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Original Research Article

Differential Expression of Alpha- Smooth Muscle Actin in Salivary Gland Tumours: Any Diagnostic Value?

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Abstract

Diagnosis of salivary gland tumours (SGTs) pose a challenge even to the experienced pathologist and immunohistochemistry may be a useful adjunct. Aim of this study is to assess the degree of differential expression of α-SMA among salivary gland tumours, and to evaluate the possible diagnostic benefits. Sections of formalin-fixed, paraffin embedded tissues blocks obtained from SGTs were stained using α-SMA monoclonal antibody. Immunoreactivity were scored based on Regezi method, with 0 indicating nonreactive, 1+ representing scattered spotty staining, 2+ indicating up to 25% of tumour cells positive, 3+ indicating between 25% to 50% tumour cells positive, and 4+ indicating more than 50%. Scores of 1+ and 2+ were regarded as low reactivity, 3+ was regarded as moderate reactivity, and 4+ was regarded as high reactivity. Data was analyzed and ***p***-value <0.05 was regarded as significant. Out of the 42 cases of SGTs studied, 33 (78.6%) expressed α-SMA. The highest α-SMA score observed in adenoid cystic carcinoma, pleomorphic adenoma and basal cell adenoma was 4+, followed by peak score of 3+ observed in polymorphous adenocarcinoma, while 2+ was the highest score in mucoepidermoid carcinoma and epimyoepithelial carcinoma. Acinic cell carcinoma did not express α-SMA in all cases studied. There was significant differential expression of α-SMA between acinic cell carcinoma and adenoid cystic carcinoma (p=0.009); mucoepidermoid carcinoma and pleomorphic adenoma (p=0.048); mucoepidermoid and adenoid cystic carcinoma (p=0.004). In conclusion, α-SMA may be useful in the exclusion of acinic cell carcinoma and in the differential diagnosis between pleomorphic adenoma and mucoepidermoid carcinoma.

Keywords: Myoepithelial cells**,** α-Smooth Muscle Actin, immunohistochemistry, salivary gland tumours, diagnostic, differential expression.

INTRODUCTION

Salivary gland tumours (SGTs) are a morphologically diverse group of neoplasms which are important to the Oral & Maxillofacial pathologists and surgeons. They may present considerable challenges for the pathologists and surgeons regarding their diagnosis and treatment [1, 2]. As a result of the rarity, the number of histologic subtypes, and the morphologic overlap and heterogeneity among these subtypes, salivary gland tumours often remain diagnostically challenging even for the experienced pathologists [3, 4].

In spite of the morphologic diversity of SGT, the neoplastic cells still differentiate into those cells that constitute the normal salivary gland. These are the acinar cells, myoepithelial cells, ductal cells, and basal cells [5]. Majority of salivary gland tumours exhibit myoepithelial differentiation albeit with varying degree of expression [6]. Some SGTs, in addition to the myoepithelial cell differentiation, also exhibit luminal cell differentiation while a few others exhibit either solely myoepithelial cells e.g. myoepithelioma, or solely acinar cell differentiation e.g. acinic cell carcinoma [7]. In this regard, immunohistochemistry can be a useful adjunctive tool in assessing the cell nature and differentiation status of the tumoral cells, thereby enhancing the diagnostic accuracy in equivocal cases [8, 9, 7].

Immunohistochemistry has been applied in the exclusion of tumours without myoepithelial differentiation from those with myoepithelial differentiation. Markers like alpha- smooth muscle actin (α-SMA), muscle specific actin (MSA), vimentin, calponin, podoplanin, are expressed by myoepithelial cells and not by acinar cells [10]. Ductal/acinar cells express EMA, CEA, whereas only acinar cells would express alpha-amylase [11]. In the differential diagnosis of myoepithelial tumours, lesions that express solely

myoepithelial markers e.g. SMA, MSA, would either be a myoepithelioma or myoepithelial carcinoma [12]. Tumours that express solely acinar cell markers e.g. alpha- amylase, would be acinic cell carcinoma [11]. This has been found useful in diagnosis of acinic cell carcinoma, although some authors have reported low sensitivity [13]. Also, identification of ductal cell differentiation in tumours with predominantly myoepithelial cells has been found useful in the differential diagnosis between pleomorphic adenoma and myoepithelioma, and between epi- myoepithelial carcinoma and myoepithelial carcinoma. The former would express markers for ductal cells in addition to myoepithelial cell markers [5].

α-SMA is an antibody that recognizes the smooth muscle isoform of actin and it does not detect other actin isoforms [14]. Antibodies to α-SMA are used in different diagnostic situations. In salivary glands, α-SMA specifically recognizes myoepithelial cells and does not react with the other isoforms expressed in various epithelial and non-epithelial cell types [14]. Because of the different level of participation of myoepithelial cells in the formation of the different subtypes of salivary gland tumours, it is logical to expect a variation in the degree of α-SMA expression among the various salivary gland tumour types. Although some studies have been done on salivary gland tumour immunohistochemistry which utilized SMA, and in some cases, in addition to other antibodies, most of the studies are mainly focused on the role of myoepithelial cells in the histiogenesis of these tumours while some only assessed the reactivity of SMA in the different tumour cells[15]. A recent study by Raman and Sherlin evaluated the expression of α-SMA looking at differences in expression between benign and malignant tumours and between epithelial and stromal components of the tumours[16]. There is paucity of studies evaluating the differences in expression between tumour types and its practical use in diagnosis[17]. The objective of this study therefore is to critically examine the diagnostic value of α-SMA with a focus on the analyses (with inferential statistics) of the differential expression between tumour types and the possible application of this antibody in a diagnostic scenario, especially in low income climes where there may be no privilege of the use of panels of antibodies in clinical settings.

MATERIALS AND METHODS

Blocks of formalin-fixed, paraffin embedded (FFPE) tissues obtained from SGTs were retrieved from the archives of the Department of Oral Pathology/Medicine and Department of Morbid Anatomy, University of Benin Teaching Hospital. The sections were stained with haematoxylin and eosine (H&E), and histopathologically re-evaluated.

A total of 42 cases of benign and malignant SGTs were selected among the cases. 5mm thick sections were made for α-SMA (Thermo scientific, USA) staining using the polymer- horseradish peroxidase (HRP) method. The sections were sequentially de-paraffinized through a series of xylene, hydrated in a decreasing ethanol series, and treated with 10% ammonium in 95^0C ethanol for removal of formalin pigments. Tissue was then immersed in heat-induced epitope retrieval citrate buffer diluted 1:10 with distilled water, and incubated at 90^0C for 1hour. Before incubation with the primary antibodies, endogenous peroxidase was blocked with hydrogen peroxide in methanol for 10 minutes. This was then followed by incubation of sections of each tissue block with the monoclonal anti-α-SMA (Thermo scientific, USA). Primary antibody enhancer was applied and incubated for 10 minutes at room temperature. The material was immersed in Tris buffer, pH 7.4, after each reaction step. The specimen was then incubated with the HRP polymer for 15 minutes at room temperature and then rinsed in buffer. The reaction was developed by adding one drop of diaminobenzidine (DAB) plus chromogen to 2ml of DAB plus substrate, mixed by swirling, and applied to tissues and incubated for 5 minutes. The material was then counterstained with Mayer's hematoxylin and rinsed in distilled water. The tissue was dehydrated and subsequently cleared in xylene and coverslipped.

Positive and negative tissue controls were obtained according to the antibodies manufacturer's datasheets and added to each run. Sections from *a uterine leiomyoma* were used as positive control while sections from a *uterine leiomyoma* with the antibody diluents *without the inclusion of the primary antibody was used as negative control.* The slides were reviewed without reference to the initial diagnosis made from H&E to eliminate bias.

Immunohistochemical signal specificity was demonstrated by the absence of immunostaining in the negative control slides and the presence of immunohistochemical staining in the positive controls. Positive tumour cells were indicated by *brown cytoplasmic* staining. Immunoreactivity of tumoral cells were scored on the basis of the Regezi method with 0 as negative or nonreactive, 1+ representing scattered spotty staining, 2+ indicating up to 25% of tumour cells positive, 3+ indicating between 25% to 50% tumour cells positive, and 4+ indicating more than 50% of tumour cells positive [2, 18]**.** Scores of 1+ and 2+ were regarded as low reactivity; a Score of 3+ was regarded as moderate reactivity, while a score of 4+ was regarded as high reactivity.

Data was analyzed using the Statistical Package for Social Science (SPSS) for Windows, version 23 software (IBM Corp., 2015). Inferential statistics used was a One Way Non-Parametric analysis of variance (ANOVA) [Kruskal Wallis test] which compared the α-SMA score across tumour types, and a

pair-wise Post-Hoc Analysis (PHA) was done to show the areas of differences that were significant. The level of significance was set at 95% (***p***-value <0.05).

RESULTS

A total of 42 cases of SGTs were used in this study, comprising of 19 cases of pleomorphic adenoma, 6 adenoid cystic carcinoma, 6 mucoepidermoid carcinoma, 6 polymorphous adenocarcinoma, 2 basal cell adenoma, 1 case of epi- myoepithelial carcinoma. The age of the patients ranged from 12 to 70 years, with a mean age of 41.6± 15.6 years (SD). There were 13 (31.0%) males and 29 (69.0%) females. Most of the cases were palatal lesions (n=20, 47.6%) and parotid lesions (n=13, 31.0%) [Table 1].

Table-1: The clinico-demographic characteristics of cases selected for the immunohistochemical study

Tumour	Mean Age ± SD (Years)	Gender		
Histology				Site/no.
		Male	Female	
ACC	54.5±7.8	1	1	Palate-1, Gingiva-1
ADCC	42.2±15.2	1	5	Parotid-1,Submand-2 Palate-1, Subling-1 Antrum-1
MEC	46±16.2	1	5	Parotid-4, Palate-2
PMA	44.8±17.3	2	4	Palate-5, Submand-1
EME CA	47	0	1	Parotid-1
PA	35.6±15.2	7	12	Parotid-4,Submand-2 Palate-11, Cheek-1 Lip-1
BCA	59±5.7	1	1	Parotid-2
Total	**41.6±15.6**	**13**	**29**	**42**

Key: ACC=Acinic cell carcinoma ADCC=Adenoid cystic carcinoma MEC=Mucoepidermoid carcinoma PMA=Polymorphous adenocarcinoma PA=Pleomorphic adenoma BCA=Basal cell adenoma EME CA=Epithelial myoepithelial carcinoma

Out of the 42 cases of SGTs studied, 33 (78.6%) cases showed varying degrees of positive expression of α-SMA (Table 2). α-SMA positive cells were showed as brown cytoplasmic staining (Figure 1-5). The highest α-SMA score observed in adenoid cystic carcinoma, pleomorphic adenoma and basal cell adenoma was 4+, followed by a peak α-SMA score of 3+ observed in polymorphous adenocarcinoma, while α-SMA score of 2+ was the highest observed in mucoepidermoid carcinoma and epimyoepithelial carcinoma. Acinic cell carcinoma did not express α-SMA in all cases studied [Table 2] [Figure 1-5].

Table-2: α-SMA score distribution of histological types of salivary gland tumours.

Tumour	α-SMA Score					
Histology	0	1+	2+	3+	4+	Total
ACC	2(100%)	-	-	-	-	2(100%)
ADCC	-	1(16.7%)	1(16.7%)	1(16.7%)	3(50%)	6(100%)
MEC	3(50%)	2(33.3%)	1(16.7%)	-	-	6(100%)
PMA	1(16.7%)	1(16.7%)	1(16.7%)	3(50%)	-	6(100%)
EME CA	-	-	1(100%)	-	-	1(100%)
PA	3(15.8%)	4(21.1%)	6(31.6%)	3(15.8%)	3(15.8%)	19(100%)
BCA	-	-	-	1(50%)	1(50%)	2(100%)
Total	**9(21.4%)**	**8(19%)**	**10(23.8%)**	**8(19%)**	**7(16.7%)**	**42(100%)**

A comparative analysis of α-SMA expression across different tumour groups using a pairwise comparison showed a statistically significant differential expression of α-SMA between acinic cell carcinoma and adenoid cystic carcinoma (p=0.009); acinic cell carcinoma and basal cell carcinoma (p=0.012); mucoepidermoid carcinoma and pleomorphic adenoma (p=0.048); mucoepidermoid and adenoid cystic carcinoma (p=0.004); mucoepidermoid and basal cell adenoma (p=0.012) [Table 3].

Table-3: Pair-wise comparison of salivary gland tumours using α-SMA

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig. (p-value)
ACC-MEC	-5.750	9.908	-0.586	0.558
ACC-PA	-16.868	8.930	-1.889	0.059
ACC-EME CA	-17.500	14.712	-1.190	0.234
ACC-PMA	-17.583	9.908	-1.793	0.073
ACC-ADCC	**-25.750**	**9.908**	**-2.625**	**0.009***
ACC-BCA	**-30.250**	**12.012**	**-2.518**	**0.012***
MEC-PA	**-11.118**	**5.625**	**-1.977**	**0.048***
MEC-EME CA	-11.750	12.975	-0.906	0.365
MEC-PMA	-11.833	6.935	-1.706	0.088
MEC-ADCC	**20.00**	**6.935**	**2.884**	**0.004***
MEC-BCA	**-24.500**	**9.808**	**-2.498**	**0.012***
PA-EME CA	0.632	12.324	0.051	0.959
PA-PMA	0.715	5.625	0.127	0.899
PA-ADCC	8.882	5.625	1.579	0.114
PA-BCA	-13.382	8.930	-1.499	0.134
EME CA-PMA	0.083	12.975	0.006	0.995
EMECA-ADCC	8.250	12.975	0.636	0.525
EME CA-BCA	-12.750	14.712	-0.867	0.386
PMA-ADCC	8.167	6.935	1.178	0.239
PMA-BCA	-12.667	9.808	-1.291	0.197
ADCC-BCA	-4.500	9.808	-0.459	0.646

A Non-Parametric One Way Analysis of Variance (One Way ANOVA) using the Independent Samples Kruskal-Wallis Test shows a significant variation in the **α**-SMA scores across different salivary gland tumour types (p = 0.022) with p< 0.05 as significant value.

ACC=Acinic cell carcinoma ADCC=Adenoid cystic carcinoma
MEC=Mucoepidermoid carcinoma PMA=Polymorphous adenocarcinoma
PA=Pleomorphic adenoma EME CA=Epithelial myoepithelial carcinoma
BCA=Basal cell adenoma *****Significant

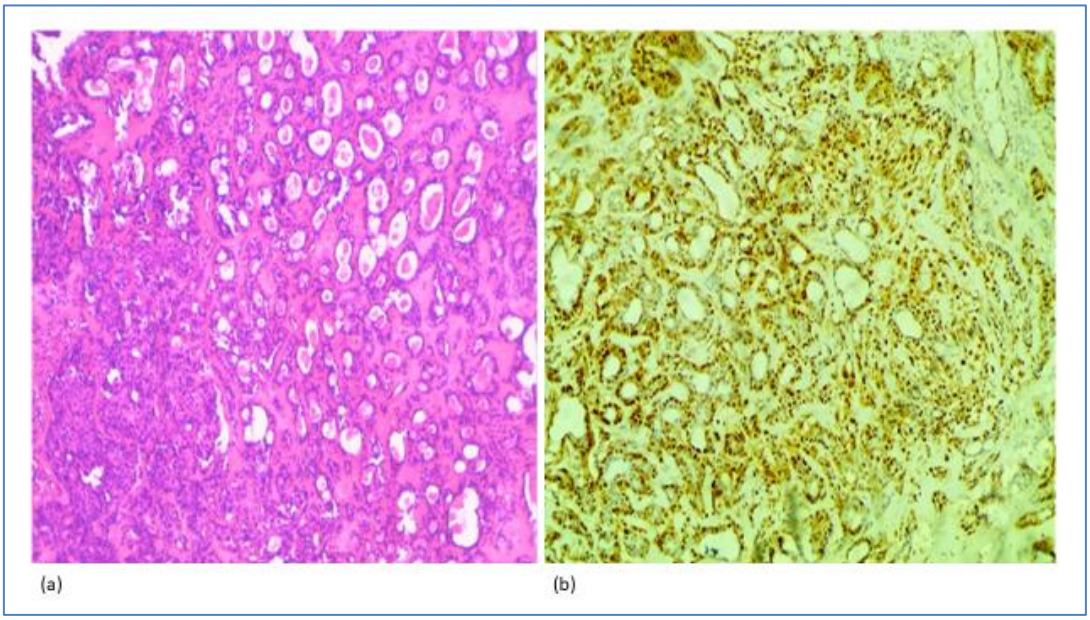

Fig-1(a): Pleomorphic adenoma, H&E x100. (b): α-SMA IHC expression in pleomorphic adenoma x100

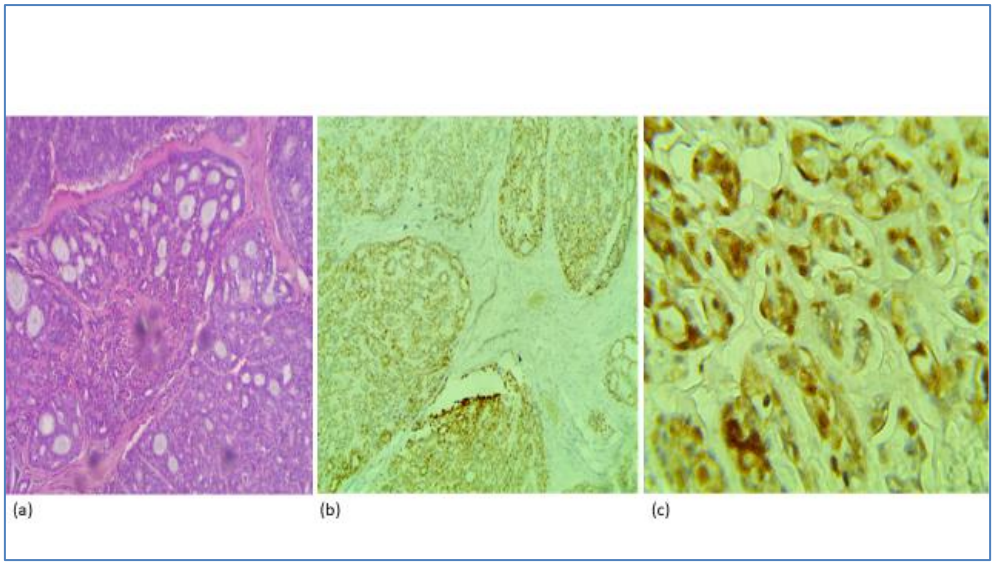

Fig-2 (a): Adenoid cystic carcinoma, H&E x100. (b) α-SMA IHC expression in adenoid cystic carcinoma x100. (c) α-SMA IHC expression in adenoid cystic carcinoma x400

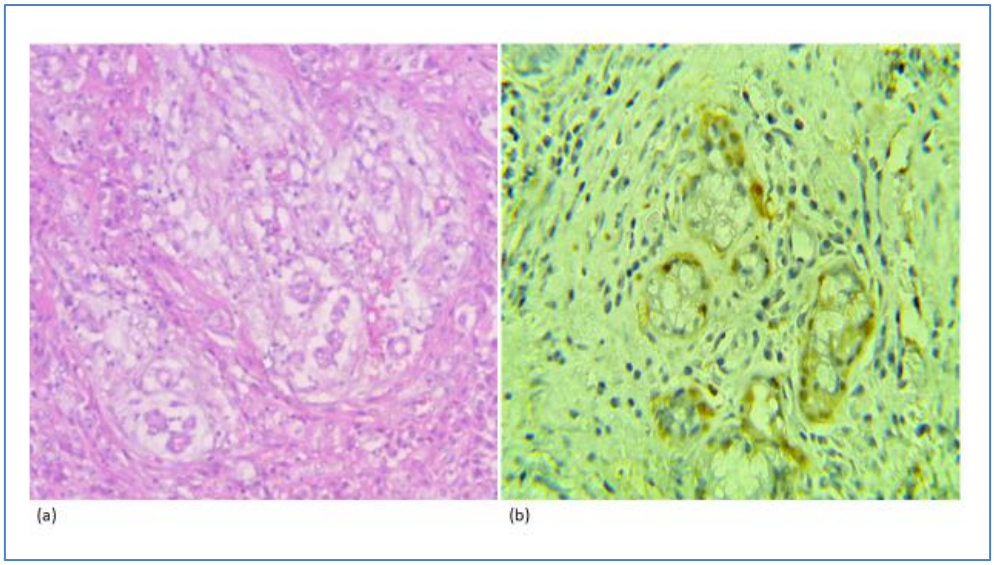

Fig-3(a): Mucoepidermoid carcinoma, H&E x400. (b) α-SMA IHC expression in mucoepidermoid carcinoma x400

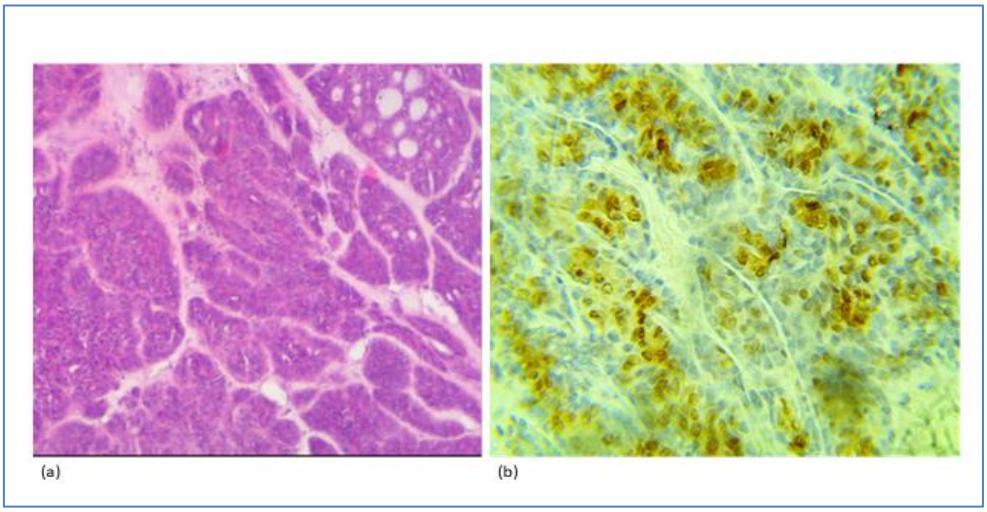

Fig-4 (a): Polymorphous adenocarcinoma, H&E×100 (b) α-SMA IHC expression in polymorphous adenocarcinoma

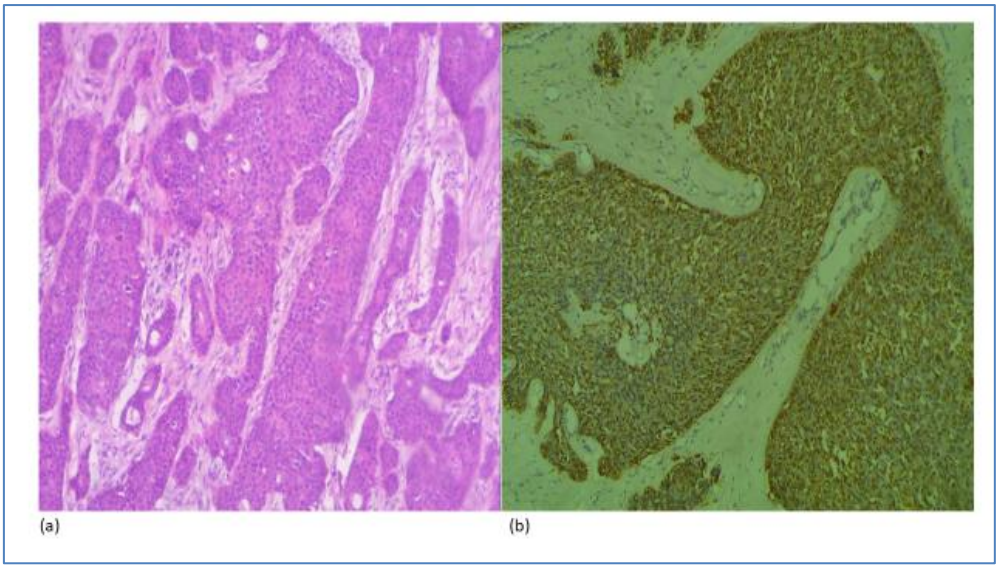

Fig-5 (a): Basal cell adenoma, H&E×100 (b) α-SMA IHC expression in basal cell adenoma

DISCUSSION

Neoplastic myoepithelial cells participate in the tumorigenesis of most SGTs, although with variation in the extent of participation in the different salivary gland tumours [19]. This study evaluates the degree of differential expression of myoepithelial cell marker (α-SMA) in different salivary gland tumours, and the possible diagnostic value.

Out of the 42 cases of salivary gland tumours in this study, 33 (78.6%) cases showed positive reactivity to α-SMA. The high expression of α-SMA in salivary gland tumours observed in this study agrees with reports in the scientific literature, which indicate that myoepithelial cells participate in the formation of most salivary gland tumours [7, 19]. Accordingly, 0% expression of α-SMA which was observed in all the cases of acinic cell carcinoma in this study is consistent with previous report by Jones *et al*.[20]. Acinic cell carcinoma is a salivary gland tumour in which myoepithelial cells do not participate in the tumorigenesis [5, 7, 19] and the histiogenesis has been traced to the transformation of terminally differentiated serous acinar cells of the salivary gland [21]. Hence, α-SMA expressivity may find a diagnostic benefit in the exclusion of an acinic cell carcinoma in difficult cases.

The 6 (100%) cases of adenoid cystic carcinoma in this study were all positively immunoreactive for α-SMA, with 3 (50%) cases showing a high reactivity (4+). This is comparable with findings from previous studies [7, 19]. The high reactivity of adenoid cystic carcinoma support the report of exuberant myoepithelial expression in these tumours [7].

There were 5 (83.3%) cases out of 6 cases of polymorphous adenocarcinoma in this study that showed positive expression of α-SMA ranging from 1+ to 3+. This agrees with previous studies which demonstrated reactivity of PLGA for α-SMA [22-24]. Although the participation of myoepithelial cells in the development of PLGA is controversial, the preponderant view is that there could be variable presence of neoplastic myoepithelial cells in PLGA [5, 19].

Out of the 6 cases of mucoepidermoid carcinoma studied, 3 (50%) cases showed positive expression of α-SMA, with all cases showing low reactivity. Mucoepidermoid carcinoma is one of the salivary gland tumours which does not classically exhibit myoepithelial cell differentiation, although some researchers have reported that myoepithelial cells could participate in the development of this tumour [7, 5, 19]. Finding from this study is comparable with the report of Prasad *et al*.[19].

Epithelial-myoepithelial carcinoma is a biphasic tumour composed predominantly of ductal structures with inner epithelial cells (ductal epithelium) and outer myoepithelial cells, which most often present as clear cells. The only case of epithelial-myoepithelial carcinoma in this study expressed a positive immunoreactivity for α-SMA. This is expected because the role of myoepithelial cells in the tumorigenesis of epi- myoepithelial carcinoma is incontrovertible. Similarly, a study by Prasad *et al*.[19] Reported a positive expression of α-SMA for this tumour.

Pleomorphic adenoma is a tumour that is predominantly of myoepithelial origin [12]. These myoepithelial cells are said to be responsible for the varied presentation in cell types and stromal nature in pleomorphic adenoma [12, 19]. In this study, 16 (84.2%) cases out of the 19 cases of pleomorphic adenoma, showed reactivity for α-SMA. Majority of the cases that were positive expressed high reactivity. This finding is comparable with previous reports [7, 19].

Also, the 2 (100%) cases of basal cell adenoma included in this study, showed positive expression to α-SMA, similar to previous reports [19].

A pair- wise comparison of the α-SMA immunoreactivity between the different salivary gland tumours show statistically significant difference in the degree of α-SMA expression between acinic cell carcinoma and adenoid cystic carcinoma; acinic cell carcinoma and basal cell adenoma; mucoepidermoid carcinoma and pleomorphic adenoma; mucoepidermoid carcinoma and adenoid cystic carcinoma; and mucoepidermoid carcinoma and basal cell adenoma. It should be noted however that the few samples of some of the tumours like basal cell adenoma may limit one from drawing conclusions from its comparison with other tumours using inferential statistics.

From the foregoing using the pairwise comparison, most of these paired tumours are histomorphologically and cytomorphologically distinct and may not require an immunohistochemical evaluation to differentiate them. However, mucoepidermoid carcinoma and pleomorphic adenoma are histologic mimics which often pose a challenge in their differential diagnosis especially with low grade mucoepidermoid carcinoma. Pleomorphic adenoma is known for its cytomorphological and architectural variability [25]. The neoplastic myoepithelial cells are thought to be pluripotent, and thus can differentiate into various cell forms or types- plasmacytoid, clear cells, basaloid cells, spindle cells etc [12]. Pleomorphic adenoma presenting with predominantly clear myoepithelial cells could resemble any other clear cell tumour of the salivary gland including low grade mucoepidermoid carcinoma [26]. Furthermore, extensive squamous metaplasia in a PA could also mimic a mucoepidermoid carcinoma [22, 27]. Thus, there is a need for an immunohistochemical marker capable of delineating between these lesions. It is documented that pleomorphic adenoma is predominantly a myoepithelial tumour with exuberant expression of myoepithelial cell markers whereas mucoepidermoid carcinoma does not classically exhibit myoepithelial differentiation [19]. Accordingly, this study revealed a significant differential expression of α-SMA between PA and MEC. This observed differential expression may find its diagnostic benefit in equivocal cases.

CONCLUSION

This study shows that α-SMA is an important immunohistochemical marker in salivary gland pathology with potential diagnostic benefits in the exclusion of acinic cell carcinoma and in the differential diagnosis between pleomorphic adenoma and mucoepidermoid carcinoma, in equivocal cases. A larger study is recommended to further substantiate this finding.

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