Immunohistochemical study of salivary gland tumors in a tertiary institution in South-South Region of Nigeria

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INTRODUCTION

Salivary gland tumors are important tumors in oral and maxillofacial practice, and malignant tumors account for about 3% of head and neck malignancies.[1]

The salivary glands consist of three major paired glands (parotid, submandibular and sublingual) and minor glands located in the mucosa of palate, lips and the respiratory tract. Each gland consists of acini (serous, mucinous or mixed) and ducts (intercalated, striated and excretory). The basic cellular components of the gland are surrounded by myoepithelial and/or basal cells. There are also ductal epithelial cells lining the lumen of the ducts.

Most of the salivary gland tumors originate from these surrounding cells and ductal epithelial cells. There are various theories regarding the morphogenesis of the various neoplasms of salivary glands particularly among biphasic tumors such as pleomorphic adenoma (PA), adenoid cystic carcinoma (ADCC) and polymorphous low-grade adenocarcinoma (PLGA).[2]

Aim: The aim of this study was to see the usefulness of immunohistochemistry in diagnosing salivary gland tumors found in a tertiary health institution.

Materials and Methods: Twenty-six formalin-fixed paraffin embedded salivary gland tumors were accessioned, and 2 µm were sectioned and processed using Streptavidin-Biotin immunoperoxidase method.

Results: Adenoid cystic carcinoma (ADCC) was positive to alpha-smooth muscle actin (α-SMA) while mucoepidermoid carcinoma (MEC), polymorphous low-grade adenocarcinoma (PLGA), squamous cell carcinoma (SCC) and oncocytic carcinoma (OCC) were all negative to it. MEC, PLGA, ADCC and the only pleomorphic adenoma (PA) were positive to Ki-67 while both SCC and OCC were negative to it. All the tumors except PA were positive to p63.

Conclusion: It appears that α-SMA may be used to distinguish ADCC from MEC and PLGA, but Ki-67 cannot be used for this purpose. Furthermore, p63 cannot help in the diagnosis of ADCC, MEC or PLGA. It was concluded that immunochemistry can be used as adjunct to routine H and E stain in the diagnosis of the various salivary gland tumors.

Keywords: Adenoid cystic carcinoma, alpha-smooth muscle actin, immunohistochemistry, Ki67, mucoepidermoid carcinoma, p63, polymorphous low-grade adenocarcinoma
Many of the tumors are easily diagnosed with routine hematoxylin and eosin stain (H and E), but a few are complicated and would require special stains to arrive at a definitive diagnosis. Various markers have been used in an attempt at differentiating the complex salivary gland tumors as adjunct to histopathological diagnosis. Such markers include monoclonal antibodies to alpha-smooth muscle actin (α-SMA), smooth muscle myosin heavy chains (SMMHCs), calponin, p63, Ki-67, c-Kit, keratin, vimentin and S100 protein.[3-13]

Acini/ductal epithelial cells are positive for keratins (CK 7 and CAM 5.2), epithelial membrane antigen but negative or focally positive for high molecular weight keratins (HMWKs, CK5/6 and 34 β12) and myoid markers (α-SMA, SMMHC and calponin).[9-11]

c-kit has been used in recent time to differentiate ADCC and PLGA because PLGA is mostly negative to this marker while ADCC is usually positive.[9] Ki-67 is a marker of proliferative activity of cells and is positive for ADCC, and its overexpression indicates poor prognosis in patients with ADCC.[4-6] Although ADCC and PLGA are both sensitive to Ki-67, ADCC was found to be more sensitive.[13] P63 is a homolog of p53, and it is used to identify basal/stem cells of stratified squamous epithelial cells and myoepithelial cells. It also plays a role in the development of epithelium and limb.[9,11]

We, therefore, undertook this study to access the role of α-SMA, Ki-67 and p63 monoclonal antibodies in the diagnosis of complex salivary gland tumors previously diagnosed with H and E stain in our center for over a period of 7 years.

**MATERIALS AND METHODS**

All the salivary gland tumors in the oral pathology file were accessioned and reviewed, and twenty-six that met the criteria for selection for immunostaining were included in this study. They were classified according to the WHO classification of 2005.

All tissue samples were previously fixed in 10% neutral buffered formalin and embedded in paraffin wax blocks. The immunostaining agents used were anti-α-SMA, Ki-67 and p63. Avidin-Biotin Complex method was used. All antibodies used were manufactured by Novocastra (Novocastra product now owned by LEICA). ELIZA method was used, and manufacturer’s instruction was strictly followed. For each reaction, it was reported either positive (when there was reaction) or negative (no reaction). When positive, it was graded mild (+1), moderate (+2) or intense (+3) depending on the intensity and extent of the positive reaction. Statistical analysis was done with SPSS version 20; descriptive statistics (frequency, tables) were done. Association between variables were accessed with Chi-square and P < 0.05.

**RESULTS**

The salivary gland tumors consisted of 25 malignant lesions (11 mucoepidermoid carcinomas [MECs, Figure 1], 7 PLGAs [Figure 2], 3 ADCCs [Figure 3], 2 squamous cell carcinomas [SCCs, Figure 4] and 2 oncocytic carcinomas [OCCs, Figure 5]) and only one benign lesion, PA, Figure 6.

Ten of the MEC were negative to α-SMA. One had intense nuclear and cytoplasmic positivity. All the PLGA were negative to α-SMA while the three ADCC were all positive to α-SMA. The two SCC and the OCC were negative to α-SMA. The difference between the ADCC positivity to α-SMA and that of MEC or PLGA was statistically significant (P = 0.01). The only PA was positive to α-SMA. The positivity was both nuclear and cytoplasmic positivities (Table 1).

One of the MECs was slightly positive to Ki-67, while eight were moderately positive and two were markedly positive. All the PLGA were positive to Ki-67. Five were moderately positive while two were markedly positive. As regards the reaction of SCC and OCC to Ki-67, one was mildly positive while the other was negative, respectively. The only PA was moderately positive to Ki-67. The difference in the positivity between MEC, PLGA and AdCC to Ki-67 was not statistically significant (Table 1).

Four of the MEC were negative to p63, one was mildly positive while six were moderately positive to p63. Five PLGAs were negative to p63 one was mildly positive, and one was moderately positive to p63. Two ADCC were

| Tumor type | n | ASMA | P | Ki-67 | P | P63 | P |
|------------|---|------|---|------|---|-----|---|
| MEC        | 11 | 10   | 1 | 0.01 | -  | 11  | 0.09 |
| PLGA       | 7  | 7    | 1 | -    | 7  | 5   | 2  |
| ADCC       | 3  | 3    | 1 | -    | 3  | 3   | -  |
| SCC        | 2  | 2    | 1 | -    | 1  | 1   | -  |
| OCC        | 2  | 2    | 1 | -    | 1  | 1   | -  |
| PA         | 1  | -    | 1 | -    | 1  | 1   | -  |

CA: Carcinoma, MEC: Mucoepidermoid CA, PLGA: Polymorphous low-grade adenocarcinoma, ADCC: Adenoid cystic carcinoma, SCC: Squamous cell carcinoma, OCC: Oncocytic CA, PA: Pleomorphic adenoma, ASMA: Alpha-smooth muscle antigen, −VE: Negative reaction, +VE: Positive reaction
moderately positive while one was markedly positive to p63. The 2 SCCs were mildly positive to p63. The 2OCCs were moderately positive to p63 while the only PA was negative to p63. The positivity of all tumors to p63 was not statistically significant [Table 1].

DISCUSSION

Myoepithelial cells are believed to play a prominent role in the histopathogenesis of some salivary gland tumors particularly ADCC, epithelial-myoeohelial carcinoma and PA. α-SMA has been used severally to identify myoepithelial cells in various tumors and to separate myoepithelial containing tumors like ADCC from nonmyoepithelial containing tumors such as MEC, SCC and to some extent PLGA[7,8].

In this study, ten MECs were negative to α-SMA while one was markedly positive to α-SMA. MEC is an epithelial tumor having epithelial, intermediate and mucous cells but with no myoepithelial cell component in its histogenesis. It has been shown in many reports that myoepithelial cells have no part to play in the histogenesis of MEC.[8,14-18] The three ADCCs were positive for α-SMA in this study. This confirms findings in many previous reports in the literature that myoepithelial cell play an prominent role in histogenesis of ADCC.[7,8,11,12]
In this study, all the PLGAs were negative to α-SMA; this is in conformity with many previous reports that myoepithelial cells do not play a part in the histogenesis of PLGA. However, some authors have reported otherwise\cite{13,17} Jones \textit{et al.}\cite{19} in a study of the immunoreactivity of α-SMA observed focal staining in 5 out of 6 PLGA. In addition, Norberg \textit{et al.} observed ultrastructural features of myoepithelial cells in 1 out of 3 PLGA\cite{20}.

Both SCC and OCC were negative to α-SMA in this study. This is in conformity with previous reports that concluded that myoepithelial cell has no role in the pathogenesis of either SCC or OCC\cite{21-23}.

In this study, the only PA was positive to α-SMA. This is in keeping with the characteristic feature of PA with myoepithelial and epithelial components playing prominent role in its histogenesis. Although while most studies have shown positivity of PA to α-SMA, few have however reported negative reaction\cite{8,24}.

In this study, all the MEC were positive to Ki-67 though to various degrees (1 mildly, 8 moderately and 2 markedly). Ki-67 is a marker for cell proliferation. It is also a prognostic indicator in malignancy. It has been shown that the degree of staining correlates with the malignant grade of the MEC\cite{17,25,26}. This may explain the different degrees of staining observed in this study.

Five PLGAs were negative to p63 while one was mildly positive and another one was moderately positive to p63. Various reports show the variability of the reaction of PLGA to p63\cite{31-34}. The difficulty of differentiating ADCC from PLGA is confirmed by this reports using many of these markers as in various other reports\cite{31-34}.

The three ADCCs were positive for p63. This is in conformity with previous reports. Edwards \textit{et al.} reported in a study comparing p63 immunoreactivity of ADCC and PLGA found that both were highly sensitive to p63\cite{31,33,34}. The difficulty of differentiating ADCC from PLGA is confirmed by this reports using many of these markers as in various other reports\cite{31-34}.

The two SCC and the two OCC were positive to p63. P63 has positive reactivity to SCC. The positivity of OCC is at variance with the report of Weinreb \textit{et al.} that showed its negativity to p63\cite{30}.

One SCC and one OCC were negative each to Ki67 while one SCC and one OCC were mildly positive to Ki67.

The only PA was moderately positive to Ki67. Positivity of PA to Ki67 have been reported\cite{28}.

In this study, out of a total of 11 MEC, 4 were negative to p63 one was mildly positive while 6 were moderately positive to p63 which is in conformity with previous reports showing MEC marked sensitivity to p63. P63 is a p53 homolog which participate in the development of epithelium and bone. Sams \textit{et al.} found the strong positivity of MEC to p63 while comparing its reactivity with acinic cell carcinoma. Fonseca \textit{et al.} also found great positivity of MEC to p63 compared to papillary cystadenoma lymphatosum and mucus retention cyst whose reactivity was limited to basal cells\cite{29}. Weinreb \textit{et al.} while comparing the positivity between OCC/oncocytoma with MEC reactivity to p63 found that MEC reactivity was more than 50% while in oncocytoima/oncocytic CA was just scanty.\cite{30}.
CONCLUSION

The α-SMA can be used to differentiate MEC and PLGA from ADCC but Ki-67, cannot be used to differentiate MEC from ADCC or from PLGA.

Immunostaining can be used as adjunct to H and E staining for diagnosis of salivary gland tumors.

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Conflicts of interest
There are no conflicts of interest.

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