Original Article

Utility of smooth muscle actin and CD117 as reliable markers in the diagnosis of salivary gland neoplasms

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Abstract

Objective: The aim of this study is to analyze the utility of immunohistochemical markers such as CD117 and smooth muscle actin (SMA) in the diagnosis of various benign and malignant salivary gland neoplasms.

Materials and Methods: The study comprises 17 samples categorized into three groups: Group I consisted of 5 histopathologically normal salivary gland tissue; Group II comprised 7 cases, of which 3 cases were pleomorphic adenoma, 3 cases were myoepithelioma and 1 case was Warthin's tumor; and Group III consisted of 5 cases, of which 1 was mucoepidermoid carcinoma and 4 cases were adenoid cystic carcinoma. The selected cases were subjected to immunohistochemistry (IHC) procedure to assess the expression pattern of CD117 and SMA.

Results: In SMA, 85.8% showed severe-to-moderate intense expression among the tumor cells in benign salivary gland tumor. All the 5 malignant tumors showed the expression of SMA and 3 cases demonstrated severe expression among the tumor cells. An intense expression pattern of SMA was observed in both benign and malignant neoplasms in the periphery and stromal components of the tumor. Only two cases were positive for CD117, and connective tissue components were completely negative in both malignant and benign salivary gland neoplasms.

Conclusion: Alpha-SMA can be utilized as reliable IHC markers for salivary gland neoplasms due to its diagnostic importance in tumors with myoepithelial origin indicative of the histogenesis of salivary gland tumors.

Keywords: Myoepithelial, myofibroblast, pleomorphic adenoma, smooth muscle actin

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INTRODUCTION

Salivary gland neoplasms are relatively uncommon and account for about 2% of all the human neoplasms.[1] It was estimated that salivary gland tumors comprise 3%–10% of all neoplasms of the head and neck,[2] with an incidence estimated at 0.4%–13.5% annually/100,000 individuals worldwide.[3] In defiance to their uncommon prevalence, they pose diagnostic difficulty in clinical course when seen early due to their gross resemblance. According to the 2005 third histological classification of the World Health Organization, there are 34 benign and malignant salivary gland epithelial tumors.[4]

Although researchers have studied a diverse group of tumors over the years, the diagnosis and treatment

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of salivary gland neoplasms remain complex and challenging problems for the head-and-neck surgeons and pathologists. This is attributed to their diverse morphology, overlapping histological features and heterogeneity among these subtypes. In spite of these overlaps, the prognosis of each type is different.[5] Although hematoxylin and eosin (H&E) staining is the gold standard method used for diagnosing the salivary gland tumor, immunohistochemistry (IHC) can provide a powerful adjunct tool for pathologists to identify the cellular differentiation and assign correct classifications in difficult tumor cases.[6]

Histologically, the salivary gland comprises ducts and acini which consist of four types of cells: ductal and acinar cells (luminal cells) and myoepithelial and basal cells (abluminal cells). There are several immunohistochemical markers that have been identified for various types of cells, but the significance has not been proved as yet.

Smooth muscle (alpha-sm) actin, an isoform typical of smooth muscle cells (SMCs) and present in high amounts in vascular SMC, plays an important role in fibrogenesis. Smooth muscle actin (SMA) is intended for laboratory use in the qualitative identification of SMA protein by IHC. Studies indicate that alpha-SMA can be used in papillary lesions of the breast and can be expressed in osteoblasts of human bone.[7,8]

The c-kit proto-oncogene (CD117) is a Type III transmembrane receptor tyrosine kinase, encoded by the c-kit gene that is located on the human chromosome segment 4q11.[9,10] The loss-of-function mutations have demonstrated the crucial role of c-kit in normal growth and/or differentiation of several cell types.[11] Literature indicates that CD117 shows positivity for luminal cells, and SMA shows positivity for myoepithelial cells in salivary gland neoplasms.[6]

Hence, the present study is designed to analyze the utility of immunohistochemical markers such as CD117 and SMA in the diagnosis of various benign and malignant salivary gland neoplasms.

MATERIALS AND METHODS

Sample selection

The study comprises 17 samples categorized into three groups, namely Group I – histologically normal salivary gland (n = 5), Group II – benign salivary gland tumors (n = 7) and Group III – malignant salivary gland tumors (n = 5). The histologically normal salivary gland specimens were derived from radical neck dissection specimens. Formalin-fixed paraffin-embedded blocks of all the salivary gland neoplasm cases were retrieved from the archives of the Department of Oral and Maxillofacial Pathology, between the years 2014 and 2016. The diagnosis was confirmed by histopathological examination of H&E-stained section and classified into three groups.

Group I consisted of 5 histopathologically normal salivary gland tissue specimens. Group II consisted of 7 cases, of which 3 cases were pleomorphic adenoma, 3 cases were myoepithelioma and 1 case was Warthin's tumor. Group III consisted of 5 cases, of which 1 was mucoepidermoid carcinoma and 4 cases were adenoid cystic carcinoma [Table 1].

Immunohistochemical analysis

For immunohistochemical study, 3 μm sections were cut from formalin-fixed paraffin-embedded blocks mounted on gelatin-coated slides. The sections were deparaffinized in xylene, dehydrated in alcohol and rinsed in distilled water. Antigen retrieval was performed using heat-induced epitope retrieval in citrate buffer (pH 6.0) for 10 min in a pressure cooker. Following which, endogenous peroxidase was blocked for 10 min and protein block for 5 min. Sections were then incubated with SMA (Clone ASM-1, Leica Biosystems, Germany) and CD117 (Clone YR 145, BioGenex, San Ramon, CA, USA) for 90 min in room temperature. The sections were then counterstained with Mayer's hematoxylin. The slides were then dehydrated and mounted. Detection was performed using the Novolink Polymer Detection System (Leica Microsystems, Newcastle, UK). The sections were then counterstained with Mayer's hematoxylin and were then dehydrated and mounted using dibutyl phthalate in xylene mountant. Negative and positive controls were used in each run.

Evaluation of slides

The presence of brown-colored reactions at the site of target antigen was indicative of positive reactivity. Immunostaining

Table 1: Salivary gland neoplasms studied

Benign tumours (Group II)	No. of cases	Malignant tumours (Group III)	No. of cases
Pleomorphic Adenoma	3	Mucoepidermoid carcinoma	1
Myoepithelioma	3	Adenoid cystic carcinoma	4
Warthin's Tumour	1		

Table 2: Smooth muscle actin expression - epithelial component

	Mild		Moderate		High		Total	
	No.	%	No.	%	No.	%	No.	%
Benign	1	14.3%	3	42.9%	3	42.9%	7	100%
Malignant	1	20%	1	20%	3	60%	5	100%
Normal	0	0%	2	40%	3	60%	5	100%
Total	2	11.8%	5	35.3%	9	52.9%	17	100%

was assessed by the evaluation of a total score obtained by combining the staining intensity and staining proportion scores of SMA-positive cells and CD117-positive cells, according to the method used by Etemad-Moghadam *et al*.[12] The scoring was based on the staining seen within the tumor islands along with adjacent stromal cells. The scores of staining intensity were recorded as 0 if no positive staining cells were seen, 1 for mild positivity, 2 indicated moderate and 3 indicated strong positivity. Based on proportion of cells, they were classified as focal and diffuse. Thus, the total score index was classified as zero (0), mild (1, 2), moderate (3, 4) and high (5). The immunohistochemical-stained sections were analyzed by two independent observers.

Statistical analysis

All the results were tabulated and assessed for statistical analysis using SPSS (IBM SPSS Statistics for Mac Version 20.0, IBM software Group, Chicago, Illinois, USA). The results of the two markers among the three groups were compared using Chi-square test, and $P = 0.05$ was statistically significant.

RESULTS

A total of 17 cases including 7 benign salivary gland tumor, 5 malignant salivary gland and 5 histologically normal salivary glands were evaluated. The IHC expression pattern of alpha-SMA and CD117 in epithelial tumor cells and the connective tissue were scored based on their staining intensity and staining proportion.

In SMA, among the 7 benign salivary gland tumor tissues, 6 cases (85.8%) showed moderate to high expression among the tumor cells [Figure 1]. All the 5 malignant tumors showed expression of SMA and 3 cases demonstrated high expression in the tumor cells [Figure 2].

A high expression pattern of SMA was observed in both benign and malignant neoplasms in the periphery of the tumor cell islands. Moderate to high expression of SMA was also seen in the stromal components of both benign and malignant salivary gland tumors [Figures 3 and 4].

The expression pattern of SMA between epithelial and connective tissue components was not found to be statistically significant ($P < 0.017$) [Tables 2 and 3].

In the case of CD117, of 7 benign neoplasms, only 2 cases showed moderate expression within the tumor islands [Figure 5] and the remaining were negative [Figure 6].

Among 5 malignant neoplasms, only 1 indicated moderate expression of tumor cells and the remaining were negative. Connective tissue components showed completely negative expression in case of both malignant and benign salivary gland neoplasms [Tables 4 and 5].

DISCUSSION

Salivary gland tumors are relatively uncommon, and there exists a considerable diagnostic difficulty owing to their diverse histological features in individual lesions and the presence of a number of types and variants, in addition to overlapping histological patterns similar to those

Table 3: Smooth muscle actin expression - connective tissue component

	Mild		Moderate		High		Total	
	No.	%	No.	%	No.	%	No.	%
Benign	0	0%	3	42.9%	4	57.1%	7	100%
Malignant	0	0%	3	60%	2	40%	5	100%
Normal	5	100%	0	0%	0	0%	5	100%
Total	5	29.4%	6	35.5%	6	35.3%	17	100%

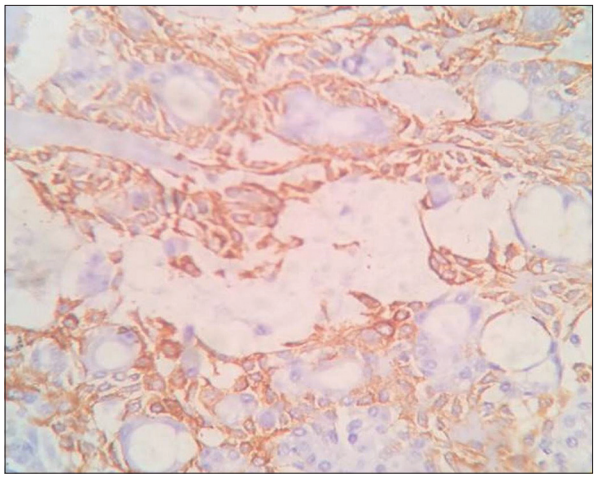

Figure 1: Strong positivity of alpha-smooth muscle actin in the epithelial component of benign salivary gland neoplasm

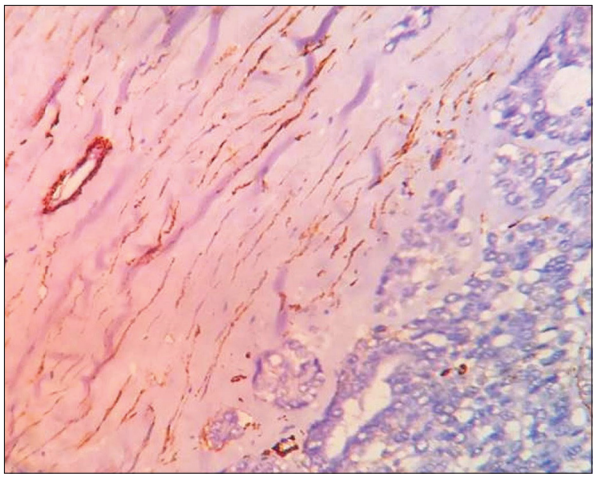

Figure 2: Strong positivity of alpha-smooth muscle actin in the connective tissue component of benign salivary gland neoplasm

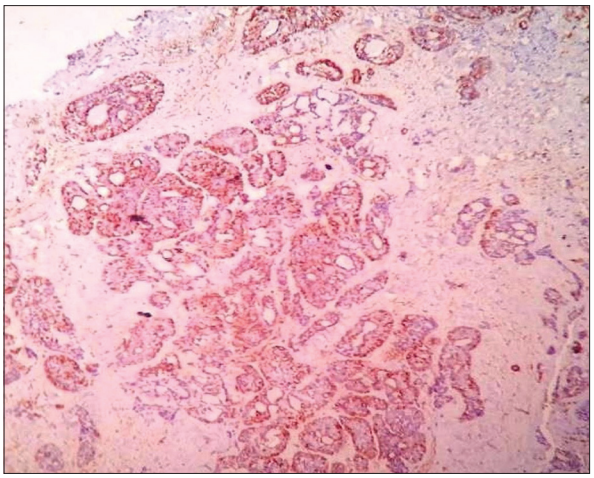

Figure 3: High expression of alpha smooth muscle actin in epithelial component of malignant salivary gland neoplasm

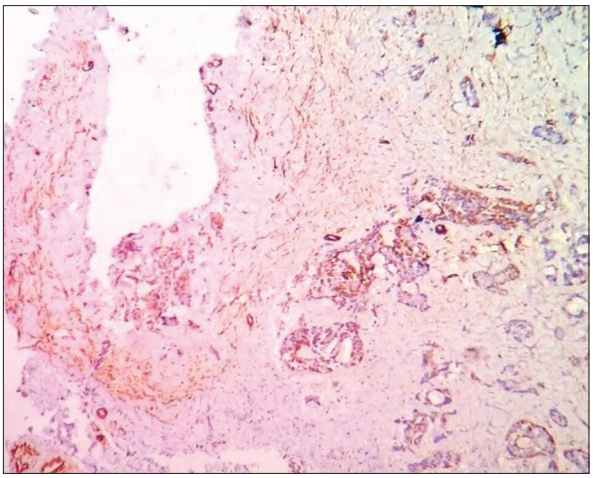

Figure 4: Alpha-smooth muscle actin-positive myofibroblasts in malignant salivary gland neoplasm

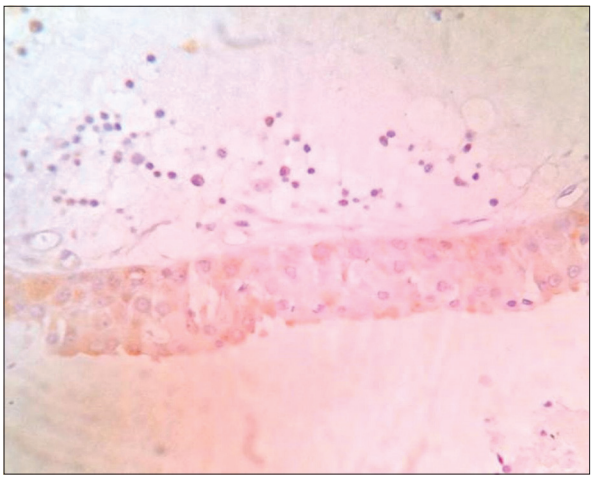

Figure 5: Mild expression of CD117 in the epithelial component of benign salivary gland neoplasm

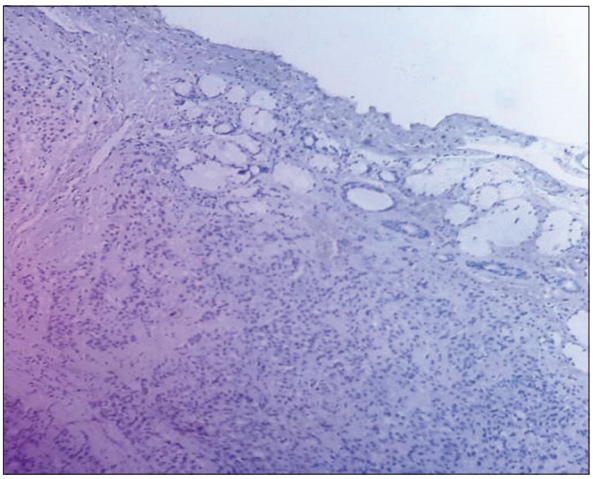

Figure 6: Negative expression of CD117 connective tissue component of benign salivary gland neoplasm

Table 4: CD117 expression - epithelial component

	Negative		Mild		Moderate		Total	
	No.	%	No.	%	No.	%	No.	%
Benign	4	57.1%	1	14.3%	2	28.6%	7	100%
Malignant	4	80%	0	0%	1	20%	5	100%
Normal	3	60%	0	0%	2	40%	5	100%
Total	11	64.7%	1	0%	5	29.4%	17	100%

Table 5: CD117 expression – connective tissue component

	Negative		Mild		Moderate		Total	
	No.	%	No.	%	No.	%	No.	%
Benign	7	100%	0	0%	0	0%	7	100%
Malignant	5	100%	0	0%	0	0%	5	100%
Normal	5	100%	0	0%	0	0%	5	100%
Total	17	100%	0	0%	0	0%	17	100%

observed in different tumor entities. The classification is complex but is closely relevant to the prognostic and therapeutic aspects.[13,14] IHC, the utilization of monoclonal and polyclonal antibodies for the detection of specific antigens in tissue sections, is an extraordinarily powerful tool in the armamentarium of the diagnostic surgical pathologist. Several studies have utilized alpha-SMA and CD117 separately, but a comparative study of the two markers by comparing their epithelial and connective tissue components has not been done. Thus, in the present study, we have compared the expression patterns of the two markers in the epithelial and connective tissue components of several benign and malignant salivary gland tumors.

In the present study, an intense (52.9%) positive expression pattern of alpha-SMA was observed in the periphery of the tumor islands in both benign and malignant tumors. These expression patterns refer to the epithelial components of the salivary gland tumors being the myoepithelial cells. These results are similar to studies by Furuse *et al.* and Savera and Zarbo, which indicated that SMA is a highly specific marker of neoplastic and nonneoplastic myoepithelial cells.[15,16]

Several immunohistochemical markers have been proposed for the identification of myoepithelial cells.[17-19] Neoplastic myoepithelium is considered a key cellular participant in morphogenetic processes, responsible for variable histological appearances of many salivary gland tumors. Neoplastic myoepithelial cells in both benign and malignant tumors can take several forms, including epithelioid, spindle, plasmacytoid and clear, and this variability largely accounts for difficulties in histopathological diagnosis.[20] However, as tumors contain myoepithelial cells in different stages of differentiation, the antibodies used show a variable affinity for these cells, especially when comparing myoepithelial cells in normal salivary glands.[15,21-24] Thus, tumors of myoepithelial origin can be recognized by their pattern in the histogenesis of the salivary gland.

A high expression of SMA (35.5%) in the connective tissue component, reflects the myofibroblasts in both benign and malignant salivary gland neoplasms indicating high invasion rate of the tumors due to the myofibroblastic reaction in the salivary gland tumors, was observed in the study. Tumor and stroma interactions are critical in determining the biological characteristics of malignancy. Myofibroblasts might be related to the aggressive growth behavior of salivary gland tumor, owing to their high levels of expression of alpha-SMA.[25] These results are synonymous to the results obtained by Savera and Zarbo.[16]

Thus, according to our study, alpha-SMA has been proved to be reliable markers of epithelial components as well as connective tissue components of the salivary gland neoplasms, indicating its participation in the histogenesis of salivary gland tumors. SMA is a highly specific marker of neoplastic and nonneoplastic myoepithelial cells. More expression of SMA indicates the aggressive nature of the tumor.

The c-kit proto-oncogene protein is a Type III transmembrane receptor tyrosine kinase that shows structural homology to the receptors of platelet-derived growth factor and macrophage colony-stimulating factor. On binding to its ligand, stem cell factor, it begins a signal cascade that contributes to the growth and differentiation of multiple hematopoietic lineages.[9] The present study results have revealed moderate positivity (29.4%) for epithelial components of both benign and malignant salivary gland tumors. This is in contrast with the previous reports in which almost all cases of both benign and malignant salivary gland tumors exhibited the expression of c-kit protein.[26-32]

However, in the present study, we observed complete negativity for connective tissue components. However, this could be due to loss of signal amplification of the protein or the decreased expression of the tumor cells itself. According to Andreadis *et al.*[29] and Penner *et al.*,[27] 50% of their examined tumors showed no expression of CD117 in connective tissue components. Thus, these results suggest that CD117 cannot be considered a reliable marker for the diagnosis of a particular salivary gland tumor.

CONCLUSION

IHC plays a limited, albeit important, role in the diagnosis of salivary gland tumors but is often useful to support the histological assessment. It is necessary to fully understand that IHC should be considered a method that can be used to assist the final diagnosis and not that can replace the H&E-based diagnosis. It should also be recognized that exceptional and unexpected results are often obtained by IHC. An IHC analysis must be performed after approximate identification of the particular tumor type by H&E staining. Thus, alpha-SMA can be utilized as reliable IHC markers for salivary gland neoplasms due to its diagnostic importance in tumors with myoepithelial origin indicative of the histogenesis of salivary gland tumors. Further studies with higher sample size can be used to derive at a more pronounced explanation of CD117 marker reliability. Furthermore, studies with more markers and more cases can derive at proper conclusive results.

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Conflicts of interest
There are no conflicts of interest.

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