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Comparison Of Immunohistochemical Expression Of Epidermal Growth Factor Receptor In Benign And Malignant Salivary Gland Tumors

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ABSTRACT

Objective

To compare the immunohistochemical expression of epidermal growth factor receptor (EGFR) in benign and malignant salivary gland tumors.

Methodology

A total of 100 cases of benign and malignant salivary gland tumors (50 each) were retrieved from histopathology lab of Peshawar Medical College, Peshawar (PMC) and Pakistan Institute of Medical Sciences, Islamabad (PIMS). Their clinical information such as gender, age and site of tumor was recorded and Hemotoxylin and eosin examination was done to confirm the diagnosis. Total immunoreactivity score of EGFR expression was determined as the sum of the staining percentage and intensity.

Results

Among 100 cases included in our study, EGFR immunopositivity was seen in 69% (n=69) of cases collectively of both Benign & Malignant Salivary Gland Tumors. Statistically, a strong correlation was observed between EGFR expression and salivary gland tumors ($p \leq 0.05$).

Conclusion

This research concludes that malignant salivary gland tumors (MSGTs) show a higher EGFR expression. Positive EGFR expression seen in benign salivary gland tumors (BSGTs) might indicate their aggressive behavior, recurrence and epithelial proliferation especially in Pleomorphic adenoma cases.

Key words: EGFR, Immunohistochemistry, Mucoepidermoid Carcinoma, Pleomorphic Adenoma, Salivary gland tumors

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INTRODUCTION

Neoplasms of the salivary glands are not very prevalent. These tumors make for 6.3% of all head and neck tumors and 1% of all body tumors.[1] Studies have shown that females are affected by tumors more frequently than males, with a male to female ratio of 1:1.36.[2] Possible risk factors which may be responsible for tumor of salivary glands include therapeutic radiations and occupational exposure to different chemicals.[3] In both benign and malignant tumors, the parotid gland is thought to be the most probable site for salivary gland tumors. Hard palate is most commonly affected site among minor salivary glands.[4] Histologically neoplasms of salivary glands are diverse group of lesions with complex clinical, histological and morphological pattern which pose challenges regarding diagnosis for the pathologists.[5] The Erythroblastosis Oncogene B (ErbB) family of tyrosine receptors includes the EGFR.[6] EGFR is expressed differently in various human tissues and is involved in cell signaling pathway that control cell proliferation, division, and mitosis, as well as in cancer development.[7] Overexpression of EGFR occurs in many tumors like head and neck cancers, glioblastoma, lungs, prostate, ovaries, bladder and colorectal carcinomas.[8] Although overexpression EGFR has been associated with low survival rate and drug resistance, studies claim that targeted therapy against EGFR has favorable results hence several inhibitors have been tested in different cancers and have shown promising results.[9]

METHODOLOGY

The experimental work was conducted at Department of Pathology, Peshawar Medical College Labs, Histopathology Division, Riphah International University; Islamabad after obtaining the ethical approval from review board committee. 50 benign and 50 malignant formalin fixed paraffin embedded tissue

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blocks of salivary gland tumors were extracted. The study lasted for six months. Relevant clinical data of cases like gender, age and site of lesion which was present in reports was entered in predesigned pro-forma. Slides of the selected cases were examined and selection of blocks was done. Two slides of about four-five micron thin sections were prepared from each block, one stained with Hemotoxylin & Eosin for histological study. Additional 6 slides for positive control were prepared and mounted having known EGFR positive carcinoma of breast.

For immunohistochemistry, incubation of sections with anti-EGFR antibody followed by application of chromogen and then counterstaining of slides was done by immersing them in hemotoxylin. EGFR Immunoreactivity was assessed by immunoreactivity score (IRS; sum of the intensity and percentage of positively stained cells).

The statistical package for the social sciences (SPSS) version 20.0 was used to conduct the statistical analysis. Using the Chi square test, the categories of benign and malignant salivary gland tumors were compared for categorical characteristics such gender and tumor behavior. In cases where the values were lesser than 5, Fisher's exact test was used. Comparing continuous data, such as mean age, proportion, and intensity between benign and malignant salivary gland tumor categories, was done using the Student's T-test. P values less than or equal to 0.05 were deemed statistically significant ($p \leq 0.05$).

RESULTS

The range of age was between 13-80 years. Mean age was 39.0±14.0 in benign and 45.7±16.5 years in MSGTs. There were 22 males and 28 females in benign tumors whereas there were 28 male and 22 females with malignant tumors. Most patients with tumors were found to be between the ages of 21 and 40, then between 41-60 years. Maximum numbers of males were in age range of 41-60 years whereas maximum numbers of females were in age range of 21-40 years. (Table 1)

	Benign tumors (n=50)	Malignant tumors (n=50)	Total (n=100)
Age categories			
1-20	4 (8%)	4 (8%)	8 (8%)
21-40	28 (56%)	13 (26%)	41 (41%)
41-60	14 (28%)	25 (50%)	39 (39%)
>60	4 (8%)	8 (16%)	12 (12%)
Age(years)			
Mean	39.0	45.7	42.4
Standard Deviation	14.0	16.5	15.6

Table 1 Age range of patients with salivary gland tumors

Site of lesion between two groups was found to be significantly variable. The parotid gland was the most often involved site in this research for both benign and malignant salivary gland tumors i.e. 56% and 30% respectively followed by submandibular gland (18%) in benign tumors. Palate was the second most prevalent site for malignant tumors (26%) followed by submandibular gland (24%) (Table 2).

Site of lesion	Benign tumors	Malignant tumors	Total
Submandibular gland	9 (18%)	12 (24%)	21
Parotid gland	28 (56%)	15 (30%)	43
Palate	4 (8%)	14 (28%)	18
Tonsillar growth	2 (4%)	1 (2%)	3
Maxilla	-	1 (2%)	1
Mandible	-	1 (2%)	1
Buccal mucosa	3 (6%)	1 (2%)	4
Nasal mucosa	-	4 (8%)	4
Lip	4 (8%)	-	4
Tongue	-	1 (2%)	1

Table 2 Comparison of site of lesion between benign and malignant salivary gland tumor

Among benign tumors pleomorphic adenoma was the most common type (40%) observed in our study whereas among malignant salivary gland tumors Adenoid Cystic Carcinoma (20%) was the commonest followed by Mucoepidermoid Carcinoma (18%). 7 cases of Polymorphous Low Grade Adenocarcinoma were also observed. 2 cases of Acinic Cell Carcinoma and C Ex-Pleomorphic Adenoma were seen and only one case belonged to Salivary Duct Carcinoma. Total staining intensity and percentage of stained cells were added to determine the final immunoreactivity score.

Total staining intensity and percentage of stained cells were summed to determine final score. All cases included in our study were evaluated for EGFR. Out of 100 cases, 31 had low EGFR expression, 19 had intermediate expression and 50 cases showed strong EGFR expression. The difference in intensity and proportion was found to be statistically highly significant.

DISCUSSION

Less than 1% of all body tumors and 2-6% of head and neck tumors are salivary gland tumors, which are rare malignancies with unparallel diversity with regards to clinical, histological and biological aspects. In our study total 100, out of which, 50 benign and 50 malignant, already diagnosed cases of salivary gland tumors from Peshawar Medical College, Pakistan Institute of Medical Sciences and City Medical Labs were included. The majority of the observed cases ranged in age from 21 to 40. There were very few younger patients; only 8 cases were below 20 years

Histological type of tumor	Final score of EGFR Expression Status			Total
	Low	Intermediate	High	
Pleomorphic Adenoma	28	10	2	40
Warthin tumor	0	0	4	4
Myoepitheloma	2	0	2	4
Oncocytoma	0	0	2	2
Mucoepidermoid carcinoma	0	4	14	18
Adenoid cystic carcinoma	1	5	14	20
Polymorphous low grade adenocarcinoma	0	0	7	7
Acinic cell carcinoma	0	0	2	2
Salivary duct carcinoma	0	0	1	1
Carcinoma ex-pleomorphic adenoma	0	0	2	2
Total	31	19	50	100

Table 3: Final score of EGFR expression in salivary gland tumors

of age whereas most of the patients were of older age as mentioned in table 1. According to study performed by Erdem Mengni in 2020, he found out that incidence of tumor increased after age of 40 years and these tumors were rarely found inchildren. Most of cases were above age of 25 years which is in line with our study.[10] A different study performed in 2020 on Brazilian population reported that the age group ranged from 20-30 years in majority of patients which is in agreement to our study.[11] The findings of study performed by Liao in 2020 revealed that most of salivary gland tumors occur above 50 years of age which contradicts with our current study.[12] Another study on salivary gland tumors was conducted in 2022, where the majority of cases were above 50, which is not consistent with our study.[13]

In our study, male and female genders were equal with male to female ratio as 1:1. Maximum numbers of males were in age range of 41-60 years of age whereas maximum numbers of females were in age range of 21-40 years of age which contradicts a study where male: female ratio was found as 1:3.[14] Study was performed on Brazilian population with male : female ratio of 1.2:1[11], which contradicts with current study. These changes found in gender distribution can be due to multiple reasons such as life style and geographical location. Use of tobacco, genetical predisposition might also be the reason. Occupational exposures to certain chemicals like nickel compounds, rubber manufacturing, employment in beauty salons; wood working and asbestos mining are considered as cause of salivary gland cancer development in male gender.

The next focus of our study was histologic type of salivary gland tumor. Pleomorphic adenoma was the most frequent benign salivary gland tumor in our research. Mucoepidermoid carcinoma is the most prevalent malignant salivary gland tumor, according to the World Health Organization.[15] Several investigations conducted in European nations as well as in our study it was found that the most prevalent malignant salivary gland tumor was Adenoid cystic carcinoma. In a study carried out in Southern Poland it was observed that with advancing age, incidence of Adenoid Cystic carcinoma also increased accounting 30.5% of malignancies. This result is in accordance with our study.[16] Pleomorphic adenoma and adenoid cystic carcinoma were the most prevalent benign and malignant salivary gland tumors, respectively, in a study conducted in Southwest China[17] which is consistent with our research. Similarly another study was carried out and the findings of this study performed on Chinese population in 2019 revealed that Mucoepidermoid carcinoma, contrary to the findings of the present research, is the most frequent malignant salivary gland tumor.[18] The most prevalent malignant salivary gland tumor, according to a completely different study conducted in 2019 on the Brazilian population, was an Adenocarcinoma not otherwise specified. His findings contradict with our current study.[19,20]

In our study, the parotid gland followed by submandibular gland was the two sites where salivary gland tumors were most frequently seen and then hard palate and other minor salivary glands. In this research the most frequent lesion site in Benign salivary gland tumors was parotid gland 28 (56%) followed by submandibular gland 9 (18%). On the other hand the most frequent site in malignant salivary gland tumors were parotid glands 14 (28%) , palate 13 (26%) and submandibular gland 12 (24%). In a study performed on Hiroshima population most common sites were parotid gland followed by submandibular and then other minor salivary glands which is similar to our study.[6] Ogle study reveals a different study pattern. Frequency of tumor in parotid salivary gland was 80% followed by submandibular and then minor salivary glands which resembles our study.[21] Another Israeli study found that the submandibular, parotid, sublingual, and other minor salivary glands were the most often affected regions. This study is different from our study.[22] Mengni concluded from his research that the submandibular gland was the most usual site for salivary gland tumor followed by parotid and then other minor salivary glands which is contradictory to our study.[15]

Number of positive cells and intensity of EGFR interpretation was assessed according to benign and malignant salivary gland tumors. In our study EGFR Immunoreactivity was assessed by immunoreactivity score (IRS; sum of the intensity and percentage of positive cells). Thirty one out of 100 salivary gland tumor cases showed weak EGFR expression, 19 cases showed intermediate expression and 50 cases showed strong EGFR expression, so 69% of cases showed positive expression. Among BSGTs that were included in study 30 cases showed negative EGFR expression and 20 cases showed positive EGFR expression. Among malignant salivary gland tumors 1 case showed negative expression and 49 cases showed positive EGFR expression. According to study conducted by Szewczyk in 2019, 92 out of 117 (78.6%) cases

showed EGFR positivity whereas 25/117 (21.4%) cases were EGFR negative.[23] According to another study conducted in November 2020, EGFR expression rate of 86.5% was observed.[12]

CONCLUSION

This study concludes that benign and malignant salivary gland tumors express EGFR in any age group and gender irrespective of their biological and histological behavior however malignant salivary gland tumor shows a higher EGFR expression Our study showed positive EGFR expression in benign salivary gland tumors as well, which might indicate their aggressive behavior, recurrence and epithelial proliferation especially in Pleomorphic adenoma cases

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CONFLICT OF INTEREST

Author declared no conflict of interest

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