Section: Pathology

Original Research Article

STUDY ON EVALUATION OF EXPRESSION OF Ki67 AND p63 MARKERS BY SALIVARY GLAND NEOPLASMS IN A TERITIARY CARE HOSPITAL

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ABSTRACT

Background: Salivary gland neoplasms are uncommonly found tumors. Although histopathology is the gold standard modality for diagnosis, immunohistochemical markers such as p63 and Ki67 are used to differentiate between malignant and benign lesion and also aid in managing these neoplasms.

Materials and Methods: 50 cases of salivary neoplasms were included in this cross-sectional study which was conducted in the Department of Pathology, Malla Reddy Medical College for Women from November 2022 to October 2024.

Results: The P value calculated for association between malignant salivary gland tumors and ki67 expression was found to be statistically significant. The association between benign salivary gland tumors and p63 expression was statistically significant.

Conclusion: This study highlights that pleomorphic adenoma is the most common benign salivary gland tumor, while mucoepidermoid carcinoma is the most frequent malignant one, with Ki67 and p63 expressions showing significant differences between benign and malignant tumors..

Keywords: Salivary gland neoplasms, benign tumors, malignant tumors, pleomorphic adenoma, mucoepidermoid carcinoma, Ki67 expression, p63 expression.

INTRODUCTION

Salivary glands are responsible for the secretion of saliva, which help in lubrication of oral cavity, mastication, speech and deglutition. There are 2 categories of salivary glands- major (Parotid, submandibular and sublingual) and numerous minor salivary glands in the tongue, palate, cheek and the lips. Depending upon its composition, they can be serous, mucus or mixed type.[1]

The incidence of salivary gland lesions is greater in age between 7 to 78 years, with a male to female ratio of 1:1.08. Parotid (56.65%) is the most common organ involved, followed by the submandibular gland (31.73%). Neoplastic diseases comprise 75% of the salivary gland lesions while non-neoplastic disease accounts for 25% of the lesions. Among the neoplastic lesions, benign neoplasms constitute 53.85% (most common being pleomorphic adenoma) and malignant neoplasms constitute 21.15 %.[2]

Pleomorphic adenoma is the commonest benign tumor of the salivary gland usually originating from parotid gland followed by submandibular gland. Its occurrence in the minor salivary glands is approximately 10%.[3] Warthin's tumor otherwise called as the "papillary cystadenoma lymphomatosum", represents about 4 to 10 % of epithelial salivary gland tumors and almost exclusively arises from the parotid gland.[4] Mucoepidermoid carcinoma constitutes 30 % of all malignant neoplasm of salivary gland and is the third most common minor salivary gland tumor (15%).[5]

With the rising incidence of salivary gland lesions, it has become important to accurately diagnose the type of tumor to initiate treatment. Although H&E staining is the gold standard method to diagnose

these tumors, IHC greatly enhances the diagnostic accuracy.

Immunohistochemistry is one of the ancillary techniques in pathology and is predominantly used to obtain a histological diagnosis of morphologically non differentiated neoplasia, to identify the primary site or tissue of origin of tumors, to differentiate between benign and malignant proliferations and to identify prognosis and therapeutic efficacies.

p63 is a member of p53 family genes. It is expressed in various normal tissues like the bronchial epithelium, squamous epithelium, myoepithelial layers of breast, salivary glands and urothelium. It also regulates growth of the salivary glands and it helps in regulation of differentiation and proliferation in epithelial progenitor cells. All salivary gland neoplasms that had myoepithelial cell differentiation also express p63.[5]

Ki 67 is large (395KD), nuclear, non-histone protein encoded by the MKI67 gene and is associated with transcription of ribosomal RNA. It is expressed in all active phase of cell cycle except in G0 phase (resting cells) and so reflects the proliferation status of the cell. Ki67 labelling index is an established prognostic marker for various tumors and is also useful in determining the recurrence rate.[6,7]

MATERIALS AND METHODS

This cross-sectional study was done to assess the expression of Ki67 and p63 in various benign and malignant salivary gland tumors and to evaluate the diagnostic and prognostic significance of these markers. Approval of ethical committee was obtained to conduct this research study.

The study was conducted at the Department of Pathology, Malla Reddy Medical College for Women from November 2022 to October 2024. All salivary gland neoplasms operated in Malla Reddy Medical College for Women, (both benign and malignant neoplasms) were included in the study. Inflammatory and other non-neoplastic diseases of salivary glands were excluded. A total of 50 cases of salivary gland neoplasms were studied.

Patient details and the histopathological diagnosis were collected from the general surgical pathology report register, clinical case sheets and from medical records department of the hospital.

The expression of Ki67 and p63 of the collected cases were assessed by Immunohistochemistry (IHC).

Ki67 SCORING

Interpretation of Ki 67 staining was done based on previous studies.[8]

Three areas of the studied tissue with highest density of tumor cells expressing Ki67 were selected using low power. 1000 malignant cells from these areas were counted manually and the following formula was applied to calculate the mitotic index (MI = n x 100/1000), where n is the number of tumor cells positive for a total of 1000 counted cells. MI score 0- <1% = score 0; MI= 2– 5 % - score 1; MI = >5% - score 2.

p63 GRADING

Interpretation of p63 was done based on previous studies. 9 Only nuclear reactivity was considered positive. Levels <10% = negative; 10 – 25% - weak positive; 26 – 75% - moderate positive; 76 – 100% - strong positive.

RESULTS

This study constituted a total of 50 cases of salivary gland neoplasms (both benign and malignant lesions) who were inpatients, admitted and operated in Malla Reddy Medical College for Women during the 2-year period.

The age of occurrence of these tumors ranges from 15 years to maximum of 72 years. The mean age of occurrence is 49.6 years. Of the total 50 cases, 24 were males (48%) and 26 were females (52%). The distribution of lesion in various salivary glands was 35 (70 %) in parotid, 9 (18%) in submandibular, 6 (12 %) in other minor salivary glands.

Out of the 50 cases, 35 (70%) were benign and 15 (30 %) were malignant. Benign tumors were common in 15-60 years of age group, with mean age of patients with benign tumors being 38.7 years.

Pleomorphic adenoma was the most common benign tumor in the present study (n=28; 56 %). It had a mean age of occurrence of 35.3 years and a male: female ratio of 1:1.8. Most common site of pleomorphic adenoma in present study was the parotid gland (60%), followed by submandibular (35%) and other minor salivary glands (5%). Pleomorphic adenoma shows significant statistical difference in relation to sex, age and site of occurrence.

Malignant tumors generally affected age group from 29 to 72 years, with a mean age of occurrence of 47.09. The commonest malignant neoplasm seen in the study was mucoepidermoid carcinoma – 9 cases (18%). The mean age of occurrence was 46.8 years. It has male: female ratio of 2.4:1

The second most common salivary gland neoplasm was acinic cell carcinoma -4 cases (8 %).

There were 4 patients with Warthin's tumor; 2 patients with Basal cell adenoma; 1 patient with Acinic cell carcinoma; 1 patient with benign oncocytoma; 2 patients with malignant oncocytoma and 1 patient with salivary duct carcinoma.

Ki67 expression was studied for all the 50 cases which showed positivity for 12 cases (24%). Amongst these 12 patients, 6 cases were benign and 6 cases were malignant. Ki67 expression showed significant statistical difference in relation to benign and malignant tumors.

P63 expression was studied for all 50 cases. Out of the 15 malignant cases 4 were negative for p63 expression. All the 34 benign cases showed positivity for p63 ranging from weak positive to strong positive.

Table 1: Distribution of cases

	NO OF CASES	PERCENTAGE
BENIGN	35	70%
MALIGNANT	15	30%

Table 2: Distribution of cases according to age group

Age group	No of benign cases	No of malignant cases
0-20 years	6	0
20-40 years	11	3
40-60 years	18	8
>60 years	0	4
Total	35	15

Table 3: Distribution of benign and malignant cases

Benign	No of cases	Malignant	No of cases
Pleomorphic adenoma	**28**	Mucoepidermoid carcinoma	**8**
Oncocytoma	**1**	Malignant oncocytoma	**2**
Warthin's tumor	**4**	Acinic cell carcinoma	**4**
Basal cell adenoma	**2**	Salivary ductal carcinoma	**1**
Total	**35**		**15**

Table 4: Ki67 expression of various cases

	CASES	No of cases	No of Ki67 positive cases	Grade 0	Grade 1	Grade 2
BENIGN (17.14% positivity for Ki67)	Pleomorphic adenoma	28	4	24	3	1
	Warthin's tumor	4	1	3	1	0
	Oncocytoma	1	1	0	1	
	Basal cell adenoma	2	0	2	0	0
MALIGNANT (40% positivity for Ki67)	Mucoepidermoid carcinoma	8	3	5	2	1
	Acinic cell carcinoma	4	1	3	1	0
	Malignant oncocytoma	2	1	1	0	1
	Salivary duct carcinoma	1	1	0		1
	TOTAL	**50**	**12**	**38**	**8**	**4**

P = 0.025805

From the above table we can see that, expression of Ki67 in malignant salivary neoplasms is significantly higher than in benign tumors.

Table 5: p63 expression in various cases

	CASES	No of cases	No of p63 positive cases	Negative	Weak	Moderate	Strong
BENIGN (100% positivity for Ki67)	Pleomorphic adenoma	28	28	0	4	14	10
	Warthin's tumor	4	4	0	2	1	1
	Oncocytoma	1	1	0	1	0	0
	Basal cell adenoma	2	2	0	1	1	0
MALIGNANT (73.3% positivity for Ki67)	Mucoepidermoid carcinoma	8	7	1	4	2	1
	Acinic cell carcinoma	4	3	1	1	1	1
	Malignant oncocytoma	2	1	1	0	1	0
	Salivary duct carcinoma	1	0	1	0	0	0
	TOTAL	**50**	**46**	**4**	**13**	**20**	**13**

P value = 0.002341

From the above table we can see that p63 expression is significantly higher in benign tumors when compared to malignant salivary neoplasms.

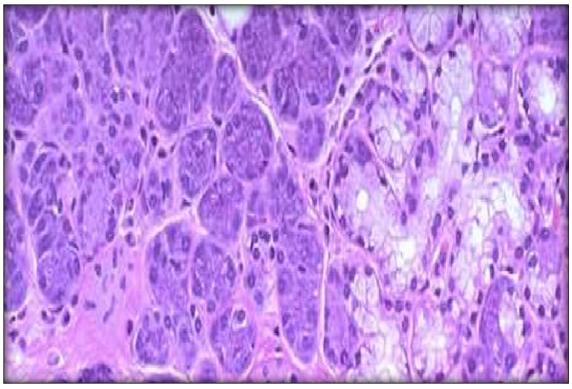

Figure 1: Submandibular salivary gland with serous and mucinous acini

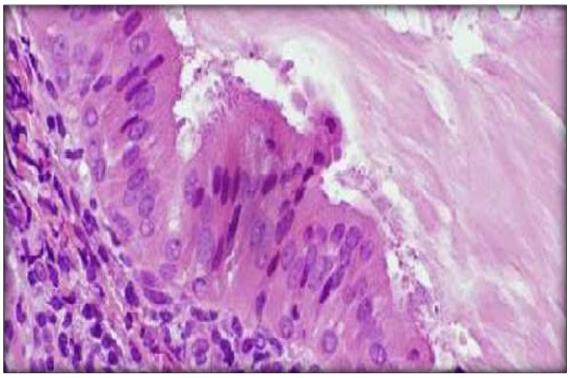

Figure 2: Warthin's tumor with double layered epithelium and lymphoid aggregates

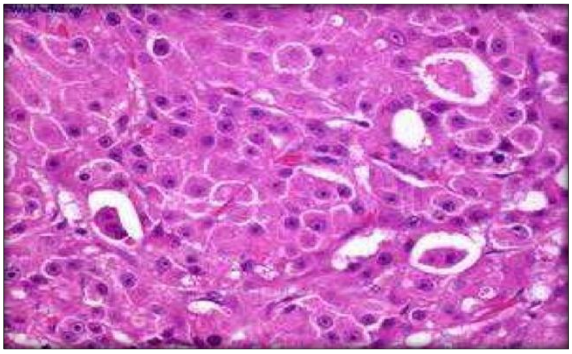

Figure 3: Oncocytoma

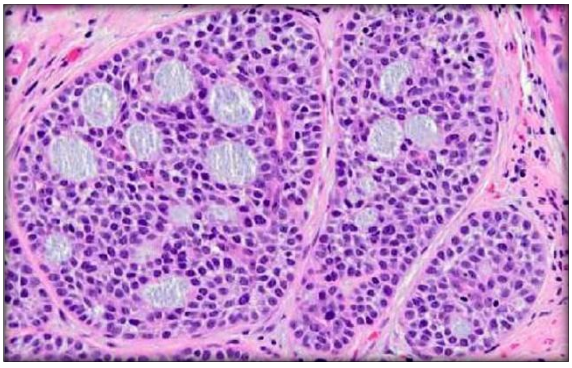

Figure 4: Adenoid cystic carcinoma

DISCUSSIONS

Salivary gland tumors are infrequent with an incidence of 2.5 to 3 per 100,000 per year.10 80% of salivary gland tumors are benign. Incidence of malignant salivary gland neoplasms range from 0.5 to 2 per 100,000 populations per year.[11] Pleomorphic adenoma which accounts for 70% of benign salivary neoplasms is the commonest benign salivary neoplasms.[12] Mucoepidermoid carcinoma is the commonest of the malignant salivary neoplasms.[13]

In our study, 35 (70%) out of the 50 salivary tumors were benign, constituting and the remaining 15 (30%) were malignant tumors. Pleomorphic adenoma was the commonest of all salivary neoplasms followed by mucoepidermoid carcinoma.

As per different studies 70 to 80% of cases occur in the parotid gland followed by submandibular salivary gland. Some authors consider minor salivary glands to be the 2nd most commonest site.[14] In our study, Parotid was the most common gland affected (70%) followed by submandibular (18%) and other minor salivary glands (12%).

Salivary gland tumors are a source of diagnostic challenge to the pathologist with their diverse morphology. IHC plays a significant role both in aiding the diagnosis of challenging cases and as a prognostic marker of salivary gland neoplasms.

Ki67 is a monoclonal antibody marker used to assess the proliferative potential of malignancy. It particularly reacts with the proliferating cells, except those cells in G0 phase of cell cycle. The strong association between the frequency of Ki67 positivity and higher degree of malignancy has been well established by various studies for various malignancies.

In our study, out of 35 benign neoplasms, 6 were positive for Ki67. Amongst these 6 patients, 4 patients had pleomorphic adenoma. According to a study conducted by Mioara Trandafirescu et al (2012),[15] Ki67 positivity in pleomorphic adenoma is taken as predictor of malignancy risk. In their study IHC profile done for 30 patients with pleomorphic adenoma, Ki67 showed a positive reaction in myxoid areas and in peripherally situated myoepithelial cell layers. Moreover according to the study, intensity of Ki67 reaction was higher in Carcinoma ex Pleomorphic adenoma. The study also states that Ki67 marker is useful in assessing the intensity of proliferation in cases of Pleomorphic adenoma and thereby gives indications of risk of malignancy. The above mentioned 4 cases of pleomorphic adenoma which showed Ki67 positivity have been kept under follow up to detect any recurrence or malignant transformation.

In present study, the germinal center of Warthin's tumor consisting of lymphoid cells showed positivity for Ki67.

In a study conducted by Wilson TC et al,[16] Ki67 expression aids in differentiation between basal cell

adenoma and its malignant counterpart (basal cell adenocarcinoma). Increased Ki67 expression (>5%) is seen in basal cell adenocarcinoma, when compared basal cell adenoma.

In case of malignant neoplasms, 6 out of 15 showed Ki67 positivity. A total of 8 mucoepidermoid carcinomas were stained for Ki67 out of which 3 were positive. Skalova et al,[17] had studied 46 cases of mucoepidermoid carcinoma and observed that low Ki67 expression had a benign clinical course whereas high Ki67 expression was associated with aggressive clinical behavior.

We had 4 cases of acinic cell carcinoma in our study and one was tested positive for Ki67 expression. Hellquist et al,[18] studied 32 acinic cell carcinomas and concluded that ki67 is a significant marker of acinic cell carcinoma and is also an independent prognostic factor for survival of patients of acinic cell carcinoma.

One case of malignant oncocytoma and one case of salivary duct carcinoma, were both positive for Ki67 expression in present study.

P63 is a member of p53 family of transcription factors and is expressed in nuclei of myoepithelial and basal duct cells in normal salivary glands. In a study conducted by Bilal H et al,[19] p63 was seen to be expressed by all salivary gland tumors that differentiated towards luminal and myoepithelial lineages.

In accordance to the above mentioned study, all the benign tumors in our study were all positive for p63 expression.

In Warthin's tumor, the basal cells were seen to express p63 positivity, which is similar to the study conducted by Bilal H et al, in which p63 is expressed by the basal cells of both Warthin's tumor and oncocytoma.

In our study which constituted of 15 malignant cases, 11 cases tested positive for p63 immunostaining, which included 7 cases of mucoepidermoid carcinoma, 3 cases of acinic cell carcinoma, and 1 case of malignant oncocytoma. According to the study conducted by Nermine M Abd Raboh et al,[19] there was no difference in the staining pattern of p63 in different tumor grades of mucoepidermoid carcinoma. In the study conducted by Sams RN et al,[20] it is said that differentiation of acinic cell carcinomas from mucoepidermoid carcinoma can be histologically difficult as both exhibit mucin production and in such cases p63 staining helps to differentiate the two entities.

CONCLUSION

At the end of this pilot study, we conclude that the expression of Ki67 was very much significant in cases of malignant salivary neoplasms and the expression increases with increasing grade of malignancy. p63 expression was mostly seen in benign tumors which was statistically significant.

This is an ongoing study and evaluation of the IHC expression of the studied markers with more number of cases could throw more light on significance of the role of these IHC markers.

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Conflicts of interest: Nil.

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