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Research Article

Role of Immunohistochemical Markers in Salivary Gland Neoplasms

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A B S T R A C T

Background: Salivary gland neoplasms are rare. In recent days its incidence is gradually increasing worldwide. Hence, it is essential to diagnose the type and grade these neoplasms for early management. Though histopathological diagnosis is the gold standard for the investigation of various salivary gland neoplasms. Immunohistochemical evaluation plays a vital role in the diagnosis of such neoplasms.

Objective: The present study is done to assess the pattern of expression of various Immunohistochemical markers (IHC) and to evaluate its diagnostic significance over histopathological diagnosis in various salivary gland neoplasms.

Materials and Methods: A total number of 57 cases of salivary gland neoplasm were studied for a period of five years between August 2012 and July 2017. All the biopsy and excised specimen sections were initially examined under routine haematoxylin-eosin stain (H&E). Later immunohistochemical markers were performed, a panel of antibodies such as P63 and HER2/ neu were performed on the sections.

Result: The pattern of expression and significance of each marker, P63 and HER2/ neu, were studied on various salivary gland neoplasms. Reports were compared with the results of conventional histopathology. It was fund that more specific results were obtained in many cases of neoplasm.

Conclusions: We found that P63, which is a nuclear marker is positive in tumours like pleomorphic adenoma, myoepithelioma, mucoepidermoid carcinoma, adenoid cystic carcinoma and carcinoma ex-pleomorphic adenoma. HER2/neu shows diffuse and strong membranous staining in salivary duct carcinoma.

Keywords: Salivary Gland Neoplasms, Histomorphology, Haematoxylin-Eosin, Immunohistochemical Markers, P63 and HER2/ neu.

Introduction

Salivary glands are exocrine organs comprising of ducto-acinar units that produce and secrete saliva. They constitute three pairs of major glands: parotid, submandibular and sublingual glands. Minor salivary glands are distributed throughout the mouth.[1] Salivary gland neoplasms constitute

about 0.4 to 13.5 cases per one lakh population,[2] which accounts about less than 3% to 10% of head and neck neoplasms.[3] The WHO classification of revised 2005 salivary gland tumours account for more than 35 distinct variants of salivary gland neoplasms.[4] Some of the common benign neoplasms are pleomorphic adenoma and warthin's tumor. Malignant neoplasms are mucoepidermoid carcinoma, adenoid cystic carcinoma, acinic cell carcinoma which can be easily identified by routine haematoxylin and eosin stain.[5] However salivary gland neoplasms exhibit diverse histomorphological patterns and numerous variants in the individual lesions. Therefore, the tumors are considered to be a diagnostic challenge for the pathologist. Immunohistochemical (IHC) staining provides a powerful tool for identification of cellular differentiation and assignment of correct categorisation of these salivary gland tumours.[6] The aim of the present study is to assess the pattern of expression of IHC markers and to evaluate its significance in various salivary gland neoplasms.

Materials and Methods

The present study is a retrospective and prospective study on salivary gland tumors, which was carried out in the Department of Pathology at Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Maduranthakam, Tamil Nadu, India. This study was conducted on biopsy and excised specimens of salivary gland tumours. A total number of 57 cases of salivary gland tumours were included in this study for a period of five years between August 2012 and July 2017. Institutional Ethics Committee approval was obtained. Inclusion criteria was patients with benign and malignant salivary gland neoplasms. Exclusion criteria was patients with inflammatory conditions of salivary gland and specimens with incomplete records and discordant diagnosis. All the biopsy and excised specimens were well fixed in 10% formalin and then processed into microscopic sections and stained with H&E. Immunohistochemical markers were performed on formalin-fixed paraffin-embedded sections, the sections were taken on positively charged or coated slides. Following panel of antibodies were used: P63 and HER2/ neu were applied. The pattern of expression and significance of each marker on various salivary gland neoplasms were studied and recorded. Data was compiled in MS Excel, checked for its correctness and analyzed using online statistical calculator.

Result

A total of 57 cases of salivary gland neoplasms, which included 40 benign and 17 malignant, were studied. Among the 40 benign cases, 33were accounted for pleomorphic adenoma (Figure 1). P63 IHC marker was applied to all the benign and malignant salivary gland neoplasms. All the 40cases (100%) of benign salivary gland neoplasms were positive. Out of 17 cases of malignant salivary gland neoplasms 14 cases(83%) were positive for p63 marker. All the 33 specimens were found positive (100%); 30 of them showed strong diffuse nuclear reactivity in myoepithelial cells and the remaining three cases showed weak reactivity. Three case of Warthin's tumour showed moderate to weak reactivity. In basal cell adenoma, a weak reactivity was observed in two cases. In the case of myoepithelial adenoma, it showed a strong and diffuse nuclear reactivity in the myoepithelial cells (Table 1).

Table 1.P63 Expression in Benign salivary gland tumours

Tumours	No. of cases	No. of Positive cases	Negative	IHC Score		
				Weak	Moderate	Strong
Pleomorphic adenoma	33	33	Nil	-	3	30
Warthin'stumour	3	3	Nil	1	2	
Basal cell adenoma	2	2	Nil	2		
Myoepithelioma	2	2	Nil			2

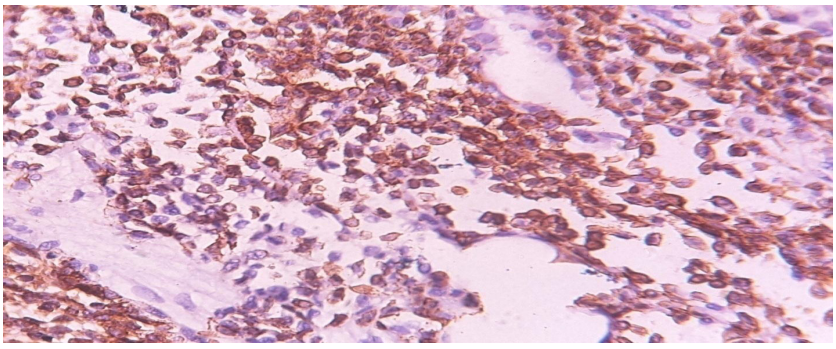

Figure 1.P63 Immunohistostaining in Pleomorphic Adenoma exhibiting Strong nuclear positivity

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Among the 17 malignant cases, six were mucoepidermoid carcinoma (Figure 2), five cases were adenoid cystic carcinoma and the remaining were one case each of polymorphous low-grade adenocarcinoma, basal cell adenocarcinoma and carcinoma ex pleomorphic adenoma. Out of six mucoepidermoid carcinoma, four cases showed strong nuclear reactivity in intermediate, squamous and clear cells, while the two others showed moderate nuclear reactivity. Out of five cases of adenoid cystic carcinoma, four cases showed moderate positivity in the nuclei of tumour cells and the remaining one case showed weak positivity. In each case of polymorphous low-grade adenocarcinoma, basal cell adenocarcinoma and carcinoma ex pleomorphic adenoma (Figure 3), showed weak nuclear reactivity for p63 and at the same time, three cases of salivary duct carcinoma were negative for P63 (Table 2).

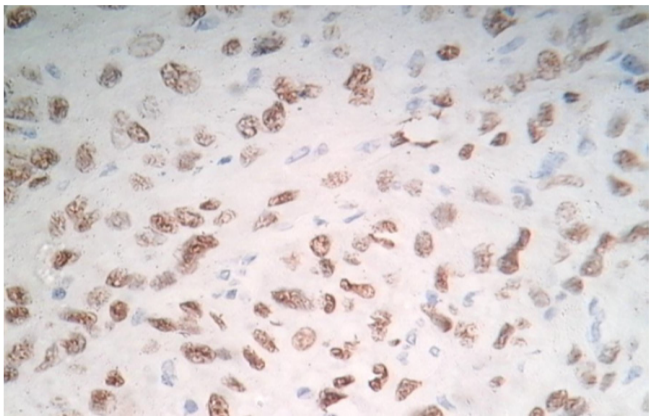

Figure 3.P63 Immunohisto staining expression in Carcinoma ex pleomorphic adenoma exhibiting nuclear positivity

Table 2.P63 Expression in Malignant salivary gland tumours

Tumours	No. of cases	No. of Positive cases	Negative	IHC SCORE		
				Weak	Moderate	Strong
Mucoepidermoid Carcinoma	6	6	Nil	-	2	4
Adenoid Cystic Carcinoma	5	5	Nil	1	4	-
Polymorphous low-grade adenocarcinoma	1	1	Nil	1		
Basal cell adenocarcinoma	1	1	Nil	1		
Salivary duct carcinoma	3	-	3	-	-	-
Carcinoma ex Pleomorphic Adenoma	1	1	Nil	-	-	1

Interpretation of P63 immunostaining

Immunohistostaining was scored as follows:

- **Negative:** less than 10% of tumour nuclear stained.
- **Weakly positive:** 10% to 25% of tumour nuclear stained.
- **Moderately positive:** 26% to 75% of tumour nuclear stained.
- **Strongly positive:** 76% to 100% of tumour nuclear stained.
- Grading was performed semi-quantitatively through double-blind study.

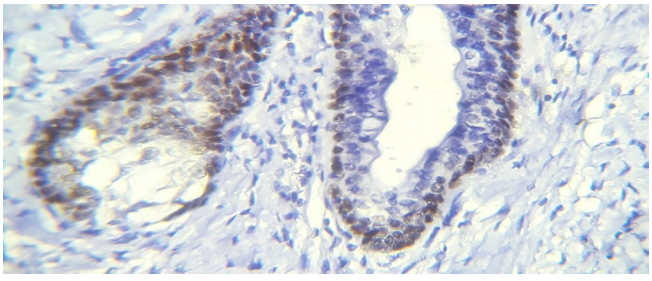

Figure 2.P63 Immunohistostaining in Mucoepidermoid carcinoma-Nuclear positivity

HER2/ neu expression

All three salivary duct carcinomas expressed diffuse and strong membrane HER2/ neu positivity in tumour cells (Figure 4).

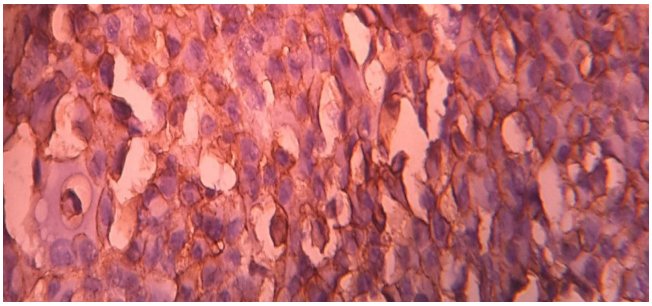

Figure 4.HER 2/ neu Immunohistostaining in Salivary duct carcinoma exhibiting membrane positivity

Discussion

Salivary gland tumours exhibit a diverse group of benign and malignant neoplasms which show multifaceted clinical presentation, variable morphological feature, architecture with unpredictable prognostic status. The present study was

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carried on 57consecutive cases of various types of salivary gland neoplasms. The main aspect of this study is to assess the pattern of expression of IHC markers and to evaluate its significance in various salivary gland neoplasms. Most of the salivary gland neoplasms arise from or differentiate towards the same cell lineage like epithelial, myoepithelial and basal cell. Similarly, each cell can also undergo different metaplastic changes, which result in overlap at all levels.[7]

A total of 57cases of salivary gland neoplasms were included in this study, out of which 40 were benign and 17 were malignant. The common benign salivary gland neoplasms observed were pleomorphic adenoma and Warthins's tumour. Similarly, in the case of malignant salivary gland neoplasms mucoepidermoid carcinoma and adenoid cystic carcinoma were commonly observed. Salivary gland tumours that exhibit diverse morphological features are a diagnostic challenge to the pathologist. IHC plays a significant role both in aiding and diagnosis of challenging cases.

Immunohistochemical Analysis of Salivary Gland Tumours

In the present study, the expression of P63 and Her2/neu in various salivary gland tumours was examined to assess their possible role in the detection and differential diagnosis of these tumours.

P63 Marker

P63 marker is a member of the P53 family of transcription factor, which is the myoepithelial marker.[8] Myoepithelial cell differentiation occurs to variable degrees in pleomorphic adenomas, adenoid cystic carcinomas, polymorphous low-grade adenocarcinomas and epithelial-myoepithelial carcinoma. Intermediate cells of mucoepidermoid carcinoma also demonstrate characteristics of modified myoepithelial cells.[9]

In the present study, all the 33 cases of pleomorphic adenoma were P63 positive (100%) of which 30 cases showed strong diffuse nuclear reactivity in abluminal myoepithelial cells and three cases showed weak reactivity. The abluminal cells took up P63marker. P63 which is the myoepithelial cell marker confirms the role of myoepithelial cells in histiogenesis of pleomorphic adenoma and myoepithelioma. This implies that the cell of origin of pleomorphic adenoma is from the intercalated duct component. In Warthin's tumour and basal cell adenoma, the basal cells were seen to express p63 positivity. There was a strong and diffuse reactivity observed in myoepithelioma. Similar results were observed by Bilal et al.,[10] who studied 32 cases of benign salivary gland neoplasm. Out of 32 cases, 15 were pleomorphic adenoma, 4 cases were myoepithelioma, 4 basal cell adenoma, 4 Warthin's tumour, 4 oncocytoma and one canalicular adenoma. All the 15 cases of pleomorphic adenoma and 4 cases of myoepithelioma were p63 positive for myoepithelial cell. Whereas the remaining benign salivary gland neoplasms showed p63 positivity for basal cells.

Out of six cases of Mucoepidermoid carcinoma in the present study, four cases showed strong nuclear reactivity in intermediate, squamous and clear cells, while two other cases showed weak positivity. Similar results were observed by Raboh et al.,[11] wherein it was reported that out of 56 cases of salivary gland neoplasms, 33 cases were mucoepidermoid carcinoma showing strong nuclear reactivity in 31 cases and moderate reactivity in 2 cases by p63 marker. Similarly, in the present study, out of five cases of adenonoid cystic carcinoma, four cases showed moderate positivity in the nuclei of tumour cells, while the other one showed weak positivity. In a study conducted by Edwards et al.,[12] out of 49 cases of malignant salivary gland neoplasms, 15 cases were adenoid cystic carcinoma, out of which 87% of cases showed nuclear reactivity by P63 marker. A solitary case of polymorphous low-grade adenocarcinoma and basal cell adenocarcinoma showed weak positivity where as the carcinoma ex-pleomorphic adenoma showed a strong nuclear positivity for P63 in the malignant squamous cells. Similar results were observed by Omitola et al.,[13] Genelhu et al.,[14] Singh et al.[15]

HER2/ neu Expression

Salivary duct carcinoma is a high-grade malignant neoplasm, which displays histological appearances similar to ductal breast carcinoma. The estrogen receptor and progesterone receptor are not detected in most salivary duct carcinomas. This finding is sometimes useful for distinguishing this tumour from breast cancer metastasis. However, more than 20% of salivary duct carcinomas show diffuse and strong membranous staining for HER2/ neu that correlate with aggressiveness of the tumour.[16] The present study reports that all the three salivary duct carcinomas encountered showed strong immunoreactivity for HER2/ neu. Our findings are in correlation with the observations made by Nagao et al.[17]

Conclusion

In the present study, we observed p63 positivity in both benign and malignant salivary gland neoplasms with exception of salivary duct carcinoma. Hence p63 can be used to exclude salivary duct carcinoma. The possible role of Immunohistochemical markers P63 and HER2/ neu were studied in the diagnosis and differential diagnosis among the various types of salivary gland tumours. P63marker confirms the role of myoepithelial cells in histogenesis of pleomorphic adenoma and myoepithelioma. This implies that the cell of origin of pleomorphic adenoma is from the intercalated duct component. In Warthin's tumour and basal

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cell adenoma, the basal cells were seen to express p63 positivity. Similarly, all the three salivary duct carcinomas encountered showed strong immunoreactivityfor HER2/ neu. However the limitations of this study included less number of available cases of each tumour type and missing of some tumour types such as acinic cell carcinomas due to its relatively infrequent occurrence. Hence, we recommend further studies to investigate the immnunohistochemical expression of p63 and HER2/ neu in other types of salivary gland tumours which are not encountered in this study.

Conflict of Interest: None

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