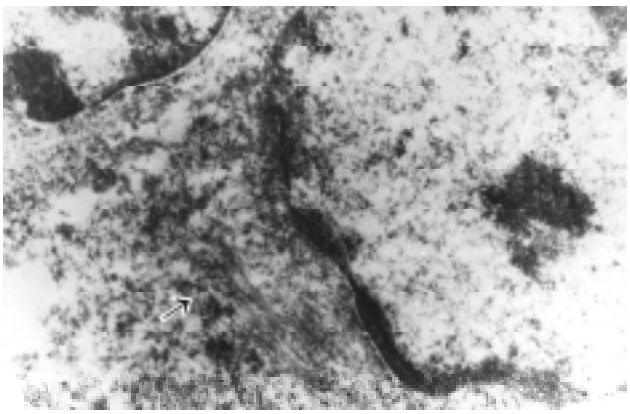

Figure 4. *Two myoepithelial cells showing abundant paranuclear filaments (arrow), mitochondriae and ribosomes.*

Table III. Immunohistochemistry.

Case	*PS100*	*VIM*	*CK2*
1	(+++)	(+++)	Focal +
2	Focal +	Focal +	Focal +
3	(+++)	(+++)	Focal +
4	Focal +	Focal +	Focal +

The four cases were EMA, MEA, CEA, SMA, COL IV negative.

Immunohistochemically, myoepitheliomas frequently give positive reactions for cytokeratins and S-100 proteins, and on occasion for vimentin, actin and myosin[9]. In our 4 cases, the most frequently positive reactions were for S-100 protein, vimentin and cytokeratin. Ultrastructurally, the most constant feature is the presence of abundant microfilaments that correspond to actin and keratin[10]. Other features that have been described are basement membranes, attachment plaques on the cell membrane and perinuclear bundles of tonofilaments reminiscent of keratinization[11]. In our cases, microfilaments, intermediate filaments and desmosome-type intercellular junctions were identified. As in other instances of surgical pathology, immunohistochemistry and electron microscopy are not useful means to separate benign from malignant myoepitheliomas[12,13]. Plain cytology from FAB, as well as routine surgical pathology, are preferable methods[14].

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Mailing address for reprint request:

Mónica B Romero Guadarrama
Hospital General de México
Unidad de Patología
Dr. Balmis No. 148,
Col. Doctores
06726, México, D.F. México