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COMPARISON OF IMMUNOHISTOCHEMICAL EXPRESSION OF INSULIN LIKE GROWTH FACTOR 1 RECEPTOR (IGF-1R) IN BENIGN AND MALIGNANT SALIVARY GLAND TUMORS

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ABSTRACT

Objectives: *To evaluate the difference in immunohistochemical expression of insulin-like growth factor-1 receptor (IGF-1R) in diagnosed cases of benign and malignant salivary gland tumors.*

Materials and Methods: *This Comparative Cross-Sectional Study was conducted at the Department of Pathology, Peshawar Medical and Dental College, Peshawar. A total of 100 Salivary Gland Tumors from 56 male and 44 female patients were included in the study, with an average age of 43.50 ± 15.21. The Immunohistochemical Expression of Insulin-like Growth Factor 1 Receptor was determined on both benign and malignant salivary gland tumors using the Bio SB Polyclonal IGF-1R antibody (rabbit, BT-AP04398). The collected data were analyzed using SPSS version 25.*

Results: *For the immunohistochemical expression of IGF-1R on benign and malignant salivary gland tumors, the Allred Scoring method was utilized. P value of <0.05 was considered significant, but no positive significance was found between the expression of IGF-1R and salivary gland tumors.*

Conclusion: *IGF1R expression was positive in various SGTs but no statistically significant difference was seen in multiple histopathological types of benign and malignant SGT*

Key words: *Insulin-like Growth Factor 1 Receptor (IGF-1R), Salivary Glands, Benign Salivary Gland Tumor, Malignant Salivary Gland tumor*

INTRODUCTION

Salivary gland tumors (SGTs) are rare neoplasms of diverse characteristics, providing pathologists with diagnostic challenges due to this extensive variation[1]. In accordance with the 2017 histological classification of salivary gland tumors by WHO, 11 types of benign SGTs and 22 types of malignant SGTs are identified[2] Every 3 patients among 100 head and neck tumor cases are affected by SGT[3]. Up to 13.5 people per 0.1 million population are affected by SGTs each year[4].

The Parotid gland is most frequently affected by SGTs, followed by the submandibular gland and palatal minor SGs while the Sublingual gland is rarely affected[5]. The most prevalent histopathological type of benign neoplasm of the salivary gland reported was Pleomorphic Adenoma (PA) followed by Warthin's tumor (WT). Among malignant tumors, Mucoepidermoid Carcinoma (MEC) was the most frequently seen salivary gland malignancy followed by Adenoid Cystic Carcinoma[6].

Diagnosis of SGTs often get difficult because of wide range of histological and biological variation of these tumors. Immunofluorescence and immunohistochemistry are use apart from hematoxylin and eosin staining for accurate diagnosis of SGTs. Different biomarkers are being used for this purpose[7].

Insulin-like growth factor 1 (IGF1) is a single chain polypeptide made up of 70 amino acids (aa)

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that plays an integral part in cell differentiation, proliferation, and apoptosis[8]. Studies show that increase signals from IGF-1R can result in the malignant transformation of non-cancerous cells.

(4) Elevated IGF-IR levels are also correlate with advanced tumor stage, HPV negativity, poor progression and reduced overall survival[9].

The aim of this study was to compare insulin like growth factor-I receptor expression in benign and malignant salivary gland tumors as studies reveal that the tumors with higher IGF 1R expression are more resistant to chemotherapy with resultant treatment failure.

MATERIALS AND METHODS

After obtaining ethical approval from the review board committee of Peshawar Medical and Dental College and Riphah International University (IRB Approval No: Prime/IRB/2021-360). Sample size was calculated using G Power software, with a one tail test, with an effective size of 0.5, α 0.05 (margin of type 1 error) with power of 80 (minimum probability acceptable for type 2 error is 20%) the experimental work was done in the Department of Pathology at Peshawar Medical and Dental College with in a time span of 1 year. 100 cases of SGTs (50 benign, 50 malignant) were taken from Pakistan Institute of Medical Sciences (PIMS), Islamabad and Peshawar Medical College, Peshawar and were processed at the histopathology and immunohistochemistry laboratory of Peshawar Medical College, Peshawar. Paraffin blocks were sectioned by using a microtome with 4-5 microns thickness. Two sections were taken from each benign and malignant tumor available. 1 for H&E staining, 1 for immune detection. To perform immunohistochemistry, sections were incubated with IGF-1R antibody, then chromogen was applied, and finally, the slides were counterstained by rinsing them in hematoxylin. This study included cases of benign and malignant salivary gland tumors diagnosed at PMC, PIMS between 2011 and 2022. Cases with a history of prior radiotherapy or chemotherapy were not considered. Allred scoring method was used to score the immunohistochemical expression of IGF-1R. Data was statistically analyzed by using SPSS version 25. To compare IGF-1R expression on benign and malignant SGTs, Chi square test was applied by using two by two contingency tables. P value ≤ 0.05 was considered as significant.

RESULT

Immunohistochemical Expression of IGF-1R was observed in salivary gland tumors of 56 male and 44 female patients who were between 13-100 years of age and had mean age of 43.50 ±

15.21. Both benign and malignant SGTS were involved in our study. Benign tumors that were observed in our study were Pleomorphic Adenoma

Table 1: Immunohistochemical expression of IGF-1R in Histopathological types of Salivary Gland Tumor

Tumor Type	Intensity of stained cells			Percentage/Frequency of stained cells per 5mm diameter				
	Negative	Weak Positive	Strong Positive	≤1% stained cells 2-3	1-10% stained cells 5-25	11-33% stained cells 27-85	34-66% Stained Cells 85-165	67-100% stained cells 167-250
Pleomorphic Adenoma	8	13	20	0	14	15	9	3
Warthin's Tumor	0	0	3	0	0	0	3	0
Myoepithelioma	0	3	1	0	0	1	2	1
Oncocytoma	0	0	2	0	0	1	0	1
Malignant Tumors								
Mucoepidermoid Carcinoma	4	8	6	0	1	6	8	3
Adenoid Cystic Carcinoma	5	10	4	1	1	13	3	1
Acinic Cell Carcinoma	0	1	2	0	0	0	1	2
Salivary Duct Carcinoma	0	1	0	0	1	0	0	0
Carcinoma Ex- Pleomorphic Adenoma	0	0	3	0	0	2	0	1
Pleomorphic Low Grade Adenocarcinoma	2	4	0	0	2	0	4	0

(PA), Warthin's Tumor (WT), Myoepithelioma (ME) and Oncocytoma(OC). While malignant tumors involved were Mucoepidermoid Carcinoma (MEC), Adenoid Cystic Carcinoma (ACC), Salivary Duct Carcinoma (SDC), Acinic Cell Carcinoma Carcinoma (AciCC), Ex-Pleomorphic Adenoma (CXPA), and Pleomorphic Low Grade Adenocarcinoma (PLGA)

Table 2: Final Score Of IGF-1R In Salivary Gland Tumors

Tumor Type	Intensity of stained cells			P-value
	Negative	Positive	Total	
Pleomorphic Adenoma	14	27	41	0.234
Warthin's Tumor	0	3	3	
Myoepithelioma	0	4	4	
Oncocytoma	0	2	2	
Malignant Tumors				
Mucoepidermoid Carcinoma	4	14	18	
Adenoid Cystic Carcinoma	2	17	19	
Acinic Cell Carcinoma	0	3	3	
Salivary Duct Carcinoma	1	0	1	
Pleomorphic Low Grade Adenocarcinoma	2	4	6	
Carcinoma Ex-Pleomorphic Adenoma	0	3	3	
Total	23	77	100	

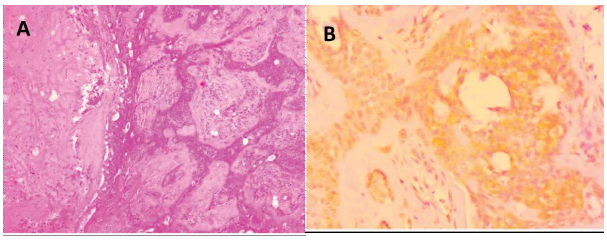

Fig 1: Hematoxylin and Eosin staining (A) and Immunohistochemical Expression of IGF1R (B) in Pleomorphic Adenoma (40X)

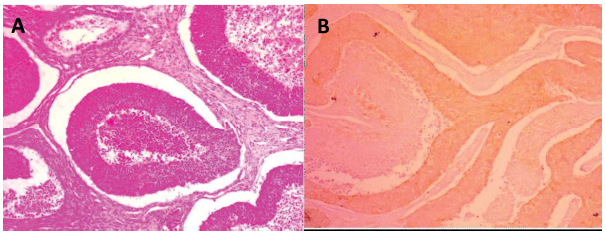

Fig 2: Hematoxylin and Eosin staining (A) and Immunohistochemical Expression of IGF1R (B) in Mucoepidermoid Carcinoma (40X)

Immunohistochemical expression of IGF-1R was scored according to Allred Scoring System.

Intensity and percentage of stained cells was observed (fig1,2) followed by addition of the 2 values to get the final score (Table 1).

Among 50 benign tumors 14 had negative expression while 36 show positive expression whereas in case of malignant SGT 9 cases were negative and 41 were found to be positive for IGF-1R. p value appeared to be 0.234, which was not statistically significant (Table 2).

DISCUSSION

SGTs are complex, heterogeneous lesions that can affect major or minor salivary gland. It is often challenging for clinicians to diagnose SGTs due to the variety in their clinical and histological presentation, as well as their growth pattern and traits. Our study was part of the effort to study different histopathological types of benign and malignant salivary gland tumors on basis of their immunohistological expression of IGF1R

In our study most cases of PA stained positive for IGF1R only few cases showed a negative result. Other benign tumors including WT, ME, OC stained positive for IGF1R.

A study done in Iran showed similar results to our study and states that higher IGF-1R expression was seen in both new and recurrent cases of PA[10]. Study results of Fırat University, Turkey were also in support of our research and showed a higher IGF1R expression in benign SGT[11].

Study performed by University of Michigan United States also show higher IGF-1R expression in malignant salivary gland tumors[12]. Study done by Mee-Young Ahn also proves the excessive expression of IGF-1R on MEC[13].

Study results of Mauro Morassi on adenoid cystic carcinoma suggest that interaction of IGF-1R with IGF2 is responsible for initiation of AKT and MAPK signaling pathway which results in proliferation of cells of ACC[14]. Another study done on ACC reveal that 50- 60% activity of 2 main oncogenic factors MYB and NFIB is dependent on IGF-1R and thus downregulating IGF- 1R decrease almost half of the activity of MYB-NFIB miRNA thus reduce protein expression and in turn cell proliferation[15]. Constant

activation of IGF-1R along with EGFR and MET is also proven in tumor cells of ACC by University of Michigan School of Dentistry, USA study[16].

In a Study done in Japan higher genetic expression of IGF-1R was observed along with genetic expression of ERBB2, VEGFA, XBP1, and RB1 on SDC and CXPA[17]. N. Chooback and teams worked on carcinoma ex-pleomorphic adenoma also show higher IGF-1R expression along with some other genes[18]. A study done by Mattias K. Andersson in University of Gothenburg, Sweden showed IGF-1R as targeted receptor in treatment of Adenoid cystic carcinoma[15].

Although, there was a significant correlation between our findings and several earlier investigations. However, our study does not demonstrate any significance (p=0.234) of IGF1R to distinguish between different histopathological types of benign and malignant salivary gland tumors.

This study was constrained by time limitations and focused solely on cases from PM&DC Peshawar and PIMS Islamabad. It is conceivable that the results could vary with more extensive timeframes and broader geographical coverage. Furthermore, the literature concerning the immunohistological expression of IGF1R on malignant salivary gland tumors is scant, indicating a significant gap for further research in this domain.

CONCLUSION

Study concluded that in most of the histopathological varients of benign and malignant SGT, IGF1R expression was positive but no statistically significant difference was seen in various histopathological types of benign and malignant SGT. Thus IGF1R might not help as a diagnostic marker for differentiation of benign SGT from malignant SGT but might indicate a prognostic significance since increased IGF1R expression in tumors is associated with more resistant to chemotherapy resulting in decreased treatment response.

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