Immunohistochemical Expression of Actin and S100 in Pleomorphic Adenoma and Mucoepidermoid Carcinoma

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ABSTRACT
Background: Tumors of the salivary glands constitute an important area in the field of oral and maxillofacial pathology. These tumors have a special status in human neoplasia and probably the most complex histopathologic features of the body organs and being heterogeneous. Pleomorphic adenoma (PA) is the most common benign; and mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumors. Many investigations have showed that myoepithelial cells play a major role in the histogenesis of PA and may be important in many MECs. The aims of the study are to determine the presence and distribution of myoepithelial cell related immunomarkers Actin and S100 in PA and MEC and to explore the histogenesis of these tumors in relation to the above mentioned markers.

Materials and methods: Seventeen formalin fixed paraffin embedded tissue blocks of PA, and other fifteen tissue blocks of MEC were included in the study. Diagnostic confirmation was performed through examination of hematoxylin and eosin sections. Both tumors were immunohistochemically analyzed for the presence of actin and S100 protein.

Results: There was a positive immunoreactivity for the expression of actin in PA (100%) with different score values, while for MEC, actin was negatively expressed in all of the cases (except in one case). There was a highly statistical significant difference in the actin immunoexpression between the two tumors \( p=0.000 \).

There was a positive immunoreactivity for S100 protein expression in all of the cases of PA and MEC (100%) with different score values; however, there was no statistically significant difference in the S100 immunoexpression between them \( p=0.545 \).

Conclusion: The myoepithelial cells are associated with the histogenesis of salivary PA, and the negative expression of actin in squamous, intermediate and mucous cells of MEC can be explained by the non-involvement of the myoepithelial cells in the histogenesis of this tumor.

Key words: Pleomorphic Adenoma, Mucoepidermoid Carcinoma, Actin, S100.

INTRODUCTION
Salivary glands tumors comprise a significant proportion of oral tumors and are the next common neoplasm of the mouth after squamous cell carcinoma (1). PA is the most common tumor of the salivary glands. Although most often found in young to middle-aged women, they can occur in either sex and at any age (2). PAs show a great variety of histological appearances with complex intermingling of epithelial components and mesenchymal-like areas (3). All are composed of a mixture of ductal epithelial cells, basal and myoepithelial cells and variable amounts of stroma, both hyaline and chondromyxoid (4). It was agreed that PAs arise from an uncommitted reserve cell of the intercalated ducts that has the capacity to differentiate into both epithelial and myoepithelial cells and that the “mesenchymal” components are a product of the myoepithelial cells (5).

MEC is one of the most common salivary gland malignancies (6). MEC is most frequently seen in the 35-65 years old age group but it’s also the commonest salivary malignancy in children, and can be seen in patients as young as 4 years old (7). Microscopically, as its name implies, the MEC is composed of a mixture of mucus-producing cells and squamous (epidermoid) cells (6). Intermediate cells, which are cells of intermediate differentiation between the other two cell types, also, can be seen (7). These tumors may be graded as low grade, intermediate grade, or high grade (8).

Cytoplasmic actins vary in amino acid sequences and can be separated by electrophoresis into six different isotopes (9). Antibodies to α-SMA are used in several diagnostic situations. These include the identification of myoepithelial cells, which are admixed, with epithelial cells (10).

S100 protein, so named because of its solubility in a saturated ammonium sulfate solution. S100 is also useful for the labeling of myoepithelial cells (10).

Immunomarkers have been studied by many researchers on the presence of actin and S100 in PA and MEC.

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Kahn et al. and Deihimy et al. (11, 12) have revealed that myoepithelial cells in normal salivary glands have immunostaining with Cytokeratin, S100, actin, and vimentin antibodies. This immunoreactivity was also present in modified myoepithelial cells in PA.

The presence and distribution of S100, glial fibrillar acidic protein in cytoplasm and nucleus of chondromyxoid and cellular areas in PA have been evaluated by Huang and Curran et al. (13,14).

In another study, MEC was nonreactive for actin, vimentin, glial fibrillar acidic protein, and S100, and various grades or subtypes of mucoepidermoid carcinoma did not exhibit any significant differences in immunohistochemical staining (15).

Foschini et al. (16) showed that the epithelial cells in mucoepidermoid carcinoma were positive for keratin, but smooth muscle actin, which is an indicator of myoepithelial cell differentiation, was negative in this tumor. These researchers also suggested that anti-mitochondrial antibody was diffusely positive in mucoepidermoid carcinoma, therefore they concluded that immunohistochemical cell profile of MEC is similar to normal striated duct cells.

According to the existing controversy regarding markers related to the myoepithelial cells in salivary gland tumors, therefore, this study was performed to determine the presence and distribution of myoepithelial cells related immunomarkers Actin and S100 in PA and MEC and to explore the histogenesis of these tumors in relation to the above mentioned markers.

**MATERIALS AND METHODS**

**Sample**

The study was performed on thirty-two formalin-fixed paraffin embedded tissue blocks, seventeen of which diagnosed as PAs, while the remaining fifteen were diagnosed as MECs. The blocks were obtained from the archives of the department of Oral & Maxillofacial Pathology/College of Dentistry/Baghdad University; Al-Shaheed Ghazi Hospital; Teaching Laboratories Department/Medical City/Baghdad; Diyala Hospital/Diyala Governorate; and private laboratories/Baghdad.

Sections of 4µm thickness of each case were mounted on normal glass slides, stained with H&E, and histopathologically re-evaluated. Histological grades were recognized for each case of MEC using the (AFIP) system according to Auclair et al. (1992) (17, 6) by two specialized pathologists. Another 4µm thick sections of each case were cut and mounted on positively charged slides (Fisher scientific and Escho superfl Frost plus, USA) for immunohistochemical staining with monoclonal antibodies Actin (US Biological, A0760-26) and S100 (US Biological S0025-03B). Demographic and clinical data provided by the surgeon were obtained from the surgical reports available with the tissue specimens, which include age, sex, and site of the tumor, unfortunately; other information regarding the size or the stage of the tumors were not available. Positive and negative tissue controls were obtained according to antibodies manufacturer’s datasheets and were added to each run.

Slides were baked in hot air oven at 60°C overnight. Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide followed by blocking the nonspecific antibody binding with normal goat serum (USBiological-I7506A); this was followed by the application of the primary antibodies with a dilution of 1:200 for actin and 1:400 for S100. For actin primary antibody; the slides were incubated within a humid chamber at room temperature for one hour, and then they were put in the refrigerator at 4°C overnight. For S100 primary antibody the slides were incubated at room temperature for overnight. Next day, after washing with PBS, biotinylated antimouse IgG (USBiological-I7506B) were applied to the sections, incubated overnight. Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide followed by blocking the nonspecific antibody binding with normal goat serum (USBiological-I7506A); this was followed by the application of the primary antibodies with a dilution of 1:200 for actin and 1:400 for S100. For actin primary antibody; the slides were incubated within a humid chamber at room temperature for one hour, and then they were put in the refrigerator at 4°C overnight. For S100 primary antibody the slides were incubated at room temperature for overnight. Next day, after washing with PBS, biotinylated antimouse IgG (USBiological-I7506B) were applied to the sections, incubated and rinsed with a stream of PBS. Conjugated antibodies were visualized with DAB chromogen. Sections were counterstained with Harris’ hematoxylin for 1–2 min, dehydrated and mounted.

**Assessment of immunohistochemical results**

Immunohistochemical signal specificity was demonstrated by the absence of immunostaining in the negative control slides and its presence in recommended positive controls. Tumor cells with clear brown cytoplasmic staining were considered positive in actin and cytoplasmic or cytoplasmic/nuclear considered positive in S100 immunostaining pattern according to the manufacturer’s data sheets and previous studies. In PA, myoepithelial cells, ductal cells, and chondromyxoid areas were immunohistochemically evaluated while in MEC, mucous cells, squamous cells, and intermediate cells were evaluated in which, at least ten representative fields for PA and MEC (except two cases in the latter where only five fields were evaluated) of each tissue section were assessed for actin and S100.

Only the number of cells that were positive for actin and positive for S100 were quantified by counting at least one thousand cells in
representative fields at 40X objective in each case. The average of the fields was counted.

The immunoreactivity of tumoral cells was scored on the basis of Regezi method with 0 as negative or nonreactive, 1+ representing scattered spotty staining, 2+ indicating up to 25% of tumor cells positive, 3+ indicating 25% to 50% tumor cells positive, and 4+ indicating more than 50% of tumor cells positive (12).

Statistical analysis
The studied parameters were scored and considered as categorical data thus they presented as count and percentage. The comparison between groups was done using T-test, Chi-square test and ANOVA. Pearson correlation was used to check the linear association between actin and S100. The level of significance was 0.05 (two-sided) in all statistical testing.

RESULTS
Immunohistochemical Findings:

Actin Expression
Actin Expression in normal salivary Gland tissue
There was a positive immunoexpression of actin found in all myoepithelial cells' surrounding acini and ducts of normal salivary gland tissue (Figure 1).

Actin Expression in PA and MEC
Collectively, all the cases of PA (Figure 2) and only one case of MEC (Figure 3) were positive for actin antibody with different score values, while the remaining fourteen cases of MEC were negative or non-reactive for actin (Figure 4). There was a statistically significant difference in the actin immunoexpression between PAs and MECs (Table 1).

S100 Expression
S100 Expression in normal salivary gland tissue
S100 protein expression was mostly luminal and negatively expressed by myoepithelial cells (Figure 5).

S100 Expression in PA and MEC:
Collectively, all the cases of PA and MEC were positive for S100 antibody with different score values as in Figures 6&7 respectively. There was no statistically significant difference in the S100 immunoexpression between PAs and MECs (Table 2).

DISCUSSION
Immunohistochemical Features
In this study, at least ten representative fields for PA and MEC of each tissue section were assessed for the expression of actin and S100 (except two cases in the latter where only five fields were evaluated). This is due to the fact that the sections of PA were larger than that of MEC and the evaluation of ten random fields were assessed so easily in comparison to MEC in which the sections were small.

Expression of Actin
Expression of Actin in normal salivary gland tissue
Functionally, myoepithelial cells are a hybrid of both smooth muscle (‘myo’) and epithelial cells. Like muscle cells, myoepithelial cells express filamentous smooth muscle actin and smooth muscle myosin, and exhibit contractile properties; like epithelial cells, myoepithelial cells express intermediate filaments (the epithelial keratins) and have cadherin-mediated cell–cell junctions (18).

In this study, the expression of actin was found in all myoepithelial cells’ surrounding acini and ducts of normal salivary glands and this was in agreement with previous studies (16, 19, and 20).

Expression of Actin in PA & MEC
The histogenesis of salivary PA is controversially discussed, although two kinds of cells have been postulated to be involved, intercalated duct cells and myoepithelial cells. The former are thought to proliferate to form the tubular, duct-like, and glandular structures seen in these tumors (21).

In the present study, α-SMA was observed in non-luminal cells of the duct-like structures, in the spindle shaped cells, star shaped cells of the myxoid, and some cells of the chondroid areas also expressed α-SMA and this was in agreement with several studies (20, 21).

Regarding MEC, the results of this study showed that all the cases of MECs were negative for actin (except one case); this was in agreement with previous studies (12, 16 and 23). It should be noted that the expression of actin was mostly stromal as well as in the endothelial cells lining the surrounding blood vessels. The false positive expression in one of the cases may be attributed to the distribution of the stain or cross reactivity.

Salivary gland tumors histogenesis is based on the ductal-acinar unity, resulting in three models of development: origin from luminal cells, from non-luminal cells, and from a mixed population of acinar and/or ductal cells with basal and/or myoepithelial cells (24). Due to the wide cellular diversity observed in MEC, its histogenesis remains controversial.

The negative expression of actin in squamous, intermediate and mucous cells of MEC in this study can be explained by the non-
involvement of the myoepithelial cells in the histogenesis of this tumor.

Expression of S100 protein:
Expression of S100 protein in normal salivary gland tissue:

Initially, S100 protein was considered the most popular marker for myoepithelial cells as it was detected in normal salivary glands. However, further studies proved that S100 protein is not a reliable myoepithelial cell marker, for it can also be expressed by ductal cells (21).

In the present study, S100 protein expression was mostly luminal and negatively expressed by myoepithelial cells and this was in agreement with previous studies (20, 25), while disagrees with others (26, 27).

This discrepancy may be attributed as it has been demonstrated that the rich autonomic nerves associated with the acini and ducts have been misconstrued as S100-positive myoepithelium (28).

REFERENCES


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Table 1, 2: Actin scores in PAs and MECs S100 scores in PA and MECs

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Figure 1-7